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

THESIS

for the

Degree of Doctor of Philosophy

by

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SUMMARY

Previous investigations have demonstrated that the cestode, Hymenolepis diminuta, is rejected immunologically from the mouse small intestine. This host-parasite relationship provides a model for the study of immunity to adult tapeworms which were formerly thought to be immunologically inert. The work described herein was undertaken to investigate the specific mechanisms underlying the response of the mouse to H. diminuta and to determine the possible effect of the response on a physiological aspect of the host-parasite relationship.

Whole-body X-irradiation of the mouse was used extensively as a tool to deplete the immune response and induce suppression of worm rejection. Initial investigations using single cysticercoid infections of H. diminuta in random-bred mice indicated that effective suppression of worm rejection was achieved with either lethal or sublethal doses of X-irradiation. Succeeding investigations (which employed five- or six-cysticercoid infections in inbred mice to reduce variation), therefore, used only sublethal doses of irradiation to eliminate the necessity for subsequent reconstitution of the haemopoietic tissues.

Growth of H. diminuta following irradiation given early in infection was found to be less than growth following irradiation given later in infection. The response inducing worm rejection was found to be biphasic with respect to radiation sensitivity: irradiation on or before day 8 post-infection effectively suppressed worm rejection, whilst irradiation on day 10 had no effect on the normal course of worm rejection.

Restoration of the irradiation-depleted response was attempted using various combinations of lymphoid and bone-marrow cells, but reconstitution was not achieved using any of the cell combinations. It is suggested that X-irradiation induces a defect additional to simple depletion of small lymphoid elements and that, in particular, local antibody production in the intestine of infected mice be investigated following irradiation.

The unresponsiveness of athymic nude mice and adult-thymectomised, irradiated, bone-marrow reconstituted mice to single H. diminuta infection demonstrates that the response inducing rejection of H. diminuta requires the presence of fully differentiated T cells. Total weight of worms recovered from nude mice infected with 10 cysticercoids of H. diminuta was less than from single worm infections in nude mice, suggesting the presence of a threshold of immunological stimulation dependent on surface area of worm rather than on worm weight.

The effect of intestinal inflammation, induced by Trichinella spiralis, on a concurrent infection with H. diminuta was investigated. It was demonstrated that no cross-immunity exists between the two parasites. The inflammatory response produced marked adverse effects on H. diminuta manifested variously by reduced worm growth, destrobilation and worm rejection from both mice and rats. The severity of the effect on H. diminuta was shown to be dependent on the timing of acute inflammation with respect to development of H. diminuta. In the mouse, if severe inflammation occurred at, or shortly following the time of infection with H. diminuta then, although many H. diminuta established and survived for at least 2 days, they did not grow and the majority were subsequently expelled. If, however, H. diminuta was allowed

to establish for 5 or 6 days prior to the appearance of severe inflammation, then although the worms destrobilated, most survived the inflammatory response. In the rat, growth of H. diminuta was stopped by the inflammatory response to T. spiralis. However, when the inflammation subsided, worm growth recommenced and eventually returned to levels found in non-inflamed controls. It is suggested that future work should investigate the specific nature of this interaction and should include careful monitoring, not only of cellular and biochemical changes occurring during the inflammatory response, but also of host dietary intake during the response.

Brief discussion is made of the possible sites of immune damage to H. diminuta and investigation of one such site, the transport of nutrients across the tapeworm tegument, is described. The transport of ^{14}C-labelled glucose, acetate and methionine, which cross the tegument by separate pathways, was investigated. Transport of these substrates by worms from six-cysticercoid infections in mice was compared to transport by worms of similar weight from mice immunosuppressed with cortisone and from rats. Transport of methionine and acetate by H. diminuta from untreated mice was depressed compared to transport by worms from immunosuppressed mice or rats. Uptake of glucose was similar, regardless of host type. The results are interpreted to demonstrate the selective blocking of membrane transport loci on the tegument of H. diminuta from mice by an immunological mediator.

The thesis adds to current concepts of immune responses to adult cestodes and suggests many lines of further investigation.

ABBREVIATIONS

B	Thymus-independent
BMC	Bone marrow cells
D	Day
GALT	Gut-associated lymphoid tissue
GVH	Graft versus host
HBSS	Hanks' balanced salt solution
Hd.	*Hymenolepis diminuta*
IBMC	Bone marrow cells from infected donors
Ig	Immunoglobulin
IMLNC	Mesenteric lymph node cells from infected donors
i.p.	Intraperitoneal(ly)
ISC	Spleen cells from infected donors
i.v.	Intravenous(ly)
K	Autopsy
KGR	Krebs' glucose ringer
MLNC	Mesenteric lymph node cells
n	Number of animals/group
ND	Not done
NLM	Phenotypically normal littermate
PFC	Plaque-forming cell(s)
PHA	Phytohaemagglutinin
p.i.	Post-infection
R	Roentgen
S	Svedberg unit
SC	Spleen cells
s.c.	Subcutaneous(ly)

SMLNC	Mesenteric lymph node cells from uninfected donors
SRBC	Sheep red blood cells
SSC	Spleen cells from uninfected donors
T	Thymus-dependent
Tsp.	*Trichinella spiralis*
1c	One cysticercoid
5c	Five cysticercoids

GENERAL INTRODUCTION

In nature, the adult form of the cyclophyllidean tapeworm, Hymenolepis diminuta, is found in the small intestine of rats. The scolex is unarmed and the entirely non-invasive worm is free to migrate anteriorly and posteriorly in a circadian rhythm in response to nutrient levels in the host intestine (Read & Kilejian, 1969; Hopkins, 1970). The rat is infected by ingestion of an intermediate host (which may be one of many arthropod species) harbouring cysticercoid larvae of H. diminuta. Intensive laboratory studies of the relationship between H. diminuta and its definitive host were begun in the 1930's by Chandler and have continued with the involvement of a great many investigators until the present day.

Until comparatively recently, adult tapeworms were thought of largely as non-immunogenic, although host immune responses developing to the tissue-invasive metacestode larvae of many species were well known (reviewed, Gemmell & MacNamara, 1972). The involvement of host immunity in the host-parasite relationship of H. diminuta was doubted largely on the strength of an early investigation by Chandler. Chandler (1939) characterised the establishment and growth of secondary infections of H. diminuta in rats. He observed that the number and size of secondary worms were inversely proportional to the size of the primary infection. When the primary worms were removed, either by administration of carbon tetrachloride, or mechanically following laparotomy of the host, the effect of the primary infection on the growth and establishment of secondary worms was removed. Chandler concluded that "premunition in tapeworm infections is due to crowding rather than to immunity in the ordinary sense". These experiments have since been quoted

(e.g., Heynemann, 1962a, b; Read, 1955) in defense of the hypothesis that adult H. diminuta is non-immunogenic.

The non-immunogenicity of H. diminuta is apparently reinforced by the fact that this cestode, which can occupy the entire small intestine of the host, may persist for many years in the rat and, indeed, has been shown to have a potential lifespan far exceeding that of its host (Read, 1967). On the other hand, in view of current knowledge of macromolecular uptake by mammalian intestinal mucosa (reviewed, Walker & Isselbacher, 1974), it would seem strange if such a large mass of actively metabolising tapeworm did not provide sufficient antigenic challenge to elicit an immune response from the host. The production of antibody by the rat in response to H. diminuta has, in fact, been observed (Coleman, Carty & Graziadei, 1968; Harris & Turton, 1973) and there is evidence (reviewed, Weinmann, 1970) that the adult stages of other cestode species are not immunologically inert. Perhaps the question now ought to be: how do persistent adult cestodes avoid the host immune response? Before this can be answered we must know more of the nature of the host immune response and it is in this respect that the use of the mouse as a definitive host for H. diminuta provides an invaluable model.

Experimental infections of H. diminuta in the mouse were first described by Read & Voge (1954). No worms were recovered by these authors from "albino" mice 20 days post-infection. However, when identical mice were injected intraperitoneally with tincture of opium before infection, recovery of H. diminuta was 100%. It was postulated that the increased recovery was due to opium slowing intestinal emptying time, thus allowing worm

establishment to take place. The results of Read (1955) tended to confirm the above hypothesis. An increased recovery of H. diminuta from "albino Swiss" mice was reported following pre-treatment of cysticercoids with acid pepsin prior to infection. However, this apparent refractoriness of mice to infection with H. diminuta was not confirmed by Turton (1968, 1971). Using Porton, CFLP and A strains of mice, Turton described successful establishment of H. diminuta using untreated or pepsin-treated cysticercoids with no significant difference between recoveries from the two groups.

Weinmann (1966) observed a high establishment (81%) of H. diminuta in mice treated with morphine and infected with either one or ten cysticercoids, but although "almost all worms persisted for at least 7 days . . . few survived 10 days in multiple infections or 12 days in single worm infections; none survived 15 days or reached full maturity". This observation on worm expulsion was confirmed by Turton (1968, 1971) and Turton noted in addition the destrobilation of many worms. Hopkins, Subramanian & Stallard (1972a) characterised the kinetics of worm expulsion from Porton and CFLP strains of mice and proposed an immunological explanation. This proposal was supported by results showing depression of the response by immunosuppressive drugs (Hopkins, Subramanian & Stallard, 1972b). Further evidence for an immune response to H. diminuta was then produced by other workers in this laboratory: delayed rejection of H. diminuta from lactating mice (Goodall, 1973); the relative unresponsiveness of young mice to H. diminuta infection (Befus & Featherston, 1974); the stunting of H. diminuta in secondary infections (Befus, 1975b). Damaged areas of the tegument of H. diminuta from mice and rats were shown to be

present (Befus & Threadgold, 1975) and it was suggested that this damage may be induced by the host's immune response, possibly as a result of the action of antibody which had been demonstrated coating the tegumental surface in concentrations increasing with time post-infection (Befus, 1974; 1975a).

There is conclusive evidence, then, that H. diminuta evokes in the mouse an immune response resulting in stunted growth, destrobilation and rejection of the parasite. However, information is lacking regarding the nature of the mechanisms involved in this response. Accordingly, the present investigation was instigated initially to provide such information. CHAPTER I of this thesis deals with this aspect specifically. It is divided into two sections. In SECTION 1, experiments designed to determine the effects of X-irradiation and subsequent cellular reconstitution are described. SECTION 2 deals with an investigation into the dependency of the response on thymus-derived (T) cells. Following on from investigation of the mechanisms involved in the response, CHAPTER II is concerned with the effect on H. diminuta of concurrent infection with the nematode Trichinella spiralis (which induces inflammation of the intestine).

Largely due to the studies of the late Clark P. Read and his numerous associates, there is a wealth of information available on the physiology, metabolism and nutrition of cestodes, in particular H. diminuta, and as Weinmann (1966) stated: "Adult cestodes would seem to be singularly suitable organisms for studies concerned with the actions of antibody on physiological processes in parasites". The effects on one such physiological process, the membrane transport of nutrients across the tegument

of H. diminuta was investigated in this study and the results are reported in CHAPTER III.

Some possible influences the present study may have on future investigations involving the immune response to H. diminuta are discussed at the end of the thesis.

GENERAL MATERIALS AND METHODS

Techniques which were used throughout the study are described in this section. Sectional MATERIALS AND METHODS describe techniques applied in particular investigations.

1. Animals used in the study.

a) Mice and rats.

Male animals were used throughout the study unless otherwise stated. The strains of mice used most frequently in the experiments described were the inbred NIH and the random-bred CFLP. These mice were purchased from Anglia Laboratory Animals Ltd. (formerly Carworth Europe Ltd.). During a period in December 1974, CFLP mice became unavailable due to an unknown infection in the breeding stock of the suppliers and mice of the outbred CD-1 strain were purchased from Charles River U.K. Ltd. (see CHAPTER III).

The congenitally athymic nude (nu/nu) mice used in experiments described in CHAPTER I, SECTION 2 were from a random-bred colony maintained in the animal house of the Wellcome Laboratories. Breeding and maintenance of these mice is described in MATERIALS AND METHODS of that section.

Rats were of the outbred CFHB strain (Wistar derived). These were bred and maintained in the animal house of the Wellcome Laboratories.

In order to minimise variation in results due to concurrent infections, all mice used were of the highest grade available from the above suppliers. Quality was assessed by reference to the 1-5 star grading system described in the Accreditation and Recognition Schemes for Suppliers of Laboratory Animals, 1974 (Manual

Series No. 1, second edition). The grading of animals from accredited breeders within this scheme is published monthly by the Medical Research Council Laboratory Animal Centre, Carshalton, Surrey. Briefly, 1 star mice under this system are not guaranteed free of cestodes; 2 star mice are free of the intermediate stages of cestodes; 3 star mice are free of all pathogenic helminths; 4 star mice are free of all helminth parasites, and 5 star mice are germ-free.

All NIH mice used were of either 2 star or 3 star category. These grades do not preclude the possibility of infection with the pinworms Aspiculuris tetraptera and Syphacia obvelata, but these nematodes were rarely observed. CFLP mice were of either 3 star or 4 star category. CD-1 mice are not graded under this scheme and as previous supplies of this strain harboured pinworms, these mice were treated with piperazine citrate on arrival (see "Anthelmintic" in this section). All experimental animals were free of cestodes other than H. diminuta at autopsy.

In most experiments mice were caged in groups of ten in polypropylene cages 45cm x 28cm x 13cm (North Kent Plastic Cages Ltd.). In some experiments using smaller numbers of mice, they were caged in groups of five in polypropylene cages 48cm x 15cm x 13cm. Sawdust litter was changed twice weekly.

The animal rooms were maintained for the most part at 20°-22°C. The lighting was maintained automatically on a 12h cycle in winter but followed day length in summer. Mice and rats were provided ad libitum with tap water, normally without additives. Diet 41B (Oxoid) or, later, Standard Rat and Mouse Breeding Diet (Grain Harvesters Ltd.) was provided ad libitum.

b) Parasites.

The strain of Hymenolepis diminuta used in all experiments was obtained from Rice University, Houston, Texas in 1963 and has been maintained in the Wellcome Laboratories since this time by regular passage through rats and the flour beetle Tribolium confusum by technical staff using the methods described below.

Tapeworms are removed from the intestines of rats into water at room temperature. The gravid segments are separated and homogenised in a blender (MSE Ltd.) to free the fully differentiated eggs. The homogenate is then allowed to settle and the supernatent drawn off. After washing, the eggs are drained free of water using filter paper and then fed to beetles which have been starved for 2-5 days at 25°-28°C. After about 24h any remaining eggs are removed, the beetles given wholemeal flour and tissues for shelter, and placed in a dark incubator at 25°-28°C. Beetle larvae and pupae are removed from these jars monthly to prevent uninfected beetles developing.

Fourteen days after infection each beetle contains on average about 10 fully differentiated cysticercoid larvae. Stock rats for passage of H. diminuta are infected with 6 cysticercoids each.

2. Infection procedures.

The age of cysticercoids used in these experiments ranged from 2 weeks to 2 months. They were dissected from flour beetles in a modified Hanks' balanced salt solution (HBSS, see Hopkins & Stallard, 1974) and administered by stomach tube to mice or rats under ether anaesthesia. The polythene stomach tubing (Portex Ltd.) was either 1.0mm O.D., 0.5mm I.D. (mice) or 1.57mm O.D., 1.14mm I.D. (rats).

3. Recovery of worms.

In experiments where total worm recovery was to be recorded, this was determined as follows.

The mouse was weighed and then killed by cervical dislocation. The small intestine was removed, measured and divided transversely into quarters. Each quarter was taken in turn and opened longitudinally in HBSS at room temperature in a 9cm diameter glass petri dish. The section of intestine and associated washings were observed under the 6x magnification of a dissecting microscope using transmitted light. Worms or parts of worms found by this method were blotted on filter paper and transferred to a small aluminium cup for weighing. All worm material recovered from a mouse was weighed together, after drying for 24h-48h in an oven at 98°-100°C. Weighing was carried out on a Stanton Unimatic balance (Stanton Instruments Ltd., London) to within 0.1mg (CHAPTER III excepted - see relevant MATERIALS AND METHODS).

If worm numbers recovered did not equal cysticercoid numbers administered, the intestinal sections were transferred to separate 5cm diameter petri dishes and incubated in HBSS at 37°C for 2h-4h. The dishes were periodically searched for worm material throughout incubation. If, at the end of this time, worm recovery was still <100% it was assumed that missing worms had either been rejected by the mouse or had not established in the first place.

On occasions (see CHAPTER III) it was more important to recover undamaged worms than to recover all worms present. In these cases worms were recovered as described in MATERIALS AND METHODS for that chapter.

4. Antibiotic.

To prevent bacterial colonisation of mice and rats immunodepressed with cortisone acetate or X-irradiation, all groups of animals including control groups in experiments using these treatments were given the antibiotic oxytetracycline hydrochloride (Terramycin, Pfizer Ltd.) in their drinking water at a concentration of 3g/l. This concentration provides a dose of the active constituent of approximately 30mg/kg/day for mice and 20-25mg/kg/day for rats.

5. Anthelmintic.

It was thought likely that the purchased CD-1 mice harboured pinworms and the original breeders of the nu/nu colony were known to be heavily infected with these parasites. Both these groups of mice were, therefore, treated with the anthelmintic piperazine citrate (Loveridge Ltd.). The CD-1 mice were given a single oral dose of 1.6mg/g in aqueous solution on arrival and all members of the nu/nu colony were similarly dosed every 7-10 days. This dose has been shown by Behnke (personal communication) to eliminate adult pinworms from mice.

6. Statistical treatment of results.

Significant deviation from the null hypothesis in the case of results involving worm weights was determined by ranking the values for individual worm weight (or weight of worm per mouse when multiple infections were used) from the relevant groups of mice, followed by application of the Wilcoxon (Mann-Whitney) test for two independent samples. Numbers of worms recovered from independent groups of mice were tested for their statistical distinction by the use of 2 x 2 contingency tables.

CHAPTER I

ANALYSIS OF THE RESPONSE

SECTION 1

X-IRRADIATION AND RECONSTITUTION OF THE RESPONSE

PREFACE

Whole body X-irradiation was used in the experiments described in the following section to deplete the response of the mouse to H. diminuta. It is, therefore, appropriate and necessary at this stage to discuss briefly some of the effects of such treatment.

As with most cytostatic and cytotoxic agents, the effects of ionising radiation on an organism are not simple. Its effect on an immune response varies considerably, depending on the sensitivity of the components active at the time of exposure. The possible specific effects and side effects must, nevertheless, be understood in order to plan experiments and to correctly interpret results. The effects can to some extent be defined by judicious timing and careful manipulation of the dosage and whole-body X-irradiation followed by selective replacement of humoral and cellular components of the response remains a vital method in investigative immunology.

The literature describing the effects of X-irradiation on biological systems is vast and continues to grow.

a) Effects on immune mechanisms

Cell death due to ionising radiation damage arises as a result of two separate defects. The first type of death occurs at metaphase of cell division as a result of the disruptive effects of the

high energy radiation on DNA metabolism and structure. Strand separation occurs within the DNA molecules and is thought to be caused by breaks in the strands and interstrand links (Rydberg & Johansen, 1975). The cell nucleus then becomes pycnotic and cell death follows.

The second type of radiation-induced cell death occurs in interphase. This is thought to be due to some form of cytoplasmic damage. For instance, Scaife & Brohée (1967) suggest that interphase death may be due to damage to the respiratory mechanism of the cell. This type of damage is very relevant to the irradiated immune response as most small lymphocytes are highly radiosensitive and will die during interphase shortly after irradiation (Trowell, 1952). There is, however, considerable variation in degree of radiosensitivity within the small lymphocytes, depending on the sub-population and on the function which is investigated.

This variation in susceptibility to interphase death may be related to the metabolic state of the cell at the time of irradiation. For example, Schrek & Stefani (1964) found that human small lymphocytes activated with phytohaemagglutinin (PHA) showed a greater degree of radioresistance to interphase death (but not to subsequent mitotic death) than unstimulated control cells. Stimulation with alloantigens has also been shown to induce radioresistance to interphase death (Sprent, Anderson & Miller, 1974) and it is assumed that such protection is afforded by the heightened metabolic state of the stimulated cells and their resulting increased ability to repair damage. Thus, some T cells which are primed for function are highly radioresistant, e.g., T helper cells (Hirst & Dutton, 1970) and fully differentiated cytotoxic T cells (Denham, Grant, Hall & Alexander, 1970). Although certain populations of small

lymphocytes, then, are to some extent radioresistant. they are not protected against metaphase death and will die if subsequently called upon to proliferate (Conrad, 1969). The thymic T cell precursor described by Kadish & Basch (1975), resistant to lethal doses of X-irradiation, would appear to be an exception to this rule. On the other hand, some T cell sub-populations are radiation sensitive. For example, Sabbadini (1974) found that the suppressor activity of T cells regulating a cell-mediated cytotoxicity reaction was sensitive to 500 rads.

As a general rule, B cells tend to be more radiation-sensitive than T cells. Their survival in culture after irradiation is low compared with T cells (Sprent et al. 1974) and their ability to home, both non-specifically to the spleen and specifically to the peripheral lymph nodes, is greatly reduced after irradiation compared with T cells (Anderson, Sprent & Miller, 1974). B cells may be protected, but to a much lesser extent than T cells, against interphase death by stimulation with mitogens and antigens (Anderson & Warner, 1975).

Macrophages are not as susceptible to irradiation-induced cell death, largely because the incidence of mitotic death is low due to their low frequency of cell division. The radiosensitivity of macrophage function in the response to antigens, however, is difficult to assess because different antigens may require different levels of macrophage involvement. Thus, depending on the antigen used, the antigen-processing capacity of irradiated macrophages has been shown to be damaged (Gallily & Feldman, 1967) or unaffected (Unanue & Askonas, 1968). The reasons for damage to the antigen-processing function, where it does occur are still controversial. Geiger & Gallily (1974) showed that although irradiated macrophages

lost their ability to co-operate in the process of antibody production to Shigella, many macrophage functions and properties remained unaffected or even stimulated after irradiation. It may be that macrophages, like small lymphocytes, are a heterogeneous population with respect to radiation sensitivity and immunologic function.

Mast cells appear to be relatively unaffected by irradiation. Kojima & Ovary (1974) looked at several aspects of mast cell function after irradiation. They found that sublethal or lethal doses of X-irradiation had no effect on the mast cell membrane receptors for antibody, on their content of vasoactive substances, or on their release of these substances during a PCA reaction.

Although there is extensive information on the effects of irradiation on isolated cell sub-populations and functions, the effects on complex immunological functions in toto such as antibody production or on the so-called cell-mediated reactions are difficult to describe in general terms because the response to different antigens will require the co-operation to a greater or lesser extent of more or less radiosensitive components.

Humoral antibody production, once begun, is reportedly unaffected by sublethal or lethal doses of irradiation (Reuter, Sassen, Kennes, 1967). This is presumably due to the remarkable radioresistance of mature splenic plasma cells (Makinodan, Nettesheim, Morita & Chadwick, 1967). The level of serum γ-globulins, however, is lowered in irradiated mice (Grabar, Kashkin, & Courcon, 1963) and the different classes of immunoglobulins were found to differ in their radiation sensitivities (Bazin & Micklem, 1967). Bazin and his colleagues have carried out perhaps the most complete study

of this phenomenon. Bazin & Malet (1969) found that catabolic loss of IgM and IgA from the serum of lethally irradiated mice was the same as in unirradiated controls. Catabolism of IgG_1, IgG_{2a} and IgG_{2b}, however, was doubled in irradiated mice. On the other hand, serum concentrations of immunoglobulins after 950R, expressed as percentages of original values were:

IgM 65%

IgA 15-20%

IgG 35-50%

The very low serum levels of IgA after irradiation may be explained by serum IgA, which is manufactured by plasma cells in the lamina propria of the intestine, leaking into the intestinal lumen because of irradiation damage to the intestinal epithelium (Bazin, Maldague & Heremans, 1970; Bazin & Doria, 1970). In addition to this loss by leakage, Bazin, Maldague, Schonne, Crabbé, Bauldon & Heremans (1971) reported a reduction in the numbers of plasma cells of all immunoglobulin classes in the lamina propria after 700R of X-irradiation. The gut is the major source of IgA, whereas other Ig classes are produced largely in the spleen. This factor, then, may contribute to the selective depletion of serum IgA after irradiation. This result for intestinal plasma cells contrasts sharply with the radioresistance of splenic plasma cells reported by Mackinodan et al. (1967) and the difference may be of particular relevance to an entirely lumen-dwelling intestinal parasite.

Implicit in the theory of Bazin et al. that selective serum IgA depletion is caused by gut leakage, is the idea of irradiation-induced damage to the intestinal epithelium. Some of these effects on the intestine are set out below.

b) Gastrointestinal effects.

The small intestinal epithelium is extremely susceptible to ionising irradiation, largely because of its high turnover rate and the resulting susceptibility to mitotic death. Quastler (1956) attributed the lethal effects of intestinal radiation injury to a loss of proliferative activity of the crypt cells. Tsubouchi & Matsuzawa (1974) stated that 80% of the proliferating cells in the crypts of the mouse are lost within 4h after irradiation at 1,000 rads.

Some of the damage and its subsequent recovery is dose dependent. Montagna & Wilson (1955) chronicled the effects of lethal (1000R) and sublethal (550R) doses of whole-body X-irradiation on the mouse small intestine. Mitosis in the cryptogenic cells ceased abruptly after either dose of irradiation. Mitosis reappeared 12h after the lethal dose but was abnormal, whereas the sublethal dose only stopped mitosis for 2h and few subsequent mitotic aberrations appeared. Villous epithelial cells showed no damage during the 48h following the lethal dose but gradual swelling and increasing karyolysis and karyorrhexis of the crypt cell nuclei occurred. Villous atrophy was maximal 96h after the lethal dose but at this point the degenerative changes had passed their peak and, in some animals, some crypts had undergone repair. Only traces of damage remained 72h after the sublethal dose. In addition to the nuclear changes due to irradiation described above, Quastler & Hampton (1962) described evidence of membrane damage to mature intestinal cells given 200 rads or more.

The fate of the Paneth cells was followed only after the lethal dose. Up to 48h post-irradiation they showed no damage. At 72h

they fragmented and their granules could be seen in the lumina of the crypts. After 96h the crypts had normal numbers of Paneth cells, though with fewer granules. Goblet cells became progressively larger after irradiation, particularly in the crypts.

In addition to the mucosal lesions, the irradiated small intestine experiences an almost immediate increase in tone and motility with doses as low as 100R (Conrad, 1951). This latter study was carried out in the rat but Conrad states that the effect also occurs in the rabbit. The tone and motility reportedly increases with dose and may be associated with changes in cholinesterase activity.

Stomach emptying time in the rat is delayed after 500R until 3 or 4 days post-irradiation and compensatory over-eating occurs after this time. Conrad (1956) states that this delay in stomach emptying time "also occurs in the mouse". Fenton & Dickson (1954) observed that intestinal and pancreatic amylase activity was normal in irradiated mice (500R) and they commented that stomach emptying time returned to normal after 24h.

INTRODUCTION

From several model systems of the host's immune response to intestinal nematodes, a pattern is emerging of the components of the host response and the sequence in which they act. In the system most extensively studied, that of *Nippostrongylus brasiliensis* in the rat, worm rejection appears to require the presence of three components. Worms are, first of all, damaged by antibodies (Ogilvie & Hockley, 1968; Lee, 1969; Jones, Edwards & Ogilvie, 1970). Antibody-damaged worms, however, will not be rejected by irradiated rats, suggesting the necessity for a lymphoid component. This component, transferrable with mesenteric lymph node cells (Keller & Keist, 1972; Dineen, Ogilvie & Kelly, 1973), acts synergistically with a third component which resides in bone marrow (Dineen & Kelly, 1973; Kelly, Dineen & Love, 1973) to cause worm rejection. Antibody action followed by a lymphoid component has also been found to be the sequence of events controlling rejection of *Trichuris muris* from the mouse (Wakelin, 1975a), although the co-operation of a bone marrow-derived component does not appear to be required in this case (Wakelin & Selby, 1976). Both antibodies and cells are also required for the expulsion of *Trichinella spiralis* from rats (Love, Ogilvie & McLaren, 1976) and from mice (Wakelin & Lloyd, 1976b) although the sequence of humoral and cellular events in this response remains undetermined.

It cannot, however, be assumed that because the time scale of the rejection process for *H. diminuta* approximates to that observed for the nematodes mentioned above, the immune mechanism is the same. Indeed, it would be strange if it were so when their differing biology is considered.

Antibody has been demonstrated bound to the tegument of H. diminuta (Befus, 1974). The protective function of this antibody is unknown but it does increase in concentration over the period of infection. It is likely that this antibody forms part of a local response to H. diminuta and that circulating antibody is unimportant in protection. In support of this, Befus (1975a) was unable to demonstrate anti-H. diminuta antibodies in the serum of infected mice; Hopkins (unpublished, personal communication) has shown that transfer of large volumes of "immune" serum has no effect on the course of worm rejection; splenectomy does not suppress worm rejection (personal observation).

The objective of the experiments described in the following section was to determine the possibility of cellular involvement in the response to H. diminuta. The experiments, to a large extent, use the technique of suppression of the response using whole-body X-irradiation followed by selective reconstitution of depleted cell populations, an approach widely used by workers investigating the host-nematode systems described above.

MATERIALS AND METHODS

1. Irradiation of mice.

Groups of up to 10 mice confined in a flat, aerated perspex box (11.5cm x 17.0cm x 4.0cm) were X-irradiated using a Siemens Stabilipan at 110 rads/min under the following operating conditions: 300kV, 12mA; Thoraeus I filtration; field size 25cm x 12.5cm; target-object distance of 52cm. All irradiated and control groups of mice were given oxytetracycline hydrochloride in their drinking water for the remainder of the experimental period (see GENERAL MATERIALS AND METHODS).

2. Cell transfer.

In all cell transfer experiments the inbred NIH strain of mouse was used so that donor cells were syngeneic with recipients.

All glassware and instruments used for cell collection were autoclaved prior to use and an aseptic technique was employed during the collection procedure.

In experiments where mesenteric lymph node cells (MLNC) or spleen cells (SC) were to be used the following procedure was followed. Donor mice were killed by cervical dislocation. The spleen and/or mesenteric lymph nodes were removed and placed in either Krebs glucose ringer (KGR) or HBSS + 1% glucose on crushed ice until all the donors were autopsied. The tissues were then pushed through a fine mesh nylon sieve into fresh saline at $4^{\circ}C$. Donors of bone marrow cells (BMC) were similarly autopsied. The long bones of their limbs were then removed and the marrow flushed into KGR or glucose-HBSS at $4^{\circ}C$.

Cell suspensions were then washed once by centrifugation at 1000g for 5 min and resuspended in a known volume of medium containing 10 i.u. heparin/ml (Evans Medical Ltd.). Cells were counted on a haemocytometer slide and the suspensions diluted as required so that each recipient mouse received approximately 0.5ml of suspension. Injections were made into the lateral tail vein. Before and after injection, tests for cell viability were carried out using a 0.1% methylene blue or 0.2% trypan blue exclusion test. In all cases cell viability as measured by these tests was at least 90%.

3. Marker antibodies.

In some experiments agglutinating antibody produced by cell recipients to sheep red blood cells (SRBC) was used as an indicator of the ability of transferred cells to initiate a humoral response. Mice were injected intraperitoneally (i.p.) with a 4% suspension of SRBC (Gibco Bio-Cult Ltd.) in 0.9% saline. Agglutinating titre, determined by a standard microtitre technique is expressed as the highest $\log_2$ dilution giving agglutination.

In one experiment the ability of primed cells to mount a response after transfer was measured with respect to agglutinating antibody titre to Salmonella typhi-O (Wellcome Ltd.). The procedure is described in the relevant passage. Ability of recipient serum to agglutinate S. typhi-O was determined as described by Campbell, Garvey Cremer and Sussdorf (1970).

RESULTS

1. Effect of X-irradiation on the response to a primary infection with H. diminuta.

a) Irradiation dose.

This preliminary experiment was designed primarily to gain experience in techniques but also to provide basic information on the comparative effects of sublethal and lethal doses of X-irradiation.

Sixty male, 4 star NIH mice, 36 days old, were infected with a single cysticercoid (1c) of H. diminuta (Table 1-1). Seventeen identical mice were left uninfected as cell donors for reconstitution of lethally irradiated mice.

Table 1-1

n[a]	DAY				
	-1	0	8	9	18
15		1c			K[b]
10		1c			K
15	530 rads	1c			K
10		1c	900 rads	BMC[c] SRBC	K
10		1c	900 rads	BMC[c] MLNC[d] SRBC	K

a. n = number of mice/group
b. K = autopsy
c. 2.0×10^7 BMC/recipient
d. 4.0×10^7 MLNC/recipient

Worm establishment determined by recovery from the control group autopsied on day 9 was 100% (Fig. 1-1) and by day 18, 70% of worms had been rejected by the mice. Sublethal whole-body X-irradiation given one day before infection or a lethal dose given on day 8 were equally effective in suppressing the rejection mechanism, effectiveness of the two dose-levels being statistically indistinguishable. In succeeding experiments, therefore, only sublethal doses of irradiation were used, thus eliminating the need for reconstitution of depleted haemopoietic tissue. There was no mortality of mice in either of the lethally irradiated groups, an indication of the viability and establishment of the transferred BMC.

The ability of the lethally irradiated mice reconstituted with MLNC in addition to BMC to produce agglutinating antibodies to SRBC suggests that the MLNC transferred contained sufficient viable mature T cells to co-operate in producing a humoral antibody response to a T cell-dependent antigen (Claman, Chaperon & Triplett, 1966). The lack of significant difference, however, between numbers of worms recovered, or dry weights of worms recovered (Fig. 1-2) from this group of mice and from the group reconstituted with BMC alone shows that although the MLNC restored the ability to mount a humoral response they afforded no protection against the already present H. diminuta infection.

b) Timing of irradiation.

In a preliminary experiment to determine the effect of X-irradiation at different times during infection, male 4 star CFLP mice were used. At 44 days old all the mice were infected

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Figure 1-1

Recovery (%) of Hymenolepis diminuta from single cysticercoid infections in NIH mice given lethal or sublethal doses of X-irradiation with or without cellular reconstitution. Solid areas of bars represent worms >0.1mg dry weight; open portions represent destrobilated worms ($\leq$0.1mg). D, day p.i.; ND, not done; BMC, 2.0×10^7 bone marrow cells from naive donors, i.v.; MLNC, 4.0×10^7 mesenteric lymph node cells from naive donors, i.v.

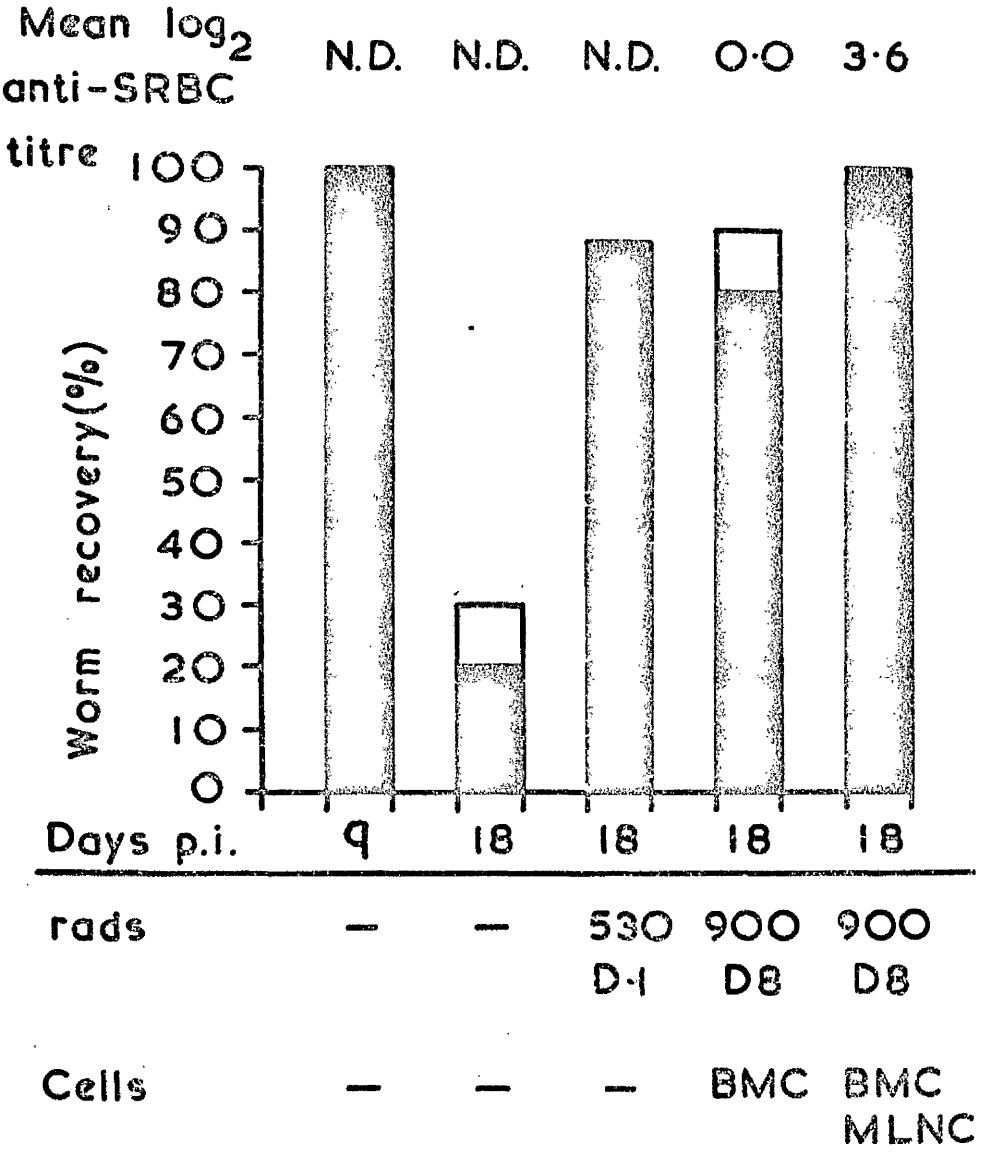
Mean log2 anti-SRBC titre
N.D.
N.D.
N.D.
0·0
3·6
Worm recovery (%)
100
90
80
70
60
50
40
30
20
10
0
Days p.i.
q
18
18
18
18
rads
—
—
530 D-1
900 D8
900 D8
Cells
—
—
—
BMC
BMC MLNC

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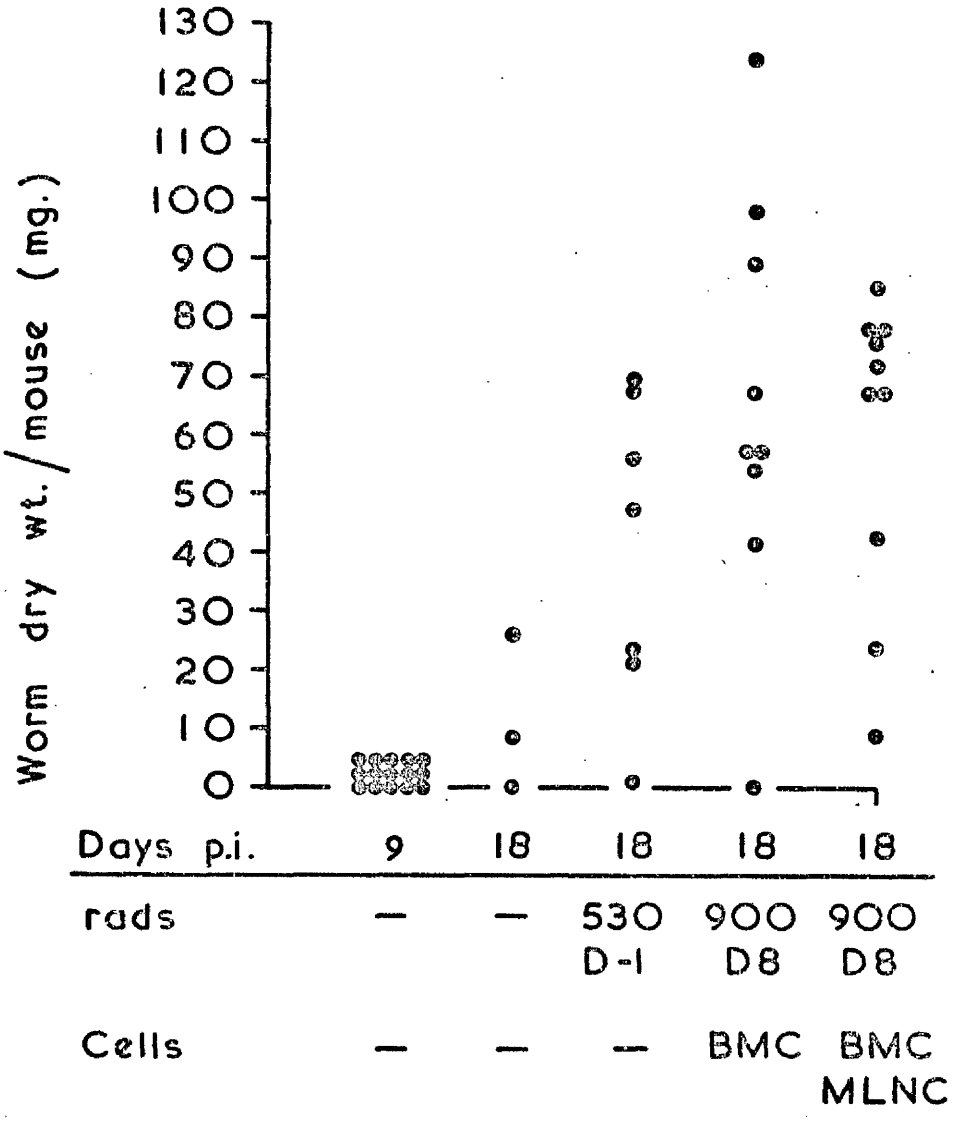
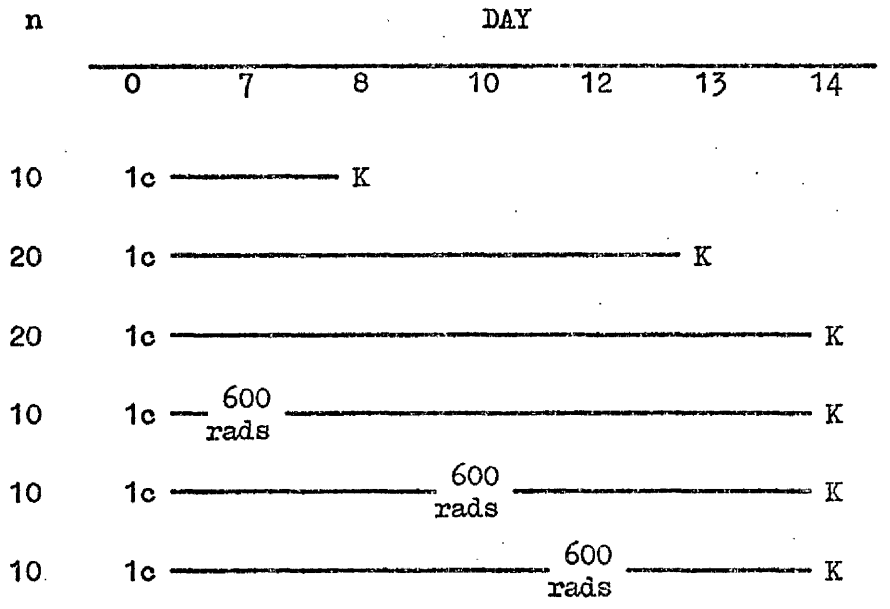
Figure 1-2

Dry weight of Hymenolepis diminuta from single cysticercoid infections in NIH mice given lethal or sublethal doses of X-irradiation with or without cellular reconstitution. Each point represents the dry weight of worm recovered from a single mouse. D, day p.i.; BMC, 2.0×10^7 bone marrow cells from naive donors, i.v.; MLNC, 4.0×10^7 mesenteric lymph node cells from naive donors, i.v.


Worm dry wt./mouse (mg.)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Days p.i.
9
18
18
18
18
rads
—
—
530
D-1
900
D8
900
D8
Cells
—
—
—
BMC
BMC
MLNC

with one cysticercoid (1c) of H. diminuta. Groups were then given a sublethal dose (600 rads) of whole-body X-irradiation at different stages of the infection (Table 1-2).

Table 1-2



Worm recovery in this experiment, given in Fig. 1-3, shows that worm establishment was good (90% recovery on day 8). A 25% recovery of worms >0.1mg on day 13 demonstrates that by this time worm destrobilation and expulsion was well advanced. The control group autopsied on the following day, however, harboured 45% of worms >0.1mg. These results give an indication of the degree of variation in time of rejection experienced when using single cysticercoid infections in random bred mice, in spite of the fact that there were 20 mice in both of these control groups. Such variation in the mean survival time (the first day on which ⩾50% of the worms administered to a group of mice have been destrobilated or lost) was discussed by

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Figure 1-3

Recovery (%) of Hymenolepis diminuta from single cysticercoid infections in CFLP mice given a sublethal dose of X-irradiation at different times after infection. Solid areas of bars represent worms $>$ 0.1mg dry weight; open portions represent destrobilated worms ($<$0.1mg). D, day p.i.

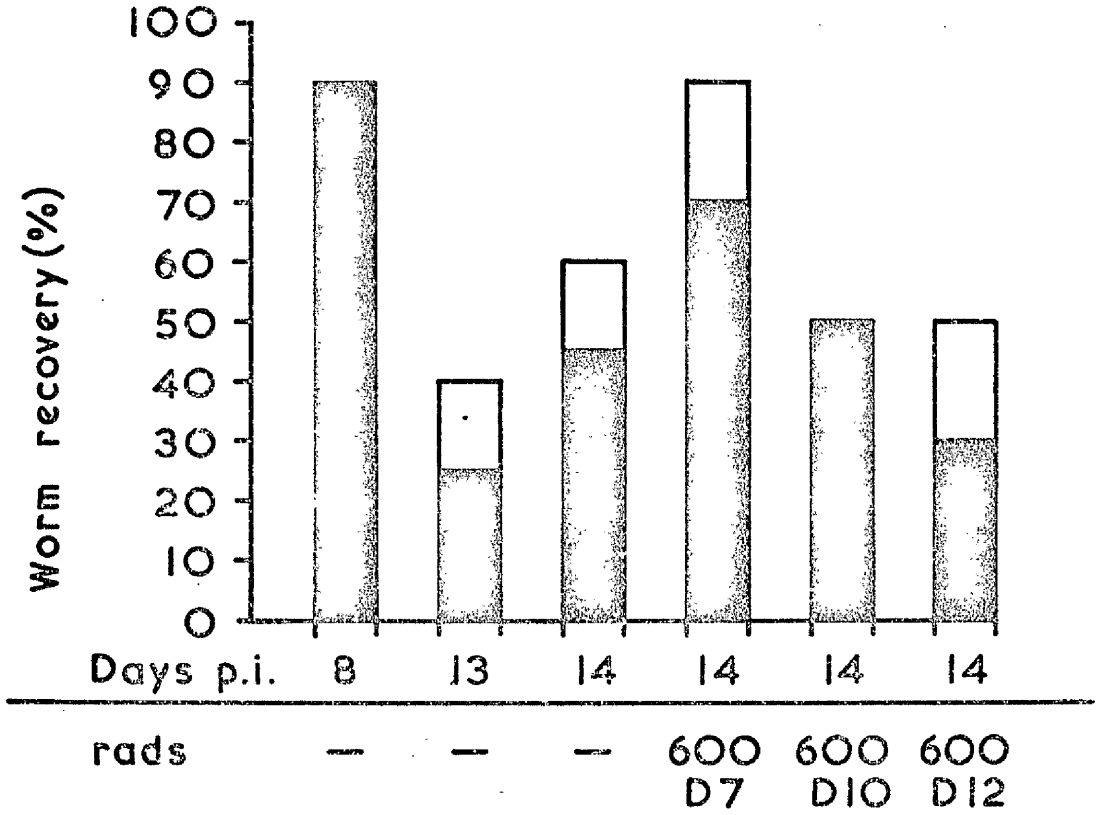
Worm recovery (%)
100
90
80
70
60
50
40
30
20
10
0
Days p.i.
8
13
14
14
14
14
rads
—
—
—
600 D7
600 D10
600 D12

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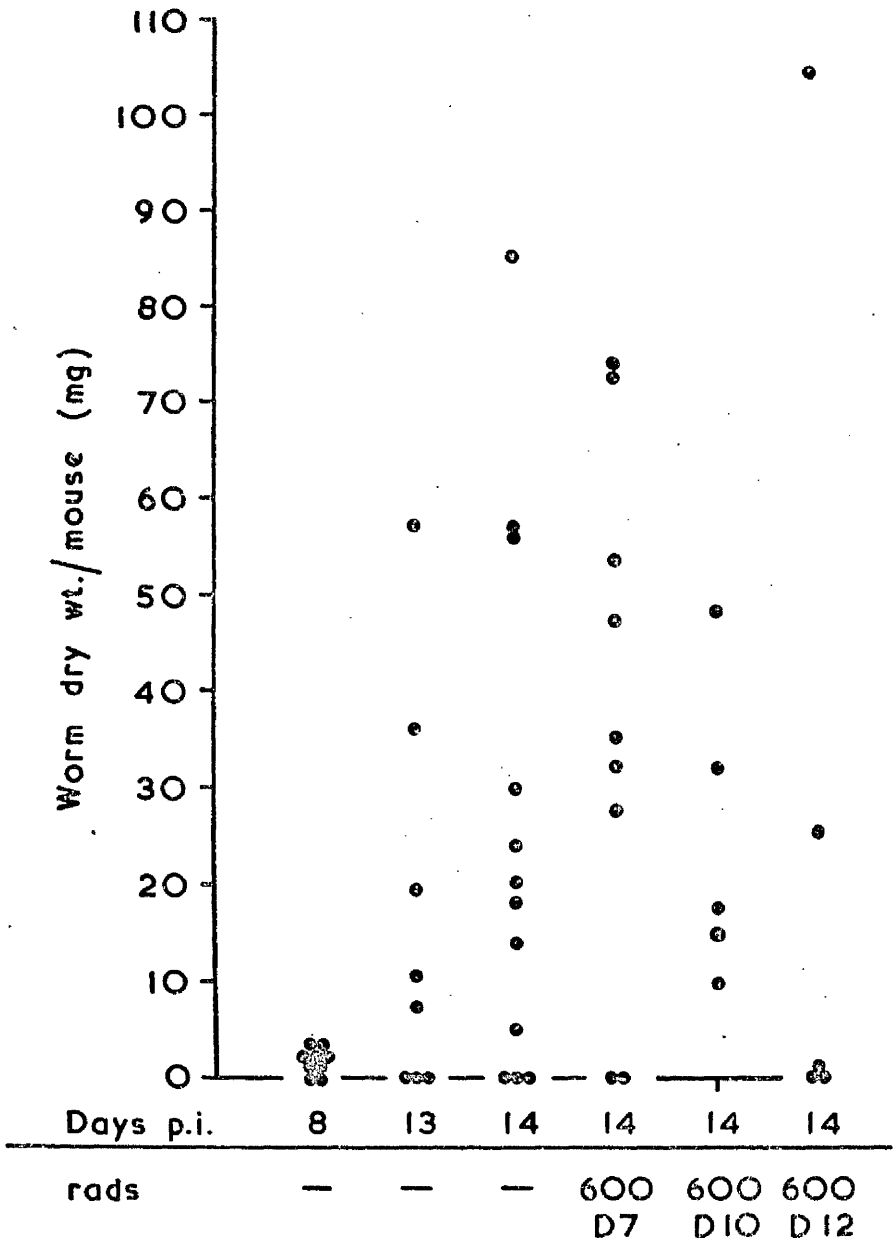
Figure 1-4

Dry weight of Hymenolepis diminuta from single cysticercoid infections in CFLP mice given a sublethal dose of X-irradiation at different times after infection. Each point represents the dry weight of worm recovered from a single mouse. D, day p.i.


Worm dry wt./mouse (mg)
110
100
90
80
70
60
50
40
30
20
10
0
Days p.i.
8
13
14
14
14
14
rads
—
—
—
600 D7
600 D10
600 D12

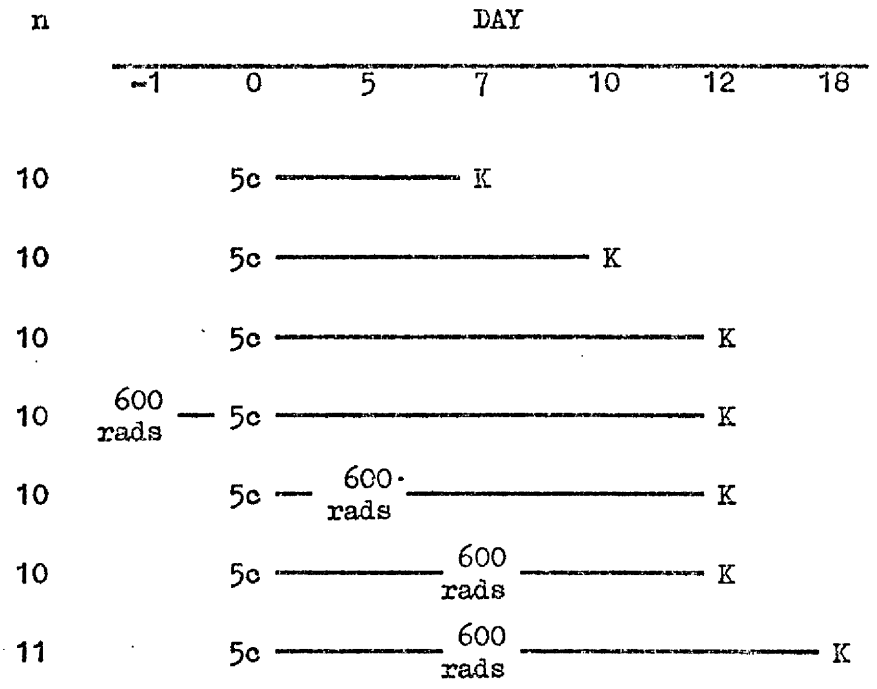
Befus (1975b) and it was shown that multiple infections are rejected more abruptly and with less variation. For this reason, multiple infections - normally of five cysticercoids - were used in all following experiments. Worm recovery on day 14 from the mice irradiated on day 7 indicates that irradiation at this time markedly depresses the response. Worm recovery from mice irradiated on days 10 or 12 did not differ significantly from controls, indicating that irradiation at this stage of the response was ineffective.

The dry weights of worms recovered from all mice in this experiment are shown in Fig. 1-4. Worms recovered from mice irradiated on day 7 had not grown significantly quicker than control worms recovered on the same day even though the mechanism causing worm rejection had been delayed in the irradiated group. The range of dry weights of worms recovered from both groups, however, is extremely variable and valid comparisons must await multiple infections in inbred mice.

At this stage it was necessary to characterise the effects of irradiation on the response to a multiple infection in an inbred strain so that a repeatable regime could be devised for syngeneic cellular reconstitution of the irradiation-depleted response.

Male, 3 star NIH mice were infected with five cysticercoids (5c) of H. diminuta when 48 days old. Groups were given a sublethal dose (600 rads) of whole-body X-irradiation at different stages during the course of the infection as shown in Table 1-3.

Table 1-3



Percentage worm recovery (Fig. 1-5) from the control mice autopsied on day 7 shows that almost all the cysticercoids administered established. This, and the consistently high recovery from irradiated groups autopsied on day 12, indicates that the technique used for infection with 5c was highly successful and repeatable.

There was little reduction in worm recovery from the control mice autopsied on day 10, but by day 12 recovery of worms >0.1mg was less than 50% showing that the response was well advanced by this time. In contrast, there was no reduction in worm numbers from any of the irradiated groups by day 12. This indicates that sublethal irradiation given either shortly before infection or as late as day 7 effectively suppresses the mechanism which normally causes worm rejection and that this mechanism is not restored by day 12. The low recovery of worms on day 18 from

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Figure 1-5

Recovery (%) of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given a sublethal dose of X-irradiation at different times before and after infection. Solid areas of bars represent worms >0.1mg dry weight; open portions represent destrobilated worms (≤ 0.1mg). D, day p.i.

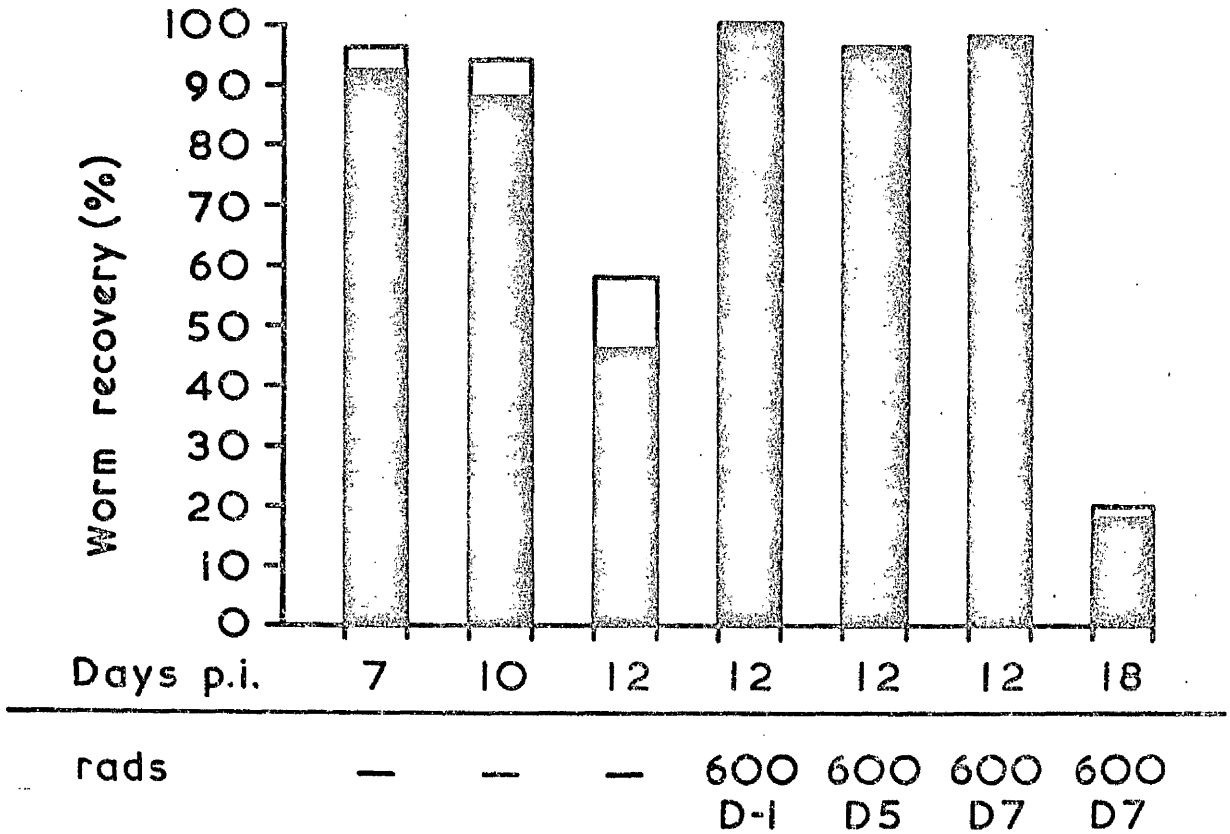
Worm recovery (%)
100
90
80
70
60
50
40
30
20
10
0
Days p.i.
7
10
12
12
12
12
18
rads
—
—
—
600 D-1
600 D5
600 D7
600 D7

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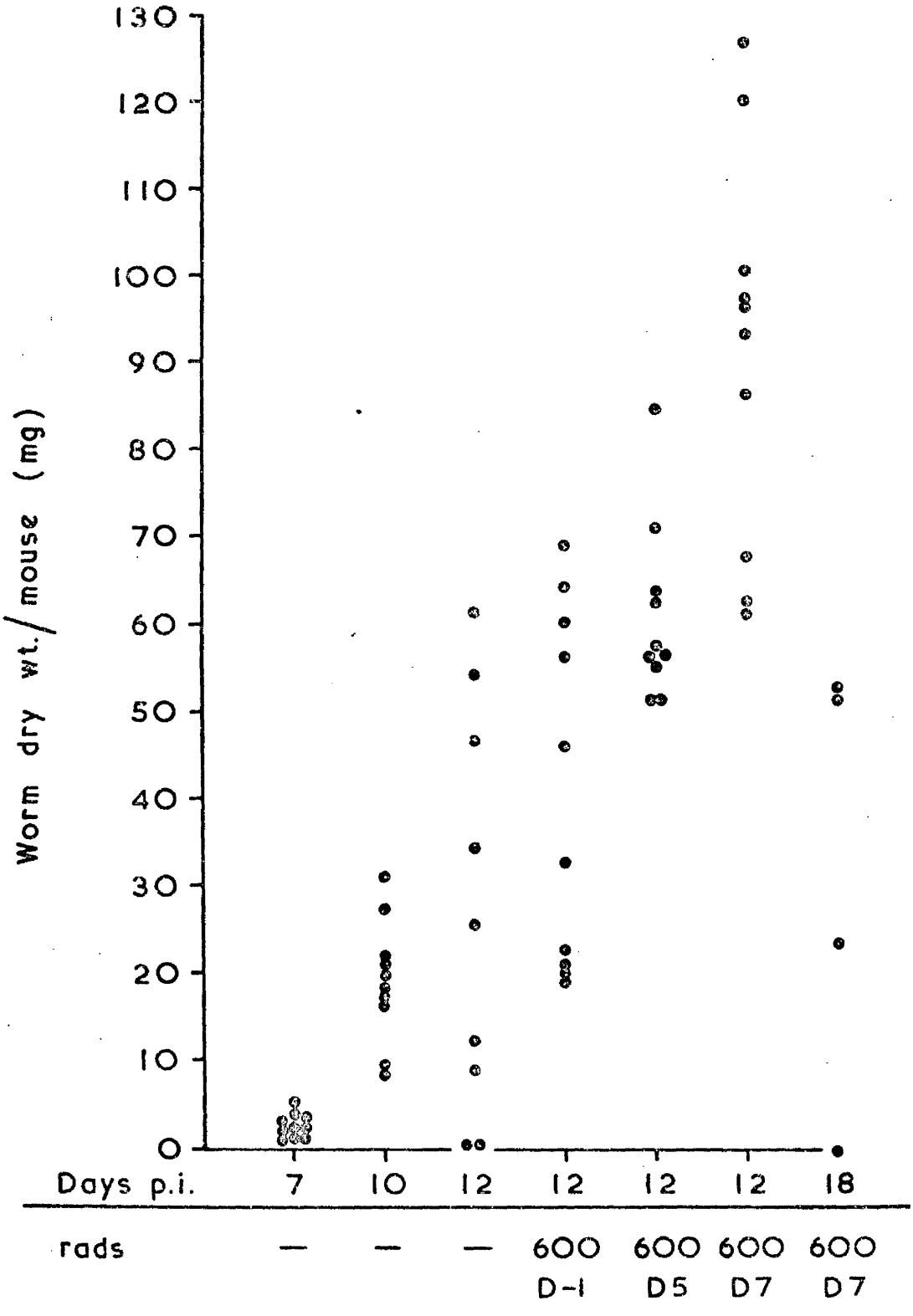
Figure 1-6

Dry weight of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given a sublethal dose of X-irradiation at different times before and after infection. Each point represents the total dry weight of worm recovered from a single mouse. D, day p.i.

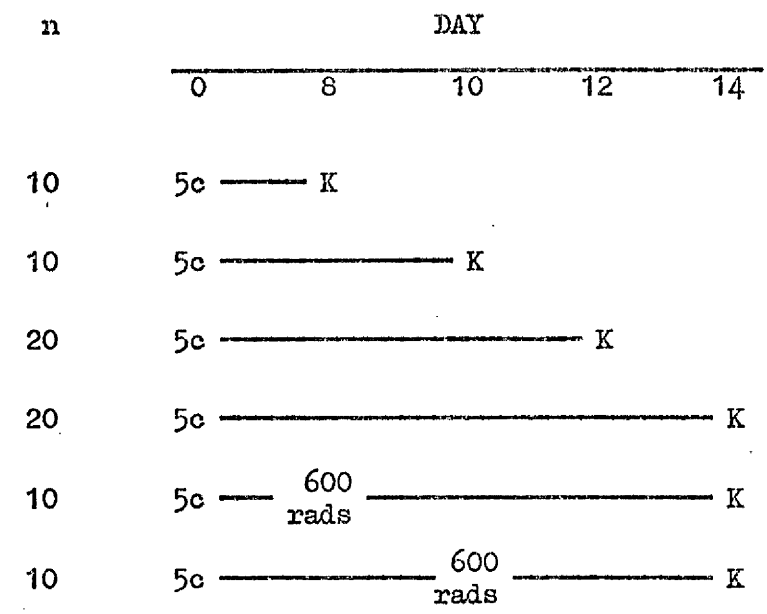

Worm dry wt./mouse (mg)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Days p.i.
7
10
12
12
12
12
18
rads
—
—
—
600 D-1
600 D5
600 D7
600 D7

mice irradiated on day 7, however, suggests that by this time the response was restored and rejection had recommenced.

Because of the method used for worm recovery, it was not possible to weigh individual worms from multiple infections. All worm material from each mouse was weighed together, giving figures for worm dry weight/mouse. The dry weight of worm tissue recovered from each mouse is shown in Fig. 1-6. This shows that although sublethal irradiation on day -1 was effective in delaying rejection of the worms, it did not affect their growth rate. Dry worm weight/mouse in this group does not differ significantly from that in control mice. Worm growth, however, in mice irradiated either on day 5 or day 7 was considerably greater than in control mice and the rate of worm growth apparently depended on the stage of the response at which mice were irradiated (control vs. irradiated day -1, N.S.; control vs. irradiated day 5, $P < 0.01$; irradiated day 5 vs. irradiated day 7, $P < 0.01$). There are several possible explanations for this increased growth rate caused by irradiation late in infection when effectors of the mechanism causing rejection are presumably building up to a peak. The implications of the observation are discussed more fully at the end of this section.

An experiment was then designed to extend these observations and investigate further the effects of sublethal X-irradiation given late in infection. The experimental design is shown in Table 1-4. Male, 40 days old, 3 star NIH mice were infected with 5 cysticercoids. One group was given 600 rads of whole-body X-irradiation on day 8 and another group was given the same dose on day 10. A larger number of controls were included so that the recovery on the days of irradiation could be accurately determined and to make possible the precise timing of worm rejection.

Table 1-4



The data expressed in Fig. 1-7 show firstly that large numbers of worms (90% - 100% of those administered) were still present on the days when experimental mice were irradiated. Destrobilation and expulsion of worms, however, commenced shortly after this time in control mice as demonstrated by the decreasing worm recoveries on day 12 and day 14. The extremely high recovery of worms >0.1mg and the absence of destrobilated worms on day 14 from the mice irradiated on day 8 clearly show that irradiation as late in the response as day 8 blocks the effector mechanism which normally causes worm rejection. Irradiation administered two days later on day 10, however, was completely ineffective in suppressing worm rejection and only a small number of strobilate worms were recovered from mice in this group on day 14.

The large range in dry weight of worm tissue recovered from each control mouse on day 12 (Fig. 1-8) occurs because almost all the mice autopsied on this day had begun to reject the worms, i.e., almost all points on this day represent weights of a combination

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Figure 1-7

Recovery (%) of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given a sublethal dose of X-irradiation on day 8 or day 10 p.i. Solid areas of bars represent worms >0.1mg dry weight; open portions represent destrobilated worms ($\leqslant 0.1$mg).
D, day p.i.

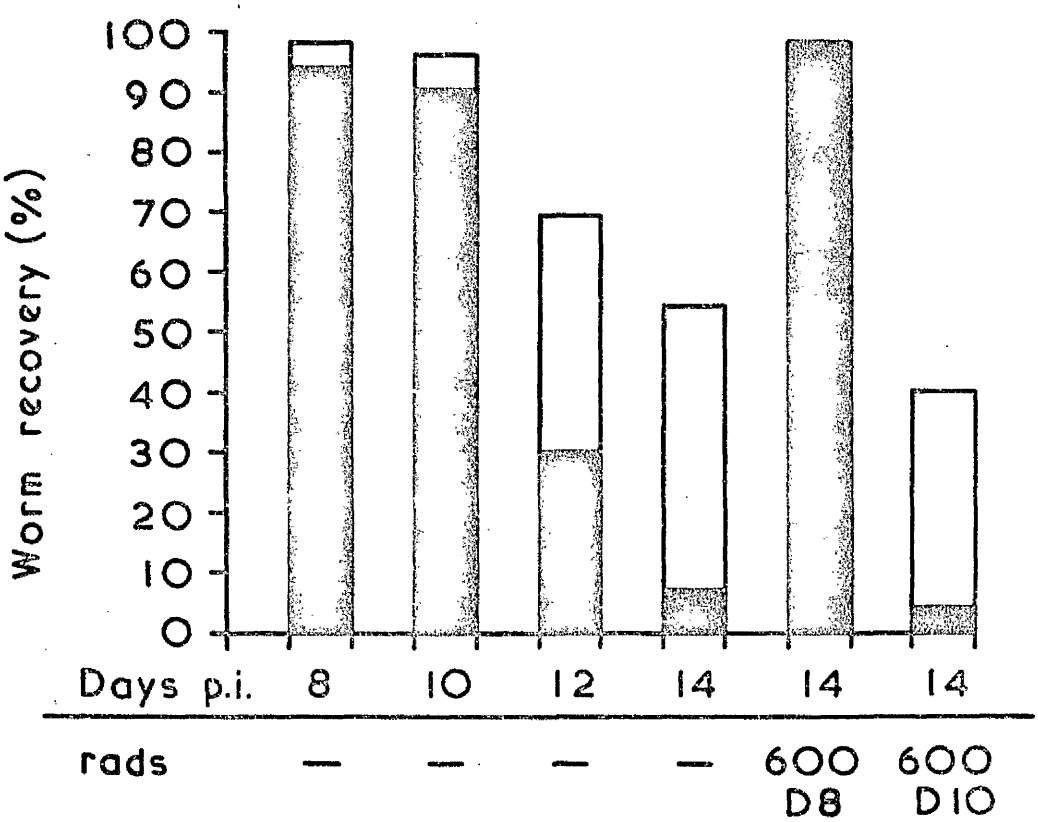
Worm recovery (%)
100
90
80
70
60
50
40
30
20
10
0
Days p.i.
8
10
12
14
14
14
rads
—
—
—
—
600
D8
600
D10

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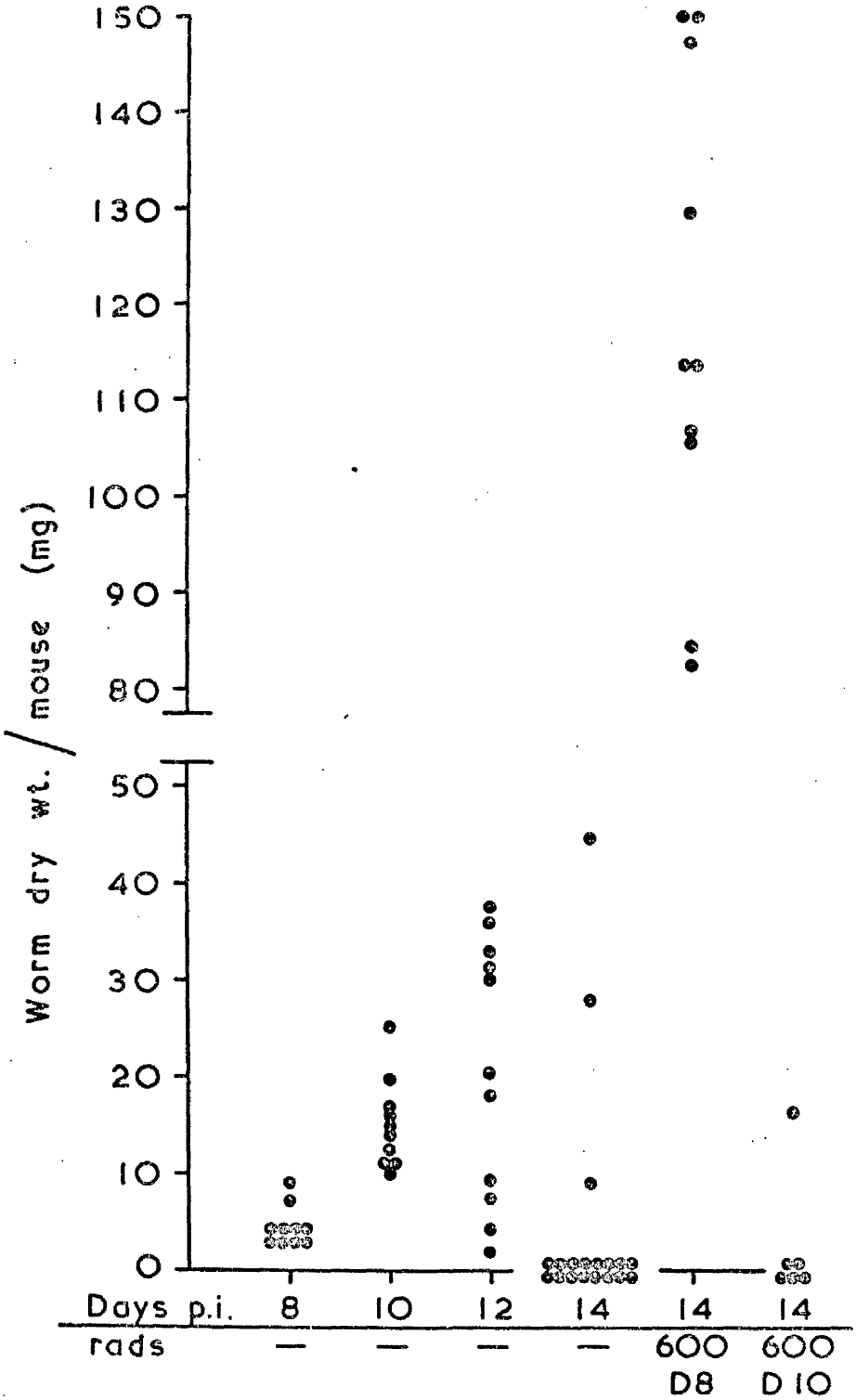
Figure 1-8

Dry weight of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given a sublethal dose of X-irradiation on day 8 or day 10 p.i. Each point represents the total dry weight of worm recovered from a single mouse. D, day p.i.


150
140
130
120
110
100
90
80
50
40
30
20
10
0
Worm dry wt. / mouse (mg)
Days p.i. 8 10 12 14 14 14
rads — — — — 600 D8 600 D10

of strobilate and destrobilated worms. Worms in mice irradiated on day 8 have evidently continued growth after this time, indicated by the very large worm burden/mouse recovered on day 14. Not only, then, has worm rejection been suppressed by irradiation on day 8, but also immunologically mediated worm stunting has been prevented. It is not known whether a single mechanism is responsible for both these effects.

2. The cellular response to a primary infection with H. diminuta.

a) Attempt at passive transfer of immunity using cells.

Investigation of the effector cells involved in the response to H. diminuta using a syngeneic cell transfer system began with an attempt to confer immunity to naive mice by transfer of cell populations from resistant mice.

Cell donors and recipients used in this experiment were 3 star, male NIH mice. As shown in Table 1-5, cell donors were given either 5c primary and 5c secondary infections or were sham infected. The donors were 35 days old when given the primary infection and were killed and the cells transferred on day 12 of the secondary infection. Recipient mice (50 days old) received either 7.4×10^7 pooled SC and MLNC from infected donors (4.0×10^7 ISC; 3.4×10^7 IMLNC) or 7.3×10^7 pooled SC and MLNC from uninfected donors (4.3×10^7 SSC; 3.0×10^7 SMLNC). All injections were carried out i.p. Control mice received no cells. Cell recipients and controls were infected with 5 cysticercoids immediately after the cell transfers had been carried out. Mice from all three experimental groups were autopsied on days 8 and 11 post-infection.

Table 1-5

CELL DONORS

n	DAY 0	9	21	33	45
10	5c —— K				
5	5c ———— K				
35	5c ————			5c —	Cell transfer
20	Sham ————			Sham —	Cell transfer

CELL RECIPIENTS

n	DAY 0	8	11
9	5c —— K		
9	SSC SMLNC 5c —— K		
9	ISC IMLNC 5c —— K		
9	5c ———— K		
9	SSC SMLNC 5c ———— K		
10	ISC IMLNC 5c ———— K		

SSC, spleen cells from naive donors
ISC, spleen cells from infected donors
SMLNC, mesenteric lymph node cells from naive donors
IMLNC, mesenteric lymph node cells from infected donors

Fig. 1-9 shows that worm establishment in the three experimental groups was slightly more variable than usual with a rather low establishment in the control group autopsied on day 8. Recovery on day 8 from both groups of cell recipients, however, was high, demonstrating that the technique of cell transfer did not result in reduced worm establishment.

Autopsy of equivalent groups of mice on day 11 showed that little, if any, reduction in total worm numbers had taken place in any group by this time. The proportion of worms $\leqslant$0.1mg dry weight recovered from all three groups was greater on day 11, this being especially true of the recipients of "primed" cells (control vs. "primed" cell recipients, $P < 0.01$, 2 x 2 contingency table). It is not possible to say whether the large proportion of worms $\leqslant$0.1mg dry weight found in this group represents worms which have destrobilated or worms with severely stunted growth.

Fig. 1-10 shows total dry weight of worm material recovered per mouse. There is no significant difference between any of the groups on day 8 or on day 11 in this respect. Comparison using the parameter of total worm weight per mouse, however, is misleading in this instance as such comparison depends on all mice having the same ratio of worms $>$ 0.1mg:worms $\leqslant$0.1mg. A more meaningful comparison in this case can be made between mean individual worm weights, calculated by dividing total weight of worms from a group of mice by total worm numbers recovered from the group.

	Mean dry wt./worm
Control mice	1.7mg
Mice receiving sham cells	1.7mg
Mice receiving primed cells	0.8mg

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Figure 1-9

Recovery (%) of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given syngeneic lymphoid cells from infected or uninfected donors on the day of infection. Solid areas of bars represent worms $>$ 0.1mg dry weight; open portions represent destrobilated worms ($\leqslant$0.1mg). D, day p.i.; SMLNC, 3.0×10^7 mesenteric lymph node cells from uninfected donors; SSC, 4.3×10^7 spleen cells from uninfected donors; IMLNC, 3.4×10^7 mesenteric lymph node cells from infected donors; ISC, 4.0×10^7 spleen cells from infected donors.

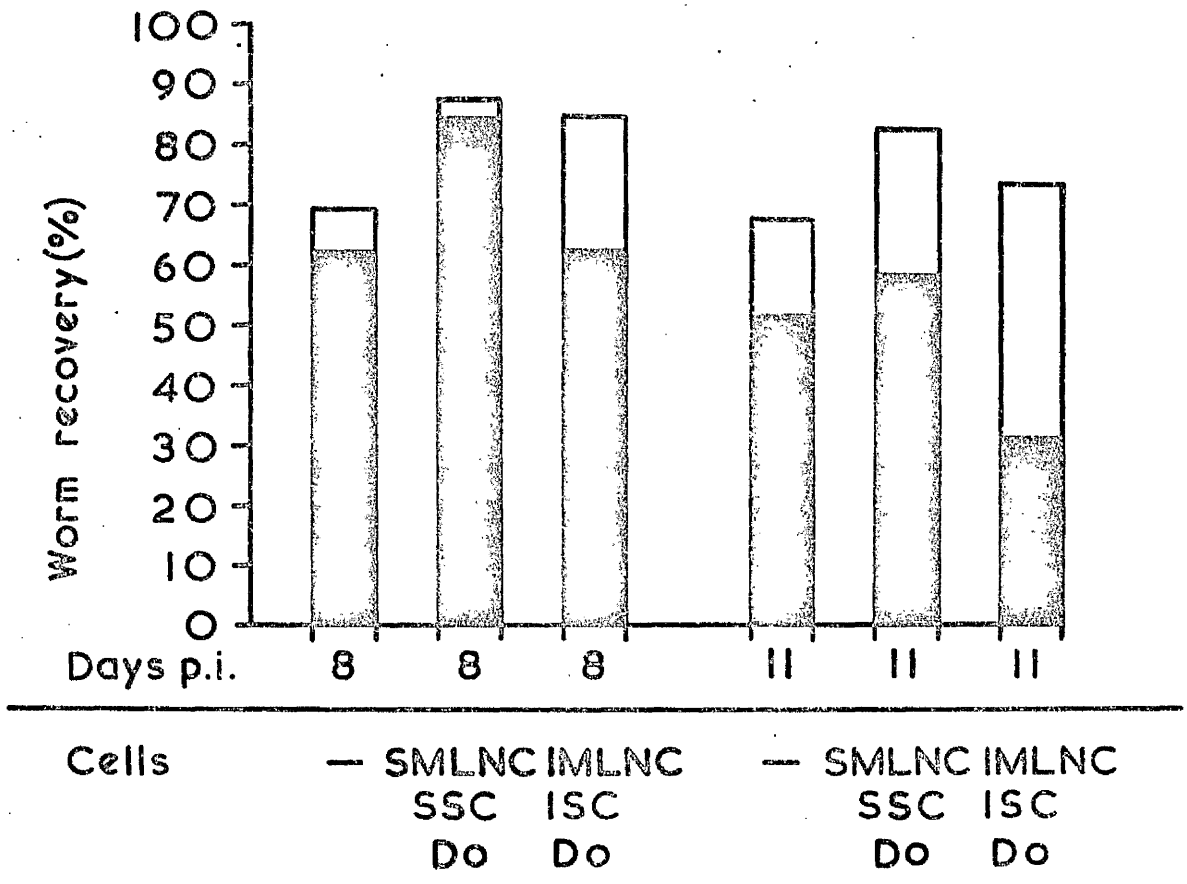
100
90
80
70
60
50
40
30
20
10
0
Worm recovery (%)
Days p.i.
8
8
8
11
11
11
Cells
— SMLNC IMLNC
SSC ISC
Do Do
— SMLNC IMLNC
SSC ISC
Do Do

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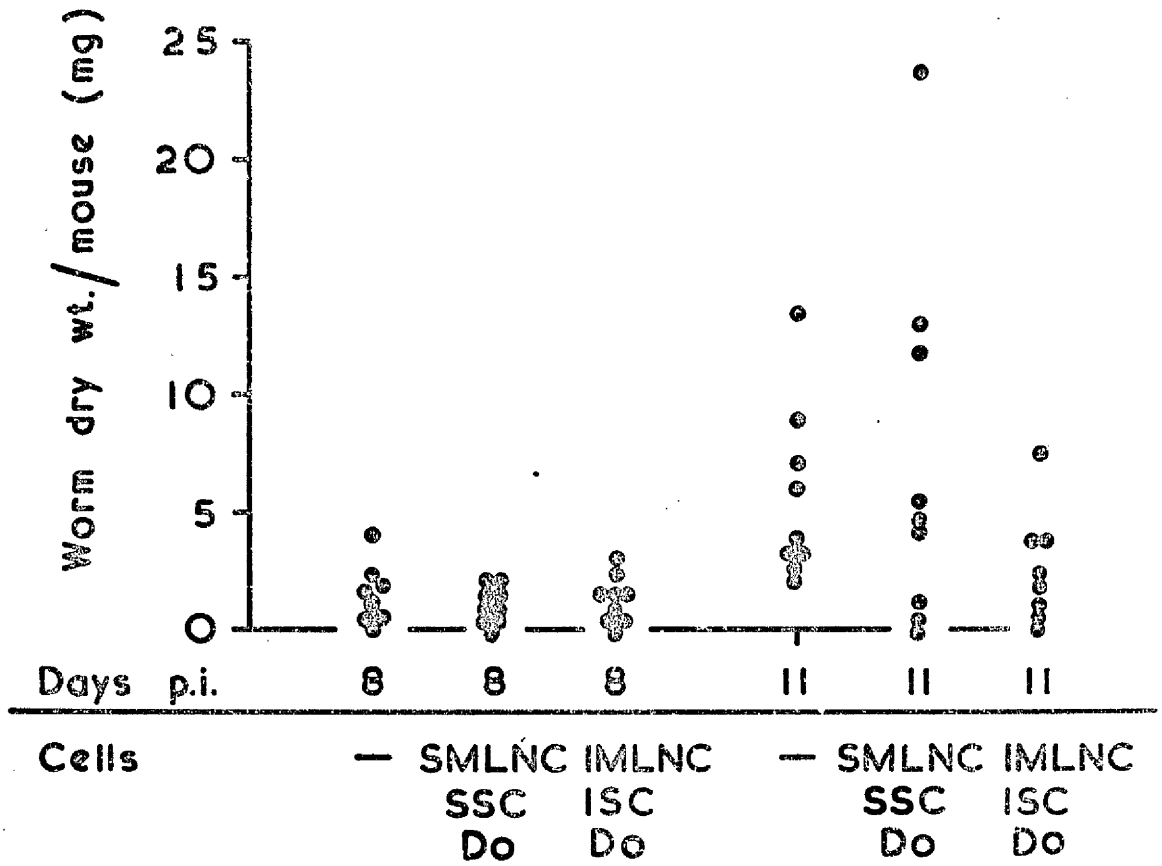
Figure 1-10

Dry weight of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given syngeneic lymphoid cells from infected or uninfected donors on the day of infection. Each point represents the total dry weight of worm recovered from a single mouse. D, day p.i.; SMLNC, 3.0×10^7 mesenteric lymph node cells from uninfected donors; SSC, 4.3×10^7 spleen cells from uninfected donors; IMLNC, 3.4×10^7 mesenteric lymph node cells from infected donors; ISC, 4.0×10^7 spleen cells from infected donors.


Worm dry wt./mouse (mg)
25
20
15
10
5
0
Days p.i.
8
8
8
11
11
11
Cells
— SMLNC IMLNC
SSC ISC
Do Do
— SMLNC IMLNC
SSC ISC
Do Do

It is possible to compare the groups using this parameter as total worm numbers in each group did not differ significantly. The values emphasise the fact that the transfer of "primed" cells had probably either stunted worm growth or advanced the process of worm destrobilation in recipient mice.

The experiment was then repeated under slightly modified conditions. Krebs glucose ringer (KGR) was used to suspend cells in this experiment. Worm recovery from the cell recipients, however, was extremely low on day 8 post-infection, indicating that for reasons unknown worm establishment was low. The results are not shown here. In this experiment, however, infected and uninfected cell donors were given primary and secondary infections of Salmonella typhi-O (0.5ml of a 1:15 suspension in 0.9% saline i.p., 14 days and 7 days before cell transfer). At cell transfer, control mice and recipients of "primed" cells were all given 0.5ml of a 1:5 suspension of S. typhi-O i.p. Recipients of cells from uninfected mice were not given S. typhi-O. Serum from all mice was prepared after autopsy and the titres of anti-S. typhi- O agglutinating antibody were measured. Mean titres for mice in each group are given below.

	Mean titre of anti-S. typhi-O agglutinating antibody
Control	$\frac{1}{10}$
Sham cell recipients	0
"Primed" cell recipients	$\frac{1}{80}$

These results demonstrate that the ability to respond to antigen given to the donor mice was retained by the transferred cells, resulting in a heightened humoral response in the cell recipients. Thus, although this experiment yielded no information on the components of the response to H. diminuta, it showed that at least one aspect of the immune potential of the transferred cells remained viable after transfer. The methods used during cell transfer, therefore, were adequate to maintain at least some cell function. KGR was used to suspend cells in all succeeding experiments.

No further passive transfer experiments involving naive recipients were attempted. Subsequent efforts were concentrated on cell transfer to restore the irradiation-depleted response.

b) Restoration of the irradiation-depleted response.

From the results described at the beginning of this Section, a procedure was devised and adopted for all X-irradiation/reconstitution experiments. The latest time after infection at which irradiation had been effective in suppressing worm rejection was 8 days. It was decided to irradiate all experimental mice on day 7 post-infection to allow for variation in this time and to ensure suppression in all irradiated groups. One group of non-irradiated control mice was always autopsied on day 7. This had the dual purpose of showing the degree of worm establishment and also showing the numbers of worms present on the day of irradiation. Thereafter, groups of control mice were autopsied at varying times to determine the course of worm rejection. Irradiated and reconstituted mice were autopsied shortly after worm rejection had taken place in the controls to determine the effectiveness of each cell function in restoring the depleted response.

Using this procedure, the effectiveness of MLNC from infected donors in restoring the depleted response was firstly investigated. The experimental plan is given in Table 1-6.

Table 1-6

n	DAY 0	7	8	12	13
10	5c	K			
10	5c			K	
10	5c				K
10	5c	600 rads			K
13	5c	600 rads	SMLNC		K
18	5c	600 rads	IMLNC		K

Male, 3 star NIH mice, to be used as cell donors were given a single infection of 5c when 45 days old. Identical mice, for use as donors of control cells were given a sham infection at the same time. Cell recipients were also infected with 5c when 45 days old. Experimental mice were irradiated on day 7 of the infection and cell transfers were carried out on the following day (day 15 of the infection in the cell donors). 4.0×10^7 MLNC from either infected or uninfected donors were injected i.v. into the respective recipient mice.

Figure 1-11 shows worm recovery. The high recovery on day 7 demonstrates that establishment was good and that mice irradiated on day 7 maintained, when irradiated, a high percentage of worms administered. Autopsy of a control group on day 12 yielded a

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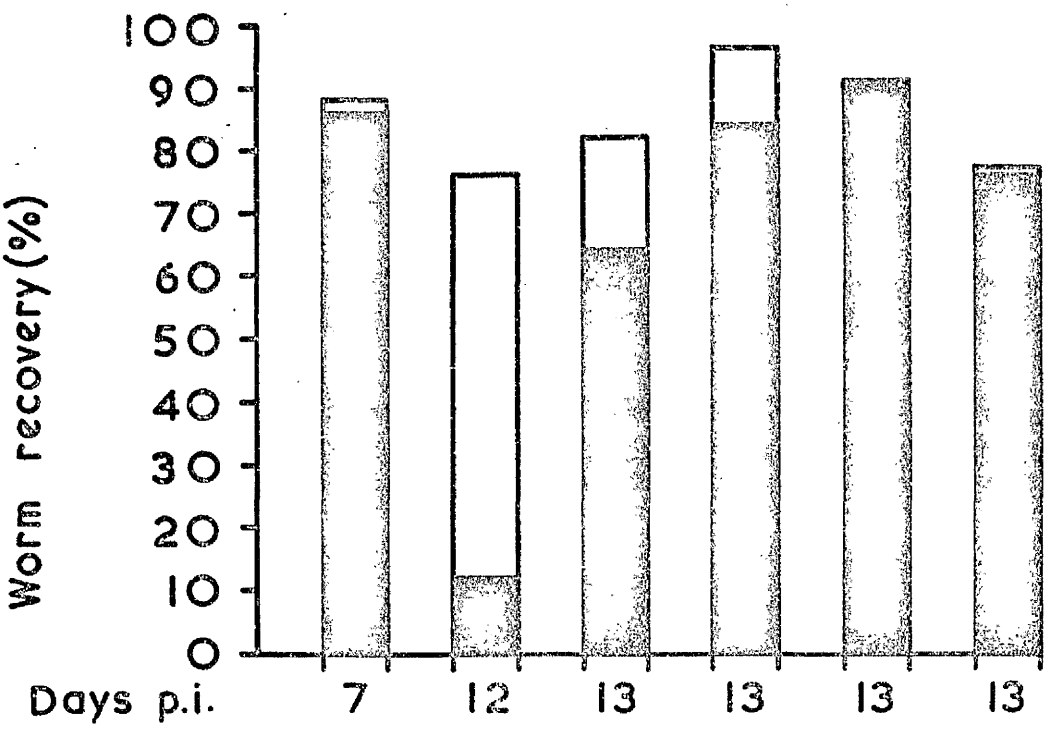
Figure 1-11

Recovery (%) of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given a sublethal dose of X-irradiation on day 7 p.i. followed by syngeneic mesenteric lymph node cells from infected or uninfected donors on day 8 p.i. Solid areas of bars represent worms $>$ 0.1mg dry weight; open portions represent destrobilated worms ($\leq$0.1mg). D, day p.i.; SMLNC, 4.0×10^7 mesenteric lymph node cells from uninfected donors; IMLNC, 4.0×10^7 mesenteric lymph node cells from infected donors.



rads	—	—	—	600 D7	600 D7	600 D7
Cells	—	—	—	—	SMLNC D8	IMLNC D8

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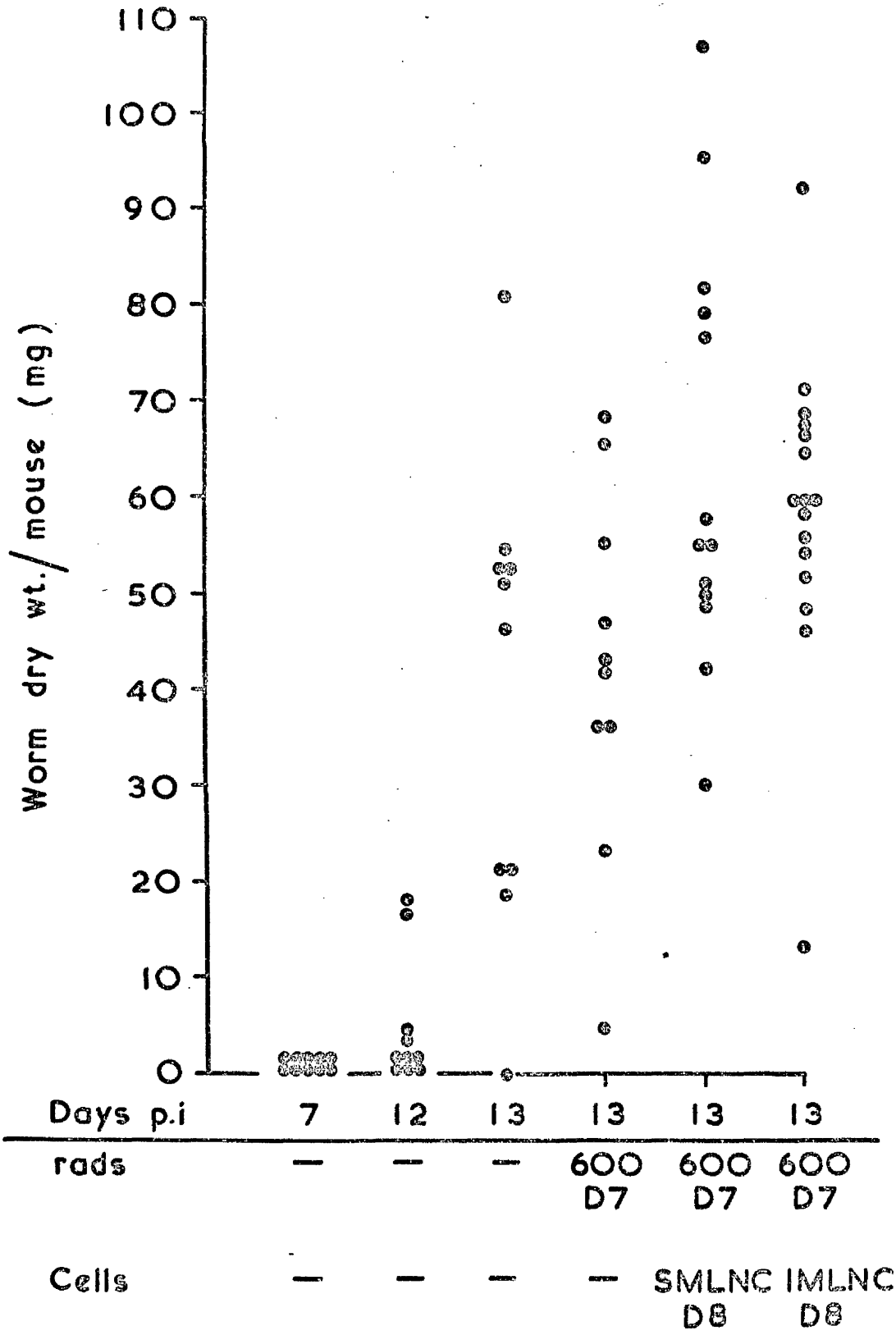
Figure 1-12

Dry weight of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given a sublethal dose of X-irradiation on day 7 p.i. followed by syngeneic mesenteric lymph node cells from infected or uninfected donors on day 8 p.i. Each point represents the total dry weight of worm recovered from a single mouse. D, day p.i.; SMLNC, 4.0×10^7 mesenteric lymph node cells from uninfected donors; IMLNC, 4.0×10^7 mesenteric lymph node cells from infected donors.


Worm dry wt./mouse (mg)
110
100
90
80
70
60
50
40
30
20
10
0
Days p.i
7
12
13
13
13
13
rads
—
—
—
600 D7
600 D7
600 D7
Cells
—
—
—
—
SMLNC D8
IMLNC D8

large number of worms $\leq$0.1mg dry weight, suggesting that worm rejection was well advanced. It was decided, therefore, to autopsy remaining groups the following day. A high proportion, however, of worms >0.1mg dry weight recovered from the control group on day 13 is difficult to explain.

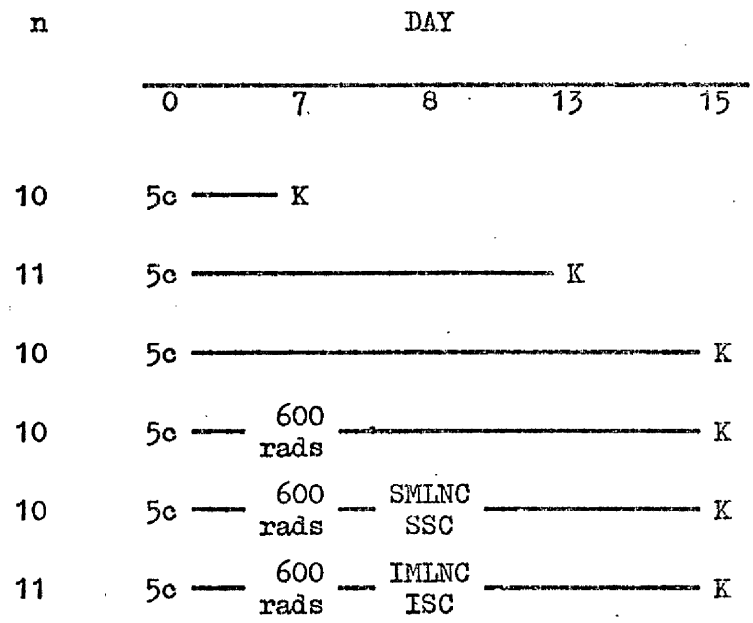
Firstly, this degree of variation between similarly treated groups is rare when multiple infections are used. Genetic variation is unlikely to account for this as inbred mice were used and variation within each group was no greater than normal. It is possible that worms $\leq$0.1mg found on day 12 were stunted rather than destrobilated worms but this is unlikely as by day 7 almost all worms in control mice were >0.1mg. The most likely explanation is that the group of control mice autopsied on day 13 had been stressed in some way and that this had delayed rejection of the worms.

Neither worm recovery from mice receiving MLNC from infected donors, nor recovery from mice receiving MLNC from uninfected donors, differed appreciably from recovery from mice which were irradiated but given no cells. Worm growth (Fig. 1-12) was also similar in all three experimental groups, indicating that MLNC alone, whether "primed" or not are ineffective in restoring the irradiation-depleted response under the conditions used.

The effect of a combination of spleen cells (SC) and MLNC on the depleted response was then determined using the regime outlined in Table 1-7.

Twenty male, 3 star NIH mice were infected with five cysticercoids when 39 days old. Cells were transferred from these mice on day 18 of the infection. Twenty identical mice were left uninfected as donors of control cells.

Table 1-7



4.0×10^7 MLNC + 4.0×10^7 SC from infected or uninfected donors were given i.v. to respective recipient groups of mice.

As the worm recoveries in Fig. 1-13 show, there was a high establishment of worms in all groups. A large number of worms were rejected from the control mice by day 13 and the group autopsied on day 15 yielded only $\frac{3}{50}$ worms > 0.1mg, the remainder having been destrobilated and/or expelled.

Irradiation on day 7, as expected, suppressed worm rejection, at least up to day 15. Worm recovery from both groups of cell recipients on day 15 shows no significant reduction compared with recovery in the irradiation-only group.

Fig. 1-14 shows that the dry weights of worms recovered from the mice reconstituted with cells from uninfected donors were significantly less ($P < 0.01$) than those recovered from mice irradiated but not reconstituted. Weights of worms recovered from mice receiving "primed" cells, however, did not differ from those recovered from the irradiation-only group.

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Figure 1-13

Recovery (%) of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given a sublethal dose of X-irradiation on day 7 p.i. followed by syngeneic mesenteric lymph node cells and spleen cells from infected or uninfected donors on day 8 p.i. Solid areas of bars represent worms >0.1mg dry weight; open portions represent destrobilated worms (≤ 0.1mg). D, day p.i.; SMLNC or IMLNC, 4.0×10^7 mesenteric lymph node cells from uninfected or infected donors respectively; SSC or ISC, 4.0×10^7 spleen cells from uninfected or infected donors respectively.

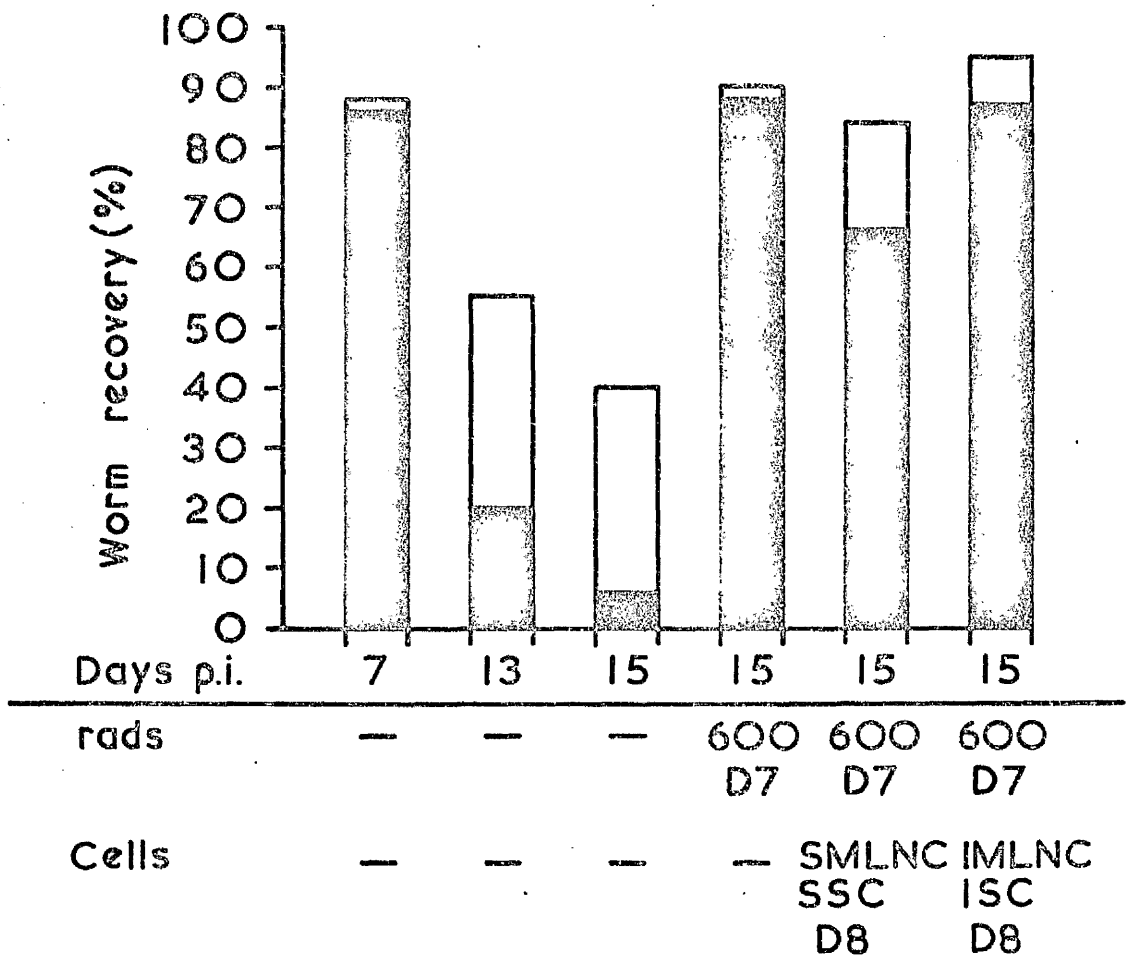
Worm recovery (%)
100
90
80
70
60
50
40
30
20
10
0
Days p.i.
7
13
15
15
15
15
rads
—
—
—
600 D7
600 D7
600 D7
Cells
—
—
—
—
SMLNC SSC D8
IMLNC ISC D8

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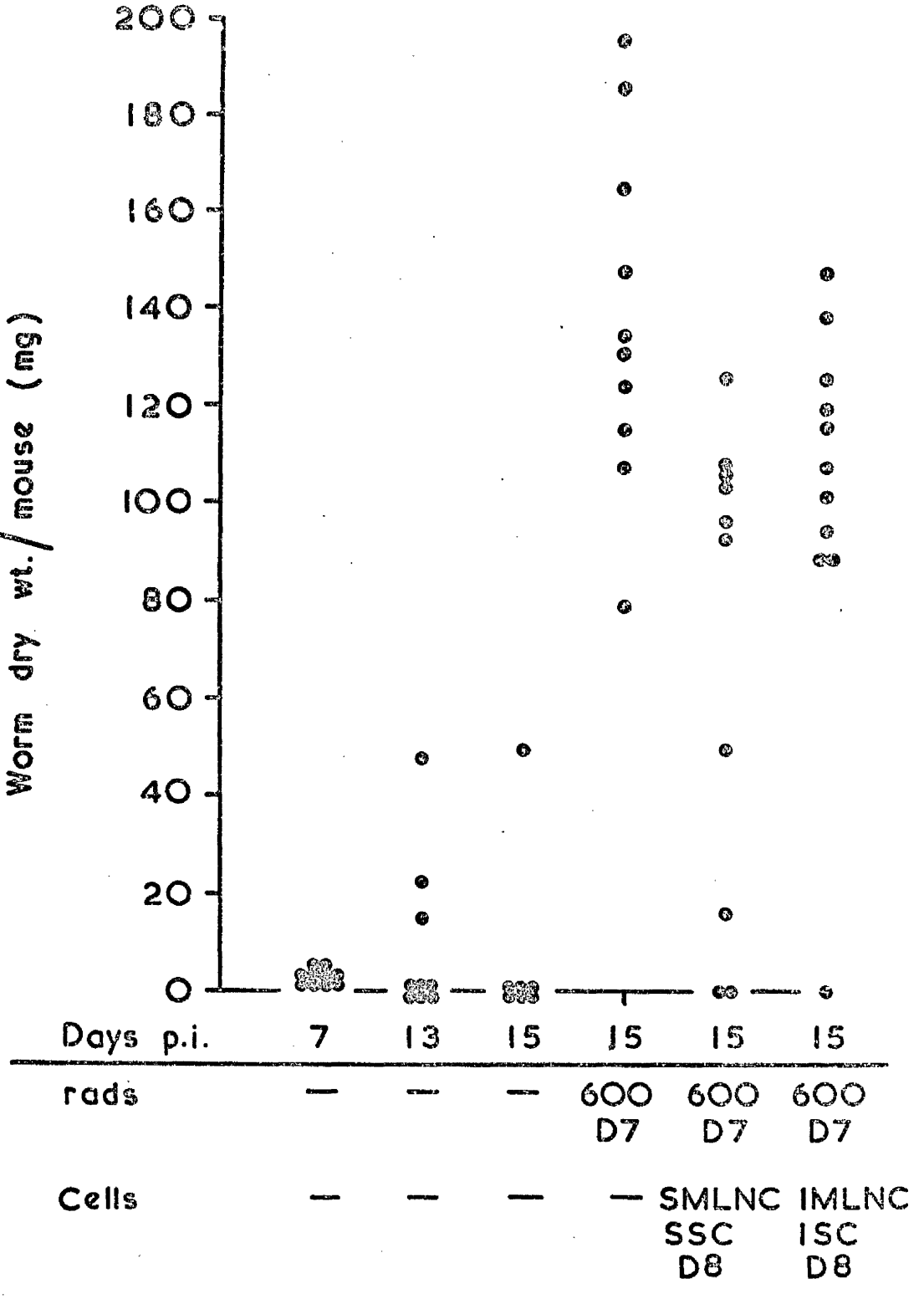
Figure 1-14

Dry weight of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given a sublethal dose of X-irradiation on day 7 p.i. followed by syngeneic mesenteric lymph node cells and spleen cells from infected or uninfected donors on day 8 p.i. Each point represents the total dry weight of worm recovered from a single mouse. D, day p.i.; SMLNC or IMLNC, 4.0×10^7 mesenteric lymph node cells from uninfected or infected donors respectively; SSC or ISC, 4.0×10^7 spleen cells from uninfected or infected donors respectively.


200
180
160
140
120
100
80
60
40
20
0
Worm dry wt./mouse (mg)
Days p.i.
7
13
15
15
15
15
rads
—
—
—
600 D7
600 D7
600 D7
Cells
—
—
—
—
SMLNC SSC D8
IMLNC ISC D8

Finally, the ability of bone marrow cells (BMC), alone and in combination with other cell types, to reconstitute the response was tested. Recipient mice were given cells only from mice which had been infected, i.e., no cells were transferred from uninfected mice. The experimental regime is given in Table 1-8.

Table 1-8

n	DAY 0	7	8	15
10	5c			K
10	5c			K
10	5c	600 rads		K
10	5c	600 rads	IMLNC	K
10	5c	600 rads	IBMC	K
10	5c	600 rads	IMLNC IBMC	K
10	5c	600 rads	IMLNC IBMC ISC	K

Cell donors (30 male, 3 star NIH mice, 44 days old) were given a primary infection only of five cysticercoids. Recipient mice were 54 days old at infection. On day 18 of the infection in donor mice, the following numbers of cells were transferred to respective recipient groups.

8.0×10^7 IMLNC
or 8.0×10^7 IBMC

or 4.0×10^7 IMLNC
4.0×10^7 IBMC

or 2.7×10^7 IMLNC
2.7×10^7 IBMC
2.7×10^7 ISC

By day 15, 80% of the worms in control mice were destrobilated and/or expelled (See Fig. 1-15). All experimental mice were autopsied on this day also. Unfortunately, suppression of worm rejection in mice receiving irradiation only was not as marked as in previous experiments, there being a recovery on day 15 of only 62% of the worms administered. This low worm recovery was distributed over most mice in the group, not confined to one or two mice rejecting all their worms. This causes some difficulty in interpretation of results as the demonstration of the ability of various components to reconstitute the response depends on the presence of a suitably depleted response. Worm recovery from all the reconstituted mice is actually greater than from the irradiation-only group in this case.

Similarly, worm growth (Fig. 1-16) was greater in all the reconstituted mice than in the mice irradiated but not reconstituted. In fact, worm growth was so great in mice reconstituted with IMLNC + IBMC that it differed significantly ($P < 0.01$) from worm growth in irradiation-only controls.

Despite this difficulty, it seems unlikely that the response was appreciably reconstituted by any of the cell treatments as all of these groups gave high recoveries of large worms when compared with untreated controls autopsied on the same day.

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Figure 1-15

Recovery (%) of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given a sublethal dose of X-irradiation on day 7 p.i. followed by syngeneic mesenteric lymph node cells, bone marrow cells and spleen cells from infected donors on day 8 p.i. Solid areas of bars represent worms >0.1mg dry weight; open portions represent destrobilated worms (≤ 0.1 mg). D, day p.i.; see text for cell numbers.

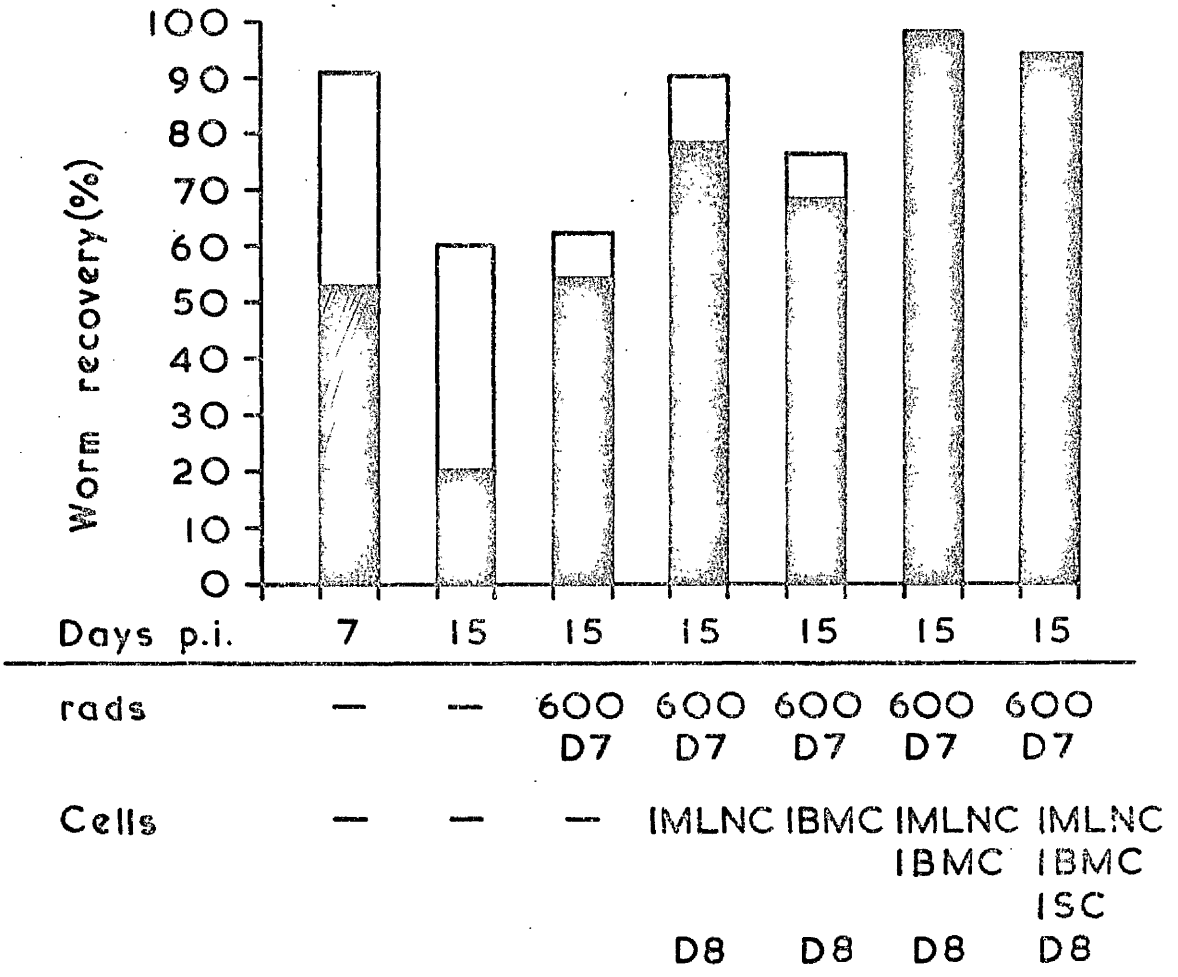
Worm recovery (%)
100
90
80
70
60
50
40
30
20
10
0
Days p.i.
7
15
15
15
15
15
15
rads
—
—
600 D7
600 D7
600 D7
600 D7
600 D7
Cells
—
—
—
IMLNC
IBMC
IMLNC IBMC
IMLNC IBMC ISC
D8
D8
D8
D8

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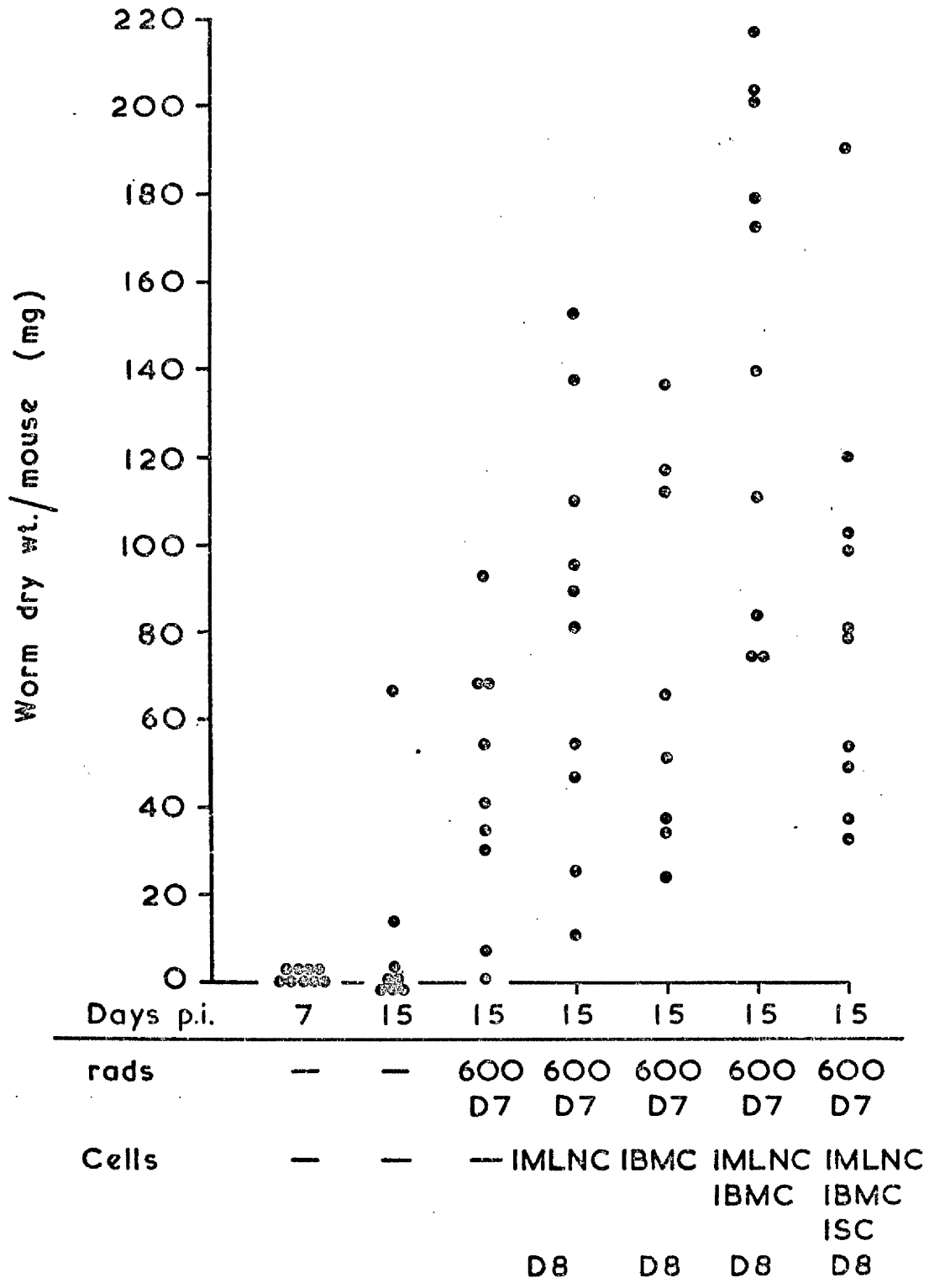
Figure 1-16

Dry weight of Hymenolepis diminuta from five-cysticercoid infections in NIH mice given a sublethal dose of X-irradiation on day 7 p.i. followed by syngeneic mesenteric lymph node cells, bone marrow cells and spleen cells from infected donors on day 8 p.i. Each point represents the total dry weight of worm recovered from a single mouse. D, day p.i.; see text for cell numbers.


Worm dry wt./mouse (mg)
220
200
180
160
140
120
100
80
60
40
20
0
Days p.i.
7
15
15
15
15
15
15
15
rads
—
—
600 D7
600 D7
600 D7
600 D7
600 D7
Cells
—
—
—
IMLNC
IBMC
IMLNC IBMC
IMLNC IBMC ISC
D8
D8
D8
D8

The ability of the transferred cells to mount a response after transfer was tested by challenging half the mice in each group with SRBC on the day of cell transfer. Anti-SRBC agglutinating antibody titres were measured at autopsy on day 15 and are given below.

Group	Mean $\log_2$ anti-SRBC titre
Control	5.3
Irradiation only	0.0
Irradiation + IMLNC	5.0
Irradiation + IBMC	0.0
Irradiation + IMLNC + IBMC	4.3
Irradiation + IMLNC + IBMC + ISC	4.8

Hence, once again the transferred cells maintained the capacity to respond to antigenic challenge by producing circulating antibody, although this did not correlate with protection towards H. diminuta.

DISCUSSION

The results show that the response to a single worm infection with H. diminuta was suppressed by administration of either a lethal dose (900 rads) or a sublethal dose (600 rads) of whole-body X-irradiation (Figs. 1-1, 1-2). A dose of 600 rads was used thereafter to deplete the response as this eliminated the necessity for bone marrow reconstitution after a lethal dose. An early experiment, using single worm infections in outbred mice, indicated that the immunosuppressive effect of 600 rads decreased markedly if irradiation was delayed until day 10 post-infection (Figs. 1-3, 1-4). The degree of variability using single worm infections was considered unacceptable and, as higher levels of infection had previously been shown to exhibit less inherent variability (Befus, 1975b), all further experiments were carried out using 5-worm infections. The results using five worms were found to parallel those from single worm infections; the response in mice irradiated on days up to and including day 8 was suppressed but irradiation two days later was ineffective. These results suggest that a radiation-sensitive component is active in the response up to day 8, after which time the response is irreversible. This result closely follows the kinetics of radiation-induced suppression of the response to T. muris (Wakelin, 1975a).

Although X-irradiation is effective in depleting the response which causes rejection of H. diminuta, the depleted component is apparently fairly rapidly restored. Suppression achieved by irradiation on day 7 was fully restored by day 18 (Fig. 1-5). This result appears to be contradicted by the 100% recovery of worms on day 12 from mice irradiated on day -1 and by the results

of the first experiment in which almost 90% of worms in a single worm infection remained on day 18 in mice also irradiated on day -1 (Fig. 1-1). Assuming that irradiation is affecting a lymphocyte-dependent phase, there are several possible explanations for this. Fully differentiated, sensitised lymphocytes are more radiation-resistant than non-sensitised lymphocytes (Denham et al. 1970; Hirst et al. 1970). Irradiation before infection would destroy small lymphocytes before sensitisation and so restoration of the response would require a longer recovery period. Alternatively, irradiation on day 7 may kill effector cells already present but would be unlikely to affect T memory cells (Playfair & Marshall-Clarke, 1973; Youdim, Stutman & Good, 1973). Upon recovery of other components a short time following irradiation, these memory cells would then initiate a rapid, anamnestic-type response inducing worm rejection.

If the response does in fact resume a short time after irradiation, this may aid explanation of the results shown in Fig. 1-6. These data indicate that less worm growth takes place after irradiation given early in infection than after irradiation given later in infection. As worm growth progresses, an increasing number of effector cells will become sensitised and the depressive effect on worm growth will increase proportionately. On day -1 no sensitised cells will be present and so irradiation will not affect the response suppressing worm growth. Even if irradiation at this time removes effector cell precursors, thus delaying the response affecting worm growth, it may be that newly excysted worms in the lag phase preceding worm growth are more sensitive to irradiation-altered gut physiology than worms which are actively growing later in infection. If these interpretations

are correct, they may explain why worm growth in mice irradiated just before infection does not differ from worm growth in unirradiated mice. By day 5, sufficient numbers of cells will have developed to exert a depressive effect on worm growth. Their removal at this stage by irradiation would allow unimpeded worm growth for a short time before recovery of the response. This would result in a worm growth somewhat greater than in unirradiated mice. The response limiting worm growth will be even greater by day 7 and its suppression by irradiation would have a more profound releasing effect on worm growth. Moreover, in this case, it is unlikely that the response would recover before autopsy of the mice on day 12.

An important point which emerges from these experiments arises from the inability of X-irradiation to suppress events occurring after day 10. Hopkins & Stallard (1976) have shown that cortisone acetate does suppress the effector arm of the response which acts at this stage. Thus, it would appear that a period of cellular sensitisation may be occurring prior to day 8, which may or may not be associated with antibody-mediated damage to the worms (Befus, 1974; Befus & Threadgold, 1975). The phase of the response occurring after this time, presumably mediated by radiation-resistant, sensitised cells is cortisone-sensitive. It was to determine the possible cellular nature of the first, radiation-sensitive component that the second series of experiments was designed.

Although the preliminary experiment suggested that the adoptive transfer of IMLNC + ISC to naive mice conferred a certain amount of response to subsequent infection, none of the combinations of cell types used in attempts to reconstitute the irradiated

response appeared to do so. There are several possible explanations for this failure.

Were the transferred cells viable? Cell viability and maintenance of immune capacity was measured in several ways. First of all, dye exclusion tests carried out before and after transfer indicated cell viability of at least 90% in all cases. These tests are not the most sensitive tests for lymphocyte viability (Tennant, 1964) and the demonstration of an *in vitro* function does not necessarily correlate to the *in vivo* situation (Ford & Hunt, 1973). Certain aspects of *in vivo* functions, however, were assessed. Cell recipients were able to produce humoral agglutinating antibody to SRBC after cell transfer. Moreover, cells sensitised to a particular antigen (*S. typhi*-O) were able to mount a response to that antigen after transfer. Thus, transferred cells had the ability to reconstitute a depleted humoral response and, if sensitised to an antigen, maintained their recognition of it and ability to proliferate in response to challenge with it. These tests measure humoral capacity of recipient mice and give no information on the ability to mount a local, intestinal response. It is possible, then, that an intestinal response could remain depleted due to, say, a reduction in the capacity of transferred cells to home to intestinal lymphoid tissue. This, however, is unlikely as Wakelin (1975a) and Wakelin & Selby (1976) successfully restored a depleted response to *T. muris* using identical techniques. The defect, then, does not seem to result from technical factors.

Is there a requirement for antibody, in addition to cells? Circulating antibody to *H. diminuta* has been demonstrated in infected rats by Coleman, Carty & Graziadei (1968) and Harris &

Turton (1973). The results of Harris & Turton show that in both 5 worm and 25 worm infections similar parasite-specific antibody levels are detectable by immunofluorescence even though no worm loss occurred at the lower infection level. Levels of anti-H. diminuta humoral antibody in the rat, then, are unlikely, in themselves to account for a protective response. Moreover, Befus (1975a) was unable to demonstrate, using immunodiffusion, specific antibody to H. diminuta in the serum of infected mice, nor was he able to demonstrate raised serum immunoglobulin levels in infected mice. This evidence, and the lack of protection afforded by "immune" serum in passive transfer studies (Hopkins, personal communication) suggests that serum antibodies are not important in the response to H. diminuta by the mouse. The work of Befus (1974, 1975a) using a direct immunofluorescence technique, suggests that anti-H. diminuta antibodies, manufactured locally in the lamina propria of the intestine, are bound to the tapeworm tegument. It would appear, then, that antibodies are produced during the protective response of the mouse to H. diminuta but that they are produced locally in the lamina propria of the small intestine and that serum antibodies, if present at all, are in such small quantities to be of little consequence in protection. The difference, in this respect, between the response to H. diminuta and the proven requirement for serum antibodies in the response to other intestinal helminths may derive from the entirely enteric nature of H. diminuta infection compared with that of nematode infections, all of which involve penetration of the mucosa to a greater or lesser extent. The differing response stimulated by parenteral or enteric challenge have been discussed by Tomasi & Grey (1972), and Targett (1973, p. 229) has emphasised this point in relation to helminth infections.

In previous studies of the component nature of responses to intestinal helminths where X-irradiation has been used to deplete the response, it has been assumed that the antibody-mediated component has been unaffected by irradiation. As discussed in the preface to this section, this assumption has been based on the observations of Makinodan et al. (1967) of the radioresistance of antibody-secreting plasma cells. This may be a fair assumption in the case of responses involving serum antibody production. However, in the response to H. diminuta, where locally produced antibody may be more important, the effect of irradiation on lamina propria plasma cells observed by Bazin et al. (1971) may have greater significance. It may be that the locally produced antibody in H. diminuta infection serves to identify the parasite antigen to other components which then act against it. If X-irradiation is reducing production of this antibody for several days as Bazin et al. (1971) suggest, then recognition of the antigen may not take place even though sensitised cells are replaced after irradiation. This also raises the possibility that the mechanism is totally dependent on antibody and that a subsequent cell-mediated phase is not required.

Were the transferred cells of the required type; were sufficient numbers of cells transferred; and were the cells sufficiently sensitised to recognise the parasite antigen? Studies on the cellular requirements of the host response to other intestinal helminths have all implicated MLNC as the cell type responsible for mediating parasite rejection. Thus, Keller & Keist (1972) induced expulsion of N. brasiliensis from sublethally irradiated rats using MLNC from peptone-treated immune or non-immune donors. Dineen, Kelly & Love (1973) however, demonstrated the importance

of using lymphocytes from infected rats to complete the rejection mechanism in this system. Wagland & Dineen (1965) and Dineen & Wagland (1966) showed that resistance to T. colubriformis could be transferred to susceptible guinea-pigs by transfer of MLNC from previously infected donors. Selby & Wakelin (1973) demonstrated transfer of resistance to T. muris with MLNC and Wakelin (1975a) successfully used MLNC from immune mice to reconstitute the depleted response in irradiated mice. Similar requirements for MLNC have been reported for transfer of immunity to T. spiralis (Larsh, Goulson & Weatherly, 1964). All of these studies used cell numbers equivalent to those used in the experiments described here. It seems unlikely that the response to H. diminuta should differ drastically in its cellular requirements from these other systems. It is possible, however, considering the apparent peculiarly local antibody response to H. diminuta, that transfer of more closely-associated intestinal lymphoid tissue (for example, Peyer's patch cells) may bear fruit. In the N. brasiliensis-rat system, a second cellular component, derived from bone marrow, is reportedly required for successful worm expulsion (Dineen & Kelly, 1973; Kelly et al. 1973). This possibility was also investigated in the present study to no added advantage.

The possibility remains that the transferred cells were taken from donors at the wrong time after infection, i.e., that insufficient numbers of sensitised cells were transferred or that transfer did not coincide with the time of requirement. It is unlikely that sufficient sensitised cells were not transferred to at least partially restore the response as the components of the response in the cell donors occur as a continuum and do not replace each other absolutely.

In conclusion, it can be said that there remains the strong probability of lymphoid cell involvement in the response of the mouse to H. diminuta but that further exhaustive experiments involving the reconstitution of the X-irradiated response must await investigation of the effects of irradiation on local antibody production in the small intestine. Fluorescent antibody studies, similar to those described by Befus, applied throughout the course of the radiation-depleted response, may yield the information required to plan further investigations of cellular involvement.

SUMMARY

1. Suppression of the immunological rejection of H. diminuta by the mouse using sublethal and lethal doses of whole-body X-irradiation is described.

2. The effects on worm growth and rejection of 600 rads X-irradiation given at varying times during a five cysticercoid infection are discussed.

3. The response to infection was shown to be biphasic with respect to radiation sensitivity: it was shown to be suppressed by irradiation administered prior to day 8 post-infection; a radiation-resistant phase followed this time. Irradiation-induced suppression was not permanent.

4. The cellular nature of the radiation sensitive phase was investigated by attempting reconstitution using various combinations of lymphoid cell populations and bone marrow cells.

5. Restoration of the radiation sensitive phase was not achieved with the cells used. Possible reasons for this lack of reconstitution are discussed.

6. Possible effects of irradiation other than the suppression of small lymphocyte action are discussed. It is suggested that analysis of side-effects, with particular emphasis on the effects on local antibody production in the small intestine (which may have particular relevance in the response to H. diminuta), be carried out before further attempts are made to reconstitute the irradiation-depleted response.

SECTION 2

THE THYMUS-DEPENDENCY OF THE RESPONSE

INTRODUCTION

a) Thymus-dependency in parasite infections.

The host response, or at least a proportion of the response, to a great many parasite species has been shown to be more or less dependent on the presence of functional thymus-derived (T) lymphocytes. In terms of present concepts on the immune regulatory functions of T cells this may not seem surprising.

The degree of thymus-dependency appears greatest in helminth infections because frequently the host response is of an "all or nothing" nature. Thymus-dependency in the host response to parasitic protozoa, however, ought to be considered briefly, not only for completeness but also because a link between thymus-dependency and evasion of the host response has been proposed.

The host response to the three most-studied genera of parasitic protozoa - Plasmodium, Trypanosoma and Leishmania - has been shown to require thymus co-operation. Brown, Allison & Taylor (1968) reported a greater mortality and higher parasitaemia in P. bergheii-infected neonatally thymectomised rats than in similarly infected controls. This was supported by the work of Spira, Silverman & Gaines (1970) using ATS-treated rats. Raised parasitaemias and heightened mortality has also been reported in experimental Trypanosoma sp. infections in T cell-depleted hosts (Schmunis, Gonzalez Cappa, Traversa,& Janovsky (1971) using T. cruzi in depleted mice; Tawil & Dusanic (1971) with T. lewisi

in ALS-treated rats; and Targett (1973) using T. musculi in T cell deprived mice). Both Plasmodium and Trypanosoma exhibit antigenic variation in successive waves of parasitaemia. Brown (1971) has suggested that T cell-B cell co-operation may be necessary to overcome the evasion of protection brought about by the production of antigenic variants. He suggests that T cells are sensitised to antigenic determinants common to all variants and that B cells respond to variant-specific antigenic determinants. Co-operation then takes place to produce variant-specific antibody. It remains to be determined whether the humoral antibody production to these protozoa is the only phase in protection which is thymus-dependent.

Bryceson & Turk (1971) showed that the initial progression of cutaneous lesions due to L. enrietti in guinea-pigs was slower in ALS-treated hosts than in untreated hosts, but that the lesions in the untreated guinea-pigs healed, whereas those in the ALS-treated animals did not. Preston, Carter, Leuchars, Davies & Dumonde (1972) observed the same type of response in thymectomised mice given L. tropica var. major. There is, however, controversy over the point of action of T cells in the protective response to Leishmania sp.

The host response to all helminth species thus studied appears to be thymus-dependent. Little work, however, has been carried out on the phase of the response which requires T cell co-operation. It is now well established that the rejection of adult Trichinella spiralis from the intestine is thymus-dependent (Machnicka, 1972 using ALS-treated mice; Ruitenberg, 1974 using ALS-treated or neonatally thymectomised rats; Ruitenberg & Steerenberg, 1974 using nude mice). In the most extensive study so far, Walls, Carter,

Leuchars & Davies (1973) using adult thymectomised, lethally irradiated, BMC reconstituted mice experienced mortality in T. spiralis-infected mice but none in uninfected mice. Adult worms were retained in the intestine for a longer period in deprived mice and they induced virtually no inflammation. Muscle larvae were studied in the diaphragm and hamstring muscles. Much higher numbers of larvae encysted in the muscles of deprived mice. Inflammatory infiltrates of eosinophils, polymorphs, lymphocytes and macrophages soon formed around larvae encysted in normal mice. In deprived mice cyst formation was not impaired but the encysted larvae induced negligible inflammatory response. The eosinophil response in deprived mice was particularly defective although the initial response was comparable to that found in controls. This thymus-dependency of T. spiralis-induced eosinophilia confirms the earlier work of Walls, Basten, Leuchars & Davies (1971). Depression of inflammation is not mandatory in T cell depleted animals. For example, Davies, Carter, Leuchars & Wallis (1969) found no depression of the inflammatory response to oxazolone in deprived mice. Eosinophils are derived from bone marrow precursors. Walls et al. (1973) found that deprived mice were still able to produce a polymorphonuclear leucocytosis on stimulation which suggests that there is no defect in the bone marrow. The defect may be in the recruitment of eosinophils by T cell chemotactic factors or in the loss of possible co-operating T cells which may act directly on the bone marrow precursors.

It is unlikely that the unresponsiveness of deprived mice to T. spiralis derives solely from a defect in the parasite-induced eosinophilia. Although there is an obvious cellular defect in the inflammatory response, this response is not necessarily the

modus operandi of protection, and humoral factors may also be involved. The recently described effector functions attributed to the eosinophil in schistosome infections, however, (see below) force renewed interest in the T cell-dependent eosinophil response to T. spiralis infections.

In neonatally thymectomised rats, Ogilvie & Jones (1967) demonstrated a reduced response to primary and challenge infections of Nippostrongylus brasiliensis. Reduced levels of reagins were demonstrated in the deprived rats. This is the only study of the effect of T cell depletion on antibodies to N. brasiliensis and so the thymus-dependency of step-one of the host response is unknown. Keller & Keist (1972) showed that neonatally thymectomised, ATS-treated rats did not expel antibody-damaged worms, indicating that the second, lymphocyte-mediated phase of the expulsion mechanism is thymus-dependent.

The host response to two other much-studied nematode parasites has been shown to be thymus-dependent but, as yet, no evidence is available on the phase of the response requiring T cell co-operation. Thus, Wakelin & Selby (1974) showed, using adult-thymectomised, irradiated mice that rejection of Trichuris muris was T cell-dependent and Dineen & Adams (1971) gave similar conclusions for Trichostrongylus colubriformis using the techniques of neonatal thymectomy and lymph duct drainage in the guinea-pig.

Adult Schistosoma mansoni was shown by Maddison, Geiger, Botero & Kagan (1970) to be unaffected by T cell depletion. Elimination of worms was not impeded by X-irradiation given before or after exposure of the parasites, by ALS treatment or by chronic thoracic duct drainage. Phillips, Reid, Bruce, Hedlund, Colvin, Campbell, Diggs & Sadun (1975), however, conferred protection to a

primary cercarial infection using either the 7S (IgG) fraction of immune serum or a nonadherent lymphoid peritoneal cell fraction. Protection with the cell fraction was removed when the cells were pretreated with ATS and complement before transfer. This work was carried out in rats which are an abnormal host for S. mansoni and are highly resistant. However, Butterworth, Sturrock, Houba & Rees (1974) report an antibody-dependent, complement-independent, cell-mediated cytotoxic effect in the sera of humans, baboons, mice and rats. The eosinophil was subsequently shown to be the effector cell in the cytotoxic reaction to the schistosomule (Butterworth, Sturrock, Houba, Mahmoud, Sher & Rees, 1975). The T cell requirement indicated by Phillips et al. (1975) may, therefore, be associated with the development of eosinophil effector cells, as the induction of eosinophilia has been shown to be T cell-dependent (Walls et al. 1971).

Rabbits treated with ALS (Dodd & O'Nuallain, 1969) and calves with congenital thymus defects (Flagstad, Anderson & Nielsen, 1972) fail to develop normal cellular responses to Fasciola hepatica.

Okamoto (1968) reported that the normal resistance of mice to challenge infection with Hymenolepis nana was depressed in neonatally thymectomised mice. The depression was abolished by subcutaneous thymus implants (Okamoto, 1970). Okamoto & Koizumi (1972) further reported that the challenge response was depressed by administration of ATS. Their suggestion that the response is cell-mediated because the ATS showed no serologic reactivity seems invalid because this does not take account of possible T cell co-operation in the production of protective antibody.

b) Nude mice.

Since its first description by Flanagan in 1966 and the discovery of its congenital athymia by Pantelouris (1968), the nude mouse has become the most widely studied mouse strain in laboratories investigating the effects of T cell depletion. It has, to a large extent, taken the place of neonatally thymectomised and adult thymectomised, irradiated animals because it presents athymia without the non-specific effects associated with these manipulations. Its level of T cell activity is also much lower than in experimentally thymectomised mice, although remnants do persist (see below).

Pantelouris (1968) showed that nude mice exhibit panleucopenia in the peripheral blood. Their phenotypically normal sibs (NLM) showed varying responses over a wide range depending on whether they were heterozygotic (nu/+) or homozygotic(+/+). The heterozygote NLM gave responses intermediate between homozygote NLM and nude mice. Because of this heterogeneity in immune response, NLM make poor controls, although they are normally used as such. For this reason, many workers are now attempting to backcross the recessive nu gene onto an inbred line so that normal mice of the inbred strain, which respond consistently, may be used as controls. Rygaard (1973) reported that although this leucopenia in nude mice results from reduced lymphopoiesis in the bone marrow, the haemopoietic ability of nude mouse bone marrow is similar to that in normal mice.

Because of their athymia, the lymphoid tissues of nude mice show severe T cell depletion (Lamelin, Lisowska-Bernstein, Matter, Ryser & Vassalli, 1972; Raff, 1973; Guy-Grand, Griscelli & Vassalli,

1975). Histologically, this depletion may be seen in clearly defined, so-called thymus-dependent areas of these tissues. Thus, in the lymph nodes, there is depletion in the paracortical area and secondary follicles do not develop (de Sousa, Parrott & Pantelouris, 1969; de Sousa, 1973; Rygaard, 1973). In Peyer's patches the depletion is confined to the subfollicular and interfollicular areas and in the spleen the periarteriolar region of the Malpighian follicles is depleted. The extent of this depletion is, to some degree, dependent on the amount of antigenic stimulation the mouse receives. Those conventionally reared will show less depletion than those reared under carefully controlled environmental conditions.

This T cell depletion is reflected in the ability of nude mice to respond to antigenic challenge. The reports of humoral immunoglobulin production in nude mice vary considerably between different workers. This variation may be due, to a large extent, to the differing genetic background of mice from different colonies. Rygaard (1973) discusses this point and gives examples of direct PFC responses to SRBC in three strains. The ratio of nude/normal values given for total serum immunoglobulins are 1.6:1, 6.1:1, and 13.5:1. Investigation of the proportion of immunoglobulin classes within this total has been made by several groups. Luzzati & Jacobson (1972) and Bloemmen & Eyssen (1973), both using radial immunodiffusion methods, obtained the same pattern of results although, as would be expected, their individual values vary considerably. Thus, they found that all classes of immunoglobulin except IgM were substantially depleted. The greatest depletion was in IgG_1. Values for IgG_{2a}, IgG_{2b}, and IgA ranged from 14%

to 59% of values for normal mice. Reaginic antibodies, if present at all in the nude mouse, are present in insufficient quantity to induce PCA. Thus, Michael & Bernstein (1973) could demonstrate no PCA in Balb-c/nu/nu mice although normal (Balb-c) and heterozygote (Balb-c/nu) mice were PCA-positive. Ability to mount a response was restored to nude mice following an injection of Balb-c thymocytes. This observation confirms the thymus-dependency of IgE production suggested by the work of Okomura & Tada (1971) who used neonatally thymectomised rats.

The thymus-dependency of 7S immunoglobulin production which the above suggests is shown in the response of nudes to antigens. Pantelouris & Flisch (1972), detecting PFC to SRBC, demonstrated a normal 19S response in nudes but very little 7S response. The initial haemolysin response in nudes followed the same pattern as in their sibs but values were lower and there was no secondary response, again reflecting the inability of nudes to mount a 7S response. Reed & Jutila (1972) confirmed this work measuring haemagglutinin titres to SRBC.

Most investigations into immunoglobulin production in nude mice have been concerned with humoral levels. Guy-Grand et al. (1975) however, in a study of the effect of thymus grafting on the development of nude Peyer's patches and mucosal IgA plasma cells, found that Balb-c/nu/nu mucosal IgA plasma cells were 4 to 7 times fewer than in normal Balb-c mice. Numbers of IgA plasma cells in the duodenum and ileum of SPF nudes were found to be approximately half of those in nudes reared conventionally. From these results, then, the low levels of serum IgA in nude mice could be predicted as Bazin et al. (1971) have proposed that the gut is the major source of serum IgA. The increase in IgA

plasma cells after thymus grafting is probably associated with a concurrent development of Peyer's patch germinal centres which may seed the lamina propria with blast cells or mature plasma cells.

The response of nude mice to polymeric antigens, previously described as being thymus-independent, has been shown to be essentially normal. This was demonstrated by Manning, Reed & Jutila (1972) and Baker, Reed, Stashak, Amsbaugh & Prescott (1973) for E. coli lipopolysaccharide and for Type III pneumococcal polysaccharide. Current theory (discussed by Kagnoff, 1974), however, regards such antigens as requiring a lower level of T cell co-operation for B cell induction rather than entirely T cell-independent. Remnants of T cell activity which may act to suppress or amplify such responses are discussed below.

Thymus-dependent cell-mediated responses are also depleted in nude mice. This was shown in vivo by Pritchard & Micklem (1972) who reported a lack of contact sensitivity in nude mice to oxazolone. This response was restored, at least partially, by carrying out a neonatal thymus transplant. Feldman, Wagner, Basten & Holmes (1972) also demonstrated this in vitro, showing a lack of cytotoxicity of nude spleen cells for ^{51}Cr-labelled mastocytoma cells.

The lack of cell-mediated response in nude mice is also reflected in their inability to reject skin grafts from allogeneic or even heterogeneic donors. There are numerous reports of allograft retention by nudes (e.g., Pantelouris, 1971; Wortis, 1971; Pennycuik, 1971; Kindred, 1971; Rygaard, 1973). The unresponsiveness is irrespective of donor-recipient strain combination and the ability to reject can be restored by neonatal

thymus transplantation or by injection of syngeneic thymus cells but not by allogeneic thymus cells (Kindred, 1974 - see below). The inability to reject allografts reflects a true deprivation of the nude cell-mediated response as cytotoxic alloantibody to the graft is produced (Koene, Gerlag, Jansen, Hagemann & Wijdeveld, 1974). Rygaard (1973) found that heterografts of rat skin were accepted more readily than mouse skin allografts, even though he found cytotoxic antibody in the former and not in the latter. Grafts from pigeon, chicken, lizard, grass snake and man were all accepted and maintained. Similarly, the nude mouse has no capacity to reject heterografts of malignant tissue. Adenocarcinomas of human colon and rectum (Povlsen & Rygaard, 1971), human melanomas (Visfeldt, Povlsen & Rygaard, 1972), human epidermoid carcinomas (Povlsen & Rygaard, 1972) and Burkitt's lymphoma (Povlsen, Fialkow, Klein, Klein, Rygaard & Wiener, 1973) were all readily accepted. In this context it is difficult to explain the lack of spontaneous tumours in nude mice and this seems to contradict the theory of immunological surveillance (Burnet, 1970).

Koene et al. (1974) suggest that the inability of the nude mouse to mount a response to grafts may be explained by low complement activity existing in nudes. They report that the ability to reject allografts was partially restored when rabbit complement was given to the grafted mice. This ability, however, was almost completely restored when complement and specific alloantiserum were given, suggesting that both of these components were at low levels. The evidence of Rygaard (1973) conflicts with this because he found levels of complement in his nude mice comparable to those in normal mice.

The presence of low T cell activity in nude mice has been mentioned above. Wortis, Nehlsen & Owen (1971) first demonstrated the presence of T cell precursors in nude mice. Nude BMC were found to repopulate thymus-dependent areas of lymphoid tissues in irradiated mice. Nude mice can be induced to reject allogeneic skin grafts by grafting a neonatal allogeneic thymus (Wortis, et al. 1971; Pantelouris, 1971). Using a chromosome marker, Pritchard & Micklem (1973) showed that the cells colonising the grafted thymus were of nude origin, suggesting that nude T cell precursors could develop into functional T cells in the presence of a grafted thymus. The nude origin of these T cells was confirmed by Loor & Kindred (1973) using fluorescent T cell markers specific for donor and host strains. Since these observations, large numbers of "T lineage" lymphocytes have been described in the nude mouse by Loor & Roelants (1974).

The number of T cell precursors which will differentiate to mature T cells may depend on the degree of antigenic experience the nude acquires. Thus, Scheid, Goldstein & Boyse (1975) report no differentiation of prothymocytes to mature T cells (measured by the T cell differentiation antigens TL and thy-1) in germ-free nude mice. However, late T cell differentiation was observed in nudes either given the thymic hormone, thymopoietin, or suffering from viral hepatitis. These workers conclude that "Prothymocytes are preprogrammed cells whose maturation to thymocytes, normally induced in the thymus by thymopoietin, can be triggered by other agents under abnormal circumstances".

The demonstration of T cell precursors in nude mice stimulated controversy over the possibility of suppressor T cell involvement in the unresponsiveness of nudes to antigenic

challenge. This possibility had previously been suggested by the work of Gershon & Kondo (1970) in thymectomised, irradiated, BMC reconstituted mice. The idea of T suppressor cells being active in nude mice, however, has been refuted by several workers (Morse, Steinberg, Schur & Reed, 1974; Monier, Sepetjian, Czyba, Ortonne & Thivolet, 1974; Kindred, 1975a).

Because the T cells populating thymus grafts in nude mice are of host origin, the establishment of competence in nudes can be achieved only in certain circumstances. Thus, allogeneic thymus grafts are only tolerated and repopulated if they are less than 4 days old on transplantation, i.e., strictly neonatal (Radov, Sussdorf & McCann, 1975). Thymus grafts from adult syngeneic donors, however, will be tolerated and will populate the nude thymus-dependent tissues (Kindred, 1971). Kindred (1975b) showed that syngeneic, but not allogeneic, thymus cell suspensions would establish competence in nude recipients. Low doses of syngeneic cells reportedly induced a higher PFC response to SRBC than low doses of allogeneic cells and, regardless of dose, the production of haemagglutinating antibody had ceased by day 10 in recipients of allogeneic cells but continued in recipients of syngeneic cells. Allogeneic T cells, then, whether given as cell suspension or as a mature allogeneic thymus graft, do not repopulate the nude tissues and are presumably rejected by a GVH reaction mounted by stimulated T cell precursors.

Although host-derived cells repopulate a grafted neonatal allogeneic thymus, the host cells do not establish competence to the level found in control mice. This was demonstrated by Kindred & Loor (1974), measuring the response of grafted mice to specific T cell-dependent antigens and mitogens. This suggests

a further functional defect in addition to simple lack of competent T cells. Gillette (1975) found that ^{51}Cr-labelled lymph node, spleen, thymus or bone marrow cells from normal mice migrated to the spleen, lymph nodes, bone marrow and small intestine of syngeneic nude mice in lower numbers than to the lymphoid organs of normal mice. Labelled nude cells migrated normally to the lymphoid organs of normal mice and recipient nude mice. Labelled cells from normal mice migrated normally in adult thymectomised, irradiated, BMC reconstituted mice. This work suggests that the defect is not in the ability of sufficient numbers of precursor T cells to differentiate fully, but rather that the subsequent homing mechanisms of mature T cells are inefficient.

c) The present investigation.

Previous investigation of the thymus-dependency of the mouse immune response to H. diminuta is limited to the observation of Hopkins et al. (1972b) that administration of ALS suppressed the response of CFLP mice to single worm infection. The experiments described here were designed to investigate this possibility using two different models. Both adult thymectomised, lethally irradiated, BMC reconstituted mice and nude mice have other defects in addition to their athymia but the results using both types ought to complement and confirm each other. Initially, single cysticercoid infections were used in the nude mouse to establish the thymus-dependency of the response. Later, multiple infections were used in an attempt to establish the degree of thymus-dependency and to determine whether the phenomenon is dependent upon antigen dose.

MATERIALS AND METHODS

1. Adult thymectomy.

Adult thymectomy was carried out after the procedure of Miller (1961) on male, 6 week old, 3 star NIH mice. Briefly, the method was as follows.

Each mouse was anaesthetised with 0.2ml 10% Nembutal (Abbott Laboratories Ltd.) in 30% ethanol injected i.p. A small glass suction pipette was introduced into the thoracic cavity through an antero-sternal incision and the thymus lobes were aspirated using suction from a water pump. Sham thymectomised mice were similarly treated but no suction was applied to the pipette. The incision was sutured using a single 7.5mm x 1.75mm Michel suture clip (Thackray Ltd.). Thymectomised mice were inspected macroscopically at autopsy for remaining thymus material and those retaining thymus remnants were not included in results. All groups were given oxytetracycline hydrochloride in the drinking water (see GENERAL MATERIALS AND METHODS).

Ten days later, thymectomised and sham-operated mice were subjected to a lethal dose of whole-body X-irradiation (850 rads). On the following day, irradiated mice were given 3.5×10^7 syngeneic BMC to reconstitute the haemopoietic tissues.

To determine the rate at which the irradiation-depleted thymus-dependent response was restored in sham-operated mice, representative mice from each group were given a single i.p. injection of 4% SRBC 4 weeks after irradiation and the anti-SRBC agglutinating response was measured in these mice 7 days later.

2. Breeding and husbandry of nude mice.

The nude mouse colony was derived from several pairs of mice heterozygous for the nu gene but of unknown strain origin which were supplied by Dr. E. M. Pantelouris in April 1973.

Several authors (e.g., Flanagan, 1966; Artzt, 1972) have reported the occurrence of small numbers of fertile nude mice, although most are sterile due to urinogenital infections (Rygaard, 1973). None of the numerous test matings carried out between nude males and nu/+ females during this study, however, produced progeny and so matings between nu/+ males and nu/+ females were used throughout.

Test matings between +/? mice and known heterozygote (nu/+) mice were carried out periodically to replace old nu/+ breeders with fresh stock. Any +/? mice failing to produce nude mice in either litter from two such test matings were considered to be homozygous for the wild type gene and were culled. Those producing litters containing nude mice were proven nu/+ and were retained for breeding stock. This system of test mating is very time-consuming and requires a great deal of record-keeping. To minimise effort in this respect, a system of harem-breeding was adopted. A maximum number of four known nu/+ males were separated for breeding at any one time. Large numbers of females were genotyped and those found to be nu/+ were caged in groups, several females to each male. Pregnant females were removed and caged individually until their litters were weaned, then they were returned to a breeding cage.

Average litter size was approximately 10 and litters of up to 15 were not uncommon. Under these conditions nude mice have difficulty in competing with their larger littermates for food,

are severely stunted and almost invariably die well before weaning. It was necessary, therefore, to separate nudes from their littermates immediately following parturition. Newborn nude mice were recognised by their absence of vibrissae. If several litters comprising both nude and nu/? mice were born concurrently, then all nude mice were fostered onto one female and the littermates pooled between the other lactating females. If this was not possible, +/? littermates were culled to allow the nude mice to thrive.

Using these methods an average of 8 nude mice/month were produced. Breeding reasonable numbers of nude mice using this system gave rise to few problems, but difficulty was encountered in keeping them alive long enough for experimental purposes. It was decided at the outset to infect mice in these experiments at six weeks of age to allow the phenotypically normal littermates (NLM), used to control infections in nude mice, to achieve immunocompetence.

Ideally, because nude mice have a lowered resistance to many pathogenic organisms, they ought to be kept isolated from other animals under barrier-maintained SPF conditions. The colony described here was maintained alongside other experimental mice under conventional animal house conditions. Under these conditions, large numbers of nude mice died before weaning. Only about 25% of those produced survived long enough for experimental purposes. Maximum survival time was about 12 weeks and this agrees closely with the reports of several authors for nude mice maintained under conventional conditions (Flanagan, 1966; Pantelouris, 1968; Kindred, 1971; Artzt, 1972). Most nude mice showed progressive symptoms of the wasting syndrome for variable periods before death. Almost

all had distinct foci of a liver disease of unknown aetiology at autopsy. Reports of similar liver disease in other nude mouse colonies are common (Flanagan, 1966; Pantelouris, 1968; Rygaard, 1973). Experimental nude mice showing excessive pathogenesis were not included in the results.

At the beginning of the study, many nude mice rapidly lost condition and died following rectal prolapse. At autopsy these mice were found to harbour enormous numbers of the pinworms Syphacea obvelata and Aspiculuris tetraptera. Subsequently, Jacobson & Reed (1975) showed that nude mice develop no natural resistance to these parasites. A programme of anthelmintic treatment was therefore initiated. All mice in the colony were treated every 7-10 days with piperazine citrate ("Citrazine", Loveridge, Ltd.) 1.6mg/g in aqueous solution administered orally. Pinworms were rarely observed after initiation of this treatment and the incidence of rectal prolapse declined to zero. Towards the end of the study the anthelmintic was given continuously in the drinking water at a concentration of 3g/l with the same effect.

RESULTS

1. Adult thymectomised mice.

A preliminary experiment studying the effect of adult thymectomy and irradiation on the response to *H. diminuta* suggested a thymus-dependency of the rejection mechanism. Only groups of mice which were thymectomised or thymectomised and irradiated retained tapeworms when autopsied 16 days post-infection. High mortality in all groups of mice due to an unknown pathogen causing pneumonic symptoms made the results of this experiment equivocal and they are not presented here.

In a repeat experiment, larger numbers of mice/group were included and all mice were given antibiotic in the drinking water on arrival. No evidence of pneumonia was found in any of the mice. The mice were divided into three groups: control untreated mice; thymectomised, lethally irradiated, BMC-reconstituted mice; and sham-operated, lethally irradiated, BMC-reconstituted mice.

Recovery of the depleted haemopoietic tissues of the sham-operated mice was measured 35 days following irradiation and BMC reconstitution by reference to their humoral response to SRBC injected 7 days previously. The titres are given below.

GROUP	MEAN LOG_2 ANTI-SRBC TITRE
Control	5.3
Thymectomised/irradiated/+BMC	1.0
Sham thymectomised/irradiated/+BMC	4.6

Reconstitution of this response in the sham-operated mice was complete and it was decided to infect the mice immediately. All mice were infected with six cysticercoids. Control and sham-operated mice were autopsied 6, 9 and 15 days after infection but groups of thymectomised mice were autopsied on day 6 and day 15 only. Figs. 2-1 and 2-2 show worm recovery and worm growth respectively.

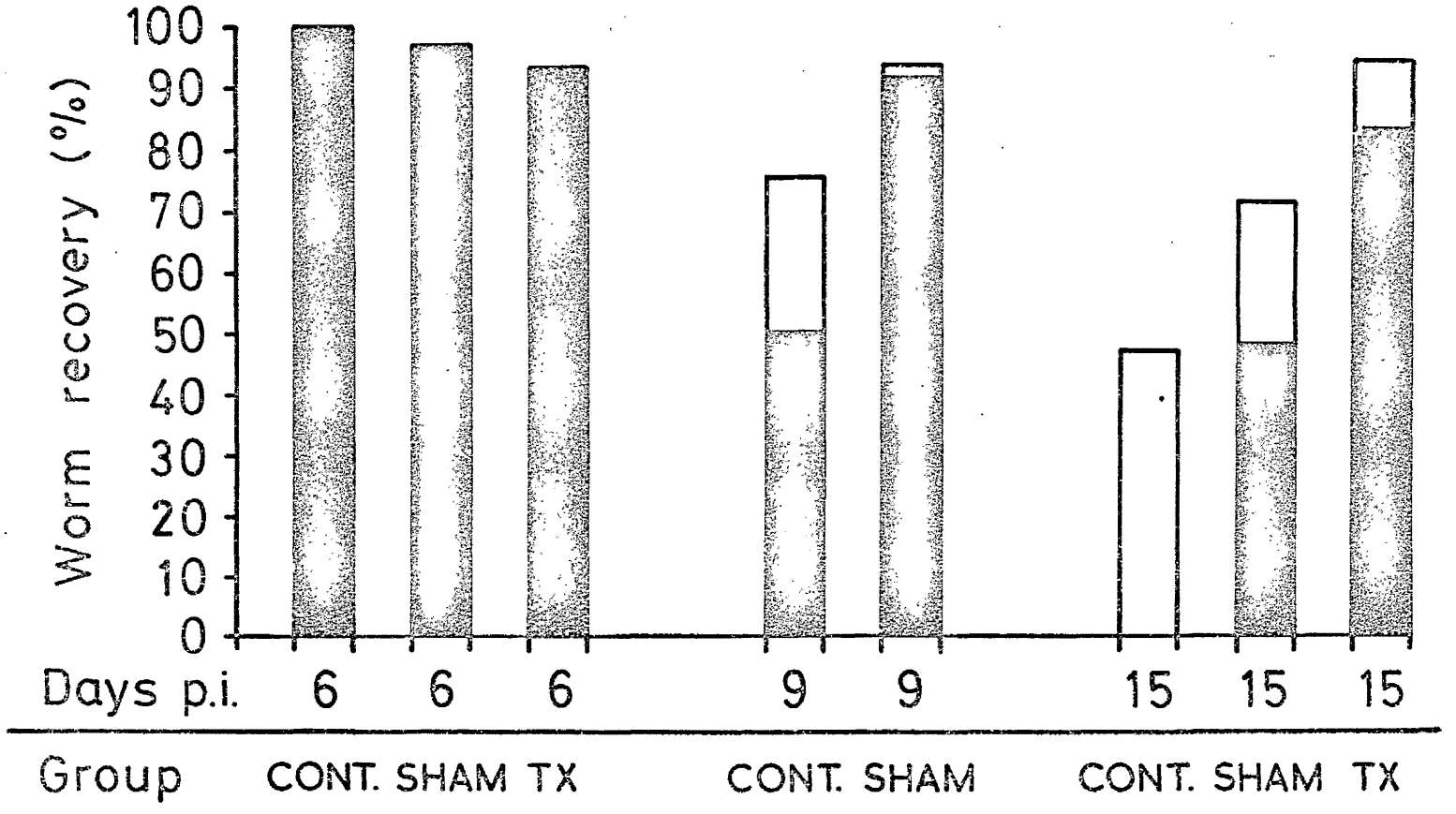
Fig. 2-1 shows that worms established equally in all groups. On day 9, only 50% of worms administered were recovered as strobilate worms from the control mice. A further 25% of destrobilated worms was also recovered from this group. In contrast, recovery of strobilate worms on the same day from sham-operated mice remained at over 90%. By day 15, only destrobilated worms remained in control mice and only 48% of worms administered to sham-operated mice remained strobilated. Six large, strobilate worms were recovered from eight of nine thymectomised/irradiated mice autopsied on day 15. Six destrobilated worms were recovered from the ninth mouse. Numbers of worms recovered from sham-operated mice differed significantly from those recovered from thymectomised mice on day 15 (2 x 2 contingency table:$P < 0.01$).

Fig. 2-2 shows that the weight of worms recovered/mouse from the sham-operated mice did not differ significantly from those recovered from the thymectomised mice on day 15. The mean dry weight of worms recovered/mouse and the mean of individual worm dry weights for these two groups autopsied on day 15 are given below. Weights of strobilate worms only are included.

Facing page 85

Figure 2-1

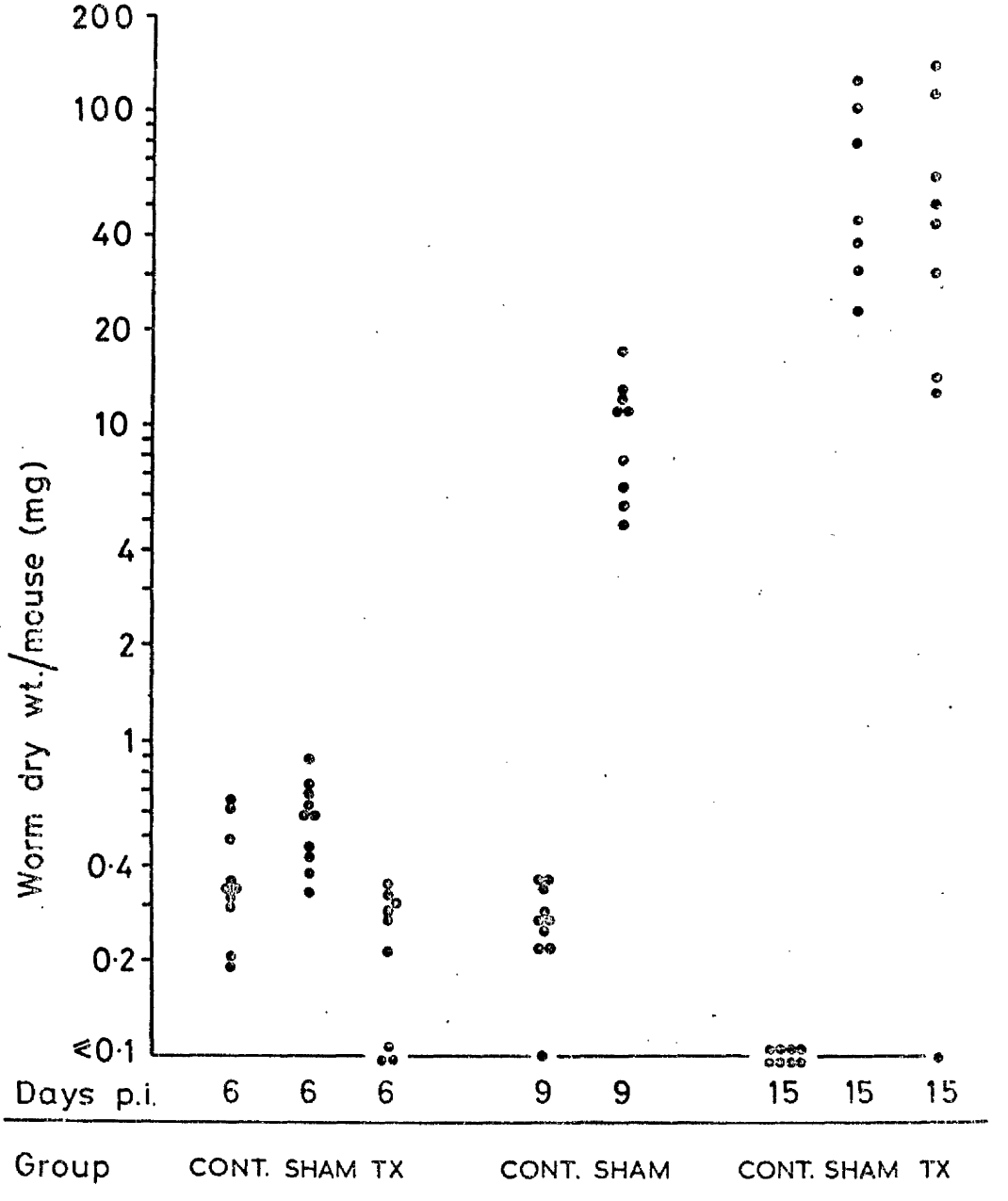
Recovery (%) of Hymenolepis diminuta from six-cysticercoid infections in NIH mice. Solid areas of bars represent worms >0.1mg dry weight; open portions represent destrobilated worms. CONT., untreated mice; SHAM, sham-thymectomised, lethally irradiated, bone marrow reconstituted mice; TX, thymectomised, lethally irradiated, bone marrow reconstituted mice.


Worm recovery (%)
100
90
80
70
60
50
40
30
20
10
0
Days p.i.
6
6
6
9
9
15
15
15
Group
CONT. SHAM TX
CONT. SHAM
CONT. SHAM TX

Facing page 86

Figure 2-2

Dry weight of Hymenolepis diminuta from six-cysticercoid infections in NIH mice. Each point represents the total dry weight of worm recovered from a single mouse. CONT., untreated mice; SHAM, sham-thymectomised, lethally irradiated, bone marrow reconstituted mice; TX, thymectomised, lethally irradiated, bone marrow reconstituted mice.


200
100
40
20
10
4
2
1
0·4
0·2
≤0·1
Worm dry wt./mouse (mg)
Days p.i.
6
6
6
9
9
15
15
15
Group
CONT.
SHAM
TX
CONT.
SHAM
CONT.
SHAM
TX

Group	Mean worm dry weight/mouse (mg) ± S.D.	Mean worm dry weight (mg) ± S.D.
Thymectomised	59.2 ± 43.3	10.5 ± 7.1
Sham	64.4 ± 37.2	20.0 ± 6.9

Total worm dry weight/mouse was measured rather than individual worm dry weight. The mean worm dry weights given above, therefore, do not take account of intra-mouse variation in worm weight. Subjectively, however, this variation appeared to be minimal. It is clear, then, that because more worms survived in the thymectomised mice, their individual weight was reduced to about half of those recovered from the sham-operated mice.

2. Nude mice.

Infections of nude mice with H. diminuta to determine the thymus-dependency of the response took place over a period of two years. Due to their inconsistent breeding and survival, it was not possible to form large groups of mice of similar age for simultaneous infection and it was necessary to infect mice as they became available from the breeding colony.

a) Single-cysticercoid infections.

The course of single-cysticercoid infections in nude mice is described in the following five pages, reproduced from Parasitology.

Parasitology (1976), 72, 93–97
With 2 figures in the text

Immunity to *Hymenolepis diminuta*: unresponsiveness of the athymic nude mouse to infection

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SUMMARY

Male and female congenitally athymic nude (nu/nu) mice infected with a single cysticercoid of *Hymenolepis diminuta* at 6 weeks of age retain the infection for at least 33 days. In the males of their phenotypically normal litter-mates, however, a single cysticercoid infection establishes and grows but is expelled between days 11 and 17. The unresponsiveness of the nude mouse to single *H. diminuta* infection is evidence that the immune rejection from normal mice is thymus-dependent.

INTRODUCTION

It is now firmly established that the tapeworm *H. diminuta* elicits an immunological response from several strains of mice causing expulsion of the worms after approximately 2 weeks of normal growth (Hopkins, Subramanian & Stallard, 1972*a*, *b*; Befus & Featherston, 1974; Befus, 1975). The mechanism of this response, however, whether it comprises cellular and/or humoral components, remains to be elucidated.

As a rule, host-protective immune responses to helminth parasites include a thymus-dependent component (Targett, 1973). Does the rejection of *H. diminuta* by the mouse involve such a component?

The congenitally athymic nude mouse has been used to establish thymus-dependency in other host–parasite systems (Jacobson & Reed, 1974*a*, *b*; Ruitenberg & Steerenberg, 1974), and it has been employed in this study because the natural impairment of thymus-dependent immunity avoids the use of immunosuppressive drugs or irradiation.

MATERIALS AND METHODS

From stock originally obtained from Dr E. M. Pantelouris of Strathclyde University, an outbred colony of nude mice was established by mating heterozygous (nu/+) females with heterozygous males. Litters were weaned 21–25 days post partum. The mice were maintained under conventional conditions and supplied with Standard Rat and Mouse Breeding Diet (Grain Harvesters Ltd) and water *ad libitum*.

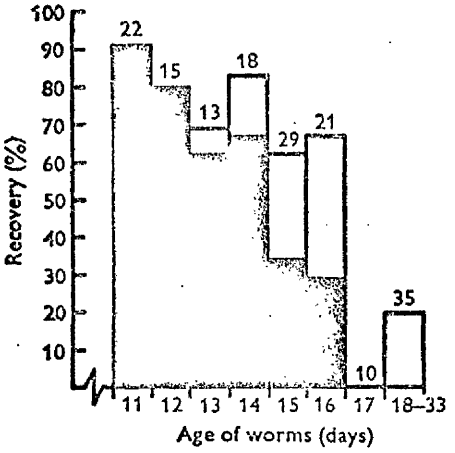

Fig. 1. Recovery (%) of *Hymenolepis diminuta* from single cysticercoid infections in male normal litter-mate mice. Solid areas of bars represent worms > 0·1 mg dry weight; open portions represent destrobilated worms (⩽ 0·1 mg). Superscripts over bars are the numbers of mice autopsied/day.

As athymic mice have an increased susceptibility to pinworm infection (Jacobson & Reed, 1974*b*), the entire colony was treated every 7–10 days with piperazine citrate ('Citrazine', Loveridge Ltd) 1·6 mg/g, in aqueous solution administered orally. This treatment proved to be effective in minimizing pinworm infection and only occasionally were pinworms observed in experimental animals. All experimental animals were free of cestodes other than *H. diminuta* at autopsy.

Male phenotypically normal litter-mates (NLM) and male and female nude mice were infected at 6 weeks of age with a single cysticercoid of *H. diminuta* by stomach tube whilst under ether anaesthesia. The Rice strain of *H. diminuta* was used throughout (see Hopkins *et al.* (1972*a*) for strain origin and laboratory maintenance). Worms were recovered and their dry weights determined using the method of Hopkins *et al.* (1972*a*). Experimental groups of NLM mice comprised both heterozygous (nu/+) and homozygous wild-type (+/+) mice, these two groups being phenotypically indistinguishable.

It is a feature of the immune rejection of *H. diminuta* by the mouse that the worms usually destrobilate prior to complete expulsion. Statement of worm recovery expressed as worm number can therefore be misleading unless the distinction is made between destrobilated and strobilate worms. Destrobilated worms weigh ⩽ 0·1 mg dry weight. Worms were, therefore, placed into one of two categories: ⩽ 0·1 mg or > 0·1 mg. The former were considered to be rejected, *i.e.* destrobilated, worms (see Befus & Featherston, 1974).

RESULTS

Time course of infection in nude mice and normal litter-mates

The number of mice autopsied on each day (Fig. 1) differs because each column represents a summation of mice from different experiments autopsied over a period of several months.

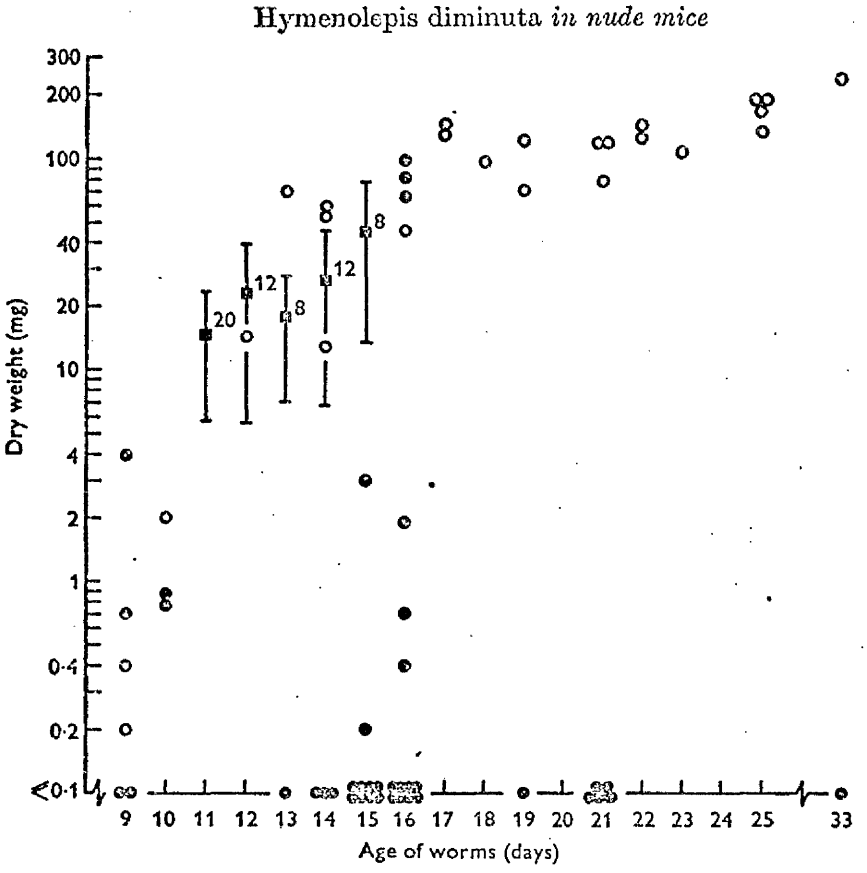

Fig. 2. Dry weight of *Hymenolepis diminuta* from single cysticercoid infections in nude mice (○) and their phenotypically normal litter-mates (●). Mean weights (± standard deviations) of worms from normal litter-mates are shown on days 11–15 (▣). Superscripts adjacent to the means are numbers of worms. See Results for worms excluded from means.

The high recovery (91 %) of worms from the NLM on day 11 indicates that *H. diminuta* establishes and survives well for 11 days in this strain of mouse. After day 11, however, the percentage recovery of worms fell, marking the onset of rejection from the intestine, and an increasing percentage of small, destrobilated worms (⩽ 0·1 mg) were recovered on subsequent days. Recovery of worms > 0·1 mg fell to zero by day 17, showing that rejection was, by then, complete. No worms in any of the 25 nude mice used in this study, however, were rejected and they continued to survive up to the latest observation made on day 33 post-infection.

Worm growth in nude mice and normal litter-mates

In Fig. 2, the dry weights of worms recovered from the NLM between days 11 and 15 are shown as the mean ± the standard deviation. From day 13 the number of destrobilated worms (⩽, 0·1 mg) increased. Other worms, very much smaller than the mean, were also found. These worms had presumably partially destrobilated, and so their weights have been excluded from the mean and shown as separate points.

Worms in both nude and NLM mice grew normally until those in the NLM were rejected. Worm weight in the nude mice appeared to stabilize shortly after this time at between 100 and 200 mg, these worms occupying the entire small intestine and stretching well back into the caecum. Although both sexes of nude mice were used in this study, there was no consistent difference between the weights of worms recovered from males and females.

DISCUSSION

The results show that the response of the NLM mice to single *H. diminuta* infection appears to be similar to that of other random-bred strains as demonstrated by Hopkins *et al.* (1972*a*). Worms establish well but are rejected from day 11 to day 17. It has been shown that *H. diminuta* is retained by the nude mouse at least until day 33 post-infection when the final observation was made, by which time the mice were almost 11 weeks old (maximum life-span of the nude mice in this colony is about 12 weeks).

Nude mice can respond to certain T cell-independent antigens (Reed, Manning, Baker & Ulrich, 1974), but because they are athymic they are unable to generate certain T cell-dependent responses such as cell-mediated immunity (Wortis, 1971). It has been demonstrated (Loor & Kindred, 1973) that T cell precursors do exist in nude mice. Although these precursors have the ability to populate neonatal thymus grafts given to nude mice, they are unable to establish fully immunological competence to the level found in normal mice (Kindred & Loor, 1974). It seems certain, then, that the potential for T cell activity in nude mice is very low, and that the unresponsiveness to *H. diminuta* infection may be attributed to a quantitative deficiency of competent T cells.

This is further supported by the results of Hopkins *et al.* (1972*b*), who demonstrated that administration of antilymphocyte serum depressed the response of CFLP mice to *H. diminuta*. Adult NIH mice which had been thymectomized, irradiated and given bone-marrow stem cells showed similar unresponsiveness (Bland, unpublished). Whether this thymus-dependent phase in the rejection of *H. diminuta* by the mouse is antibody- and/or cell-mediated has yet to be established, and work is now in progress on the reconstitution of irradiated mice with selective populations of cells in order to clarify the rejection mechanism.

I am grateful to Professor C. A. Hopkins and Dr D. Befus for critically reviewing the manuscript. Mr J. Keys and Mr H. Campbell provided expert technical assistance. The work was supported by the Medical Research Council, London (grant no G77/4593).

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Printed in Great Britain

b) Five- and ten-cysticercoid infections.

Nude mice, infected with either five cysticercoids or ten cysticercoids were autopsied between day 7 and day 26 post-infection. The growth of worms in these multiple worm infections is indicated in Fig. 2-3. The weights of worms from single worm infections are included for comparison. Shortage of mice unfortunately precluded investigation of five-worm infections after day 17. No significant worm loss was observed in either group. Five worms were recovered from all mice infected with five cysticercoids and nine or ten worms from all mice infected with ten cysticercoids.

The five-cysticercoid infections were not controlled. NIM mice were infected with ten cysticercoids and autopsied on days 7, 10 and 13 to monitor the response to this level of infection in these mice. Values for worm recovery and mean worm weight/mouse are given below.

Days p.i.	No. of mice per group	Worm recovery	Mean worm day weight/mouse (mg) $\pm$ S.D.
7	9	$\frac{82(+4)}{90}$ [a]	1.5 $\pm$ 0.8
10	9	$\frac{71(+8)}{90}$	4.5 $\pm$ 4.1
13	10	$\frac{46(+34)}{100}$	14.5 $\pm$ 12.3

a. $\frac{\text{Number of worms } > 0.1\text{mg (+ number of worms } \leq 0.1\text{mg)}}{\text{Number of cysticercoids administered}}$

Thus, by day 13 in the NIM mice substantial worm destrobilation had taken place.

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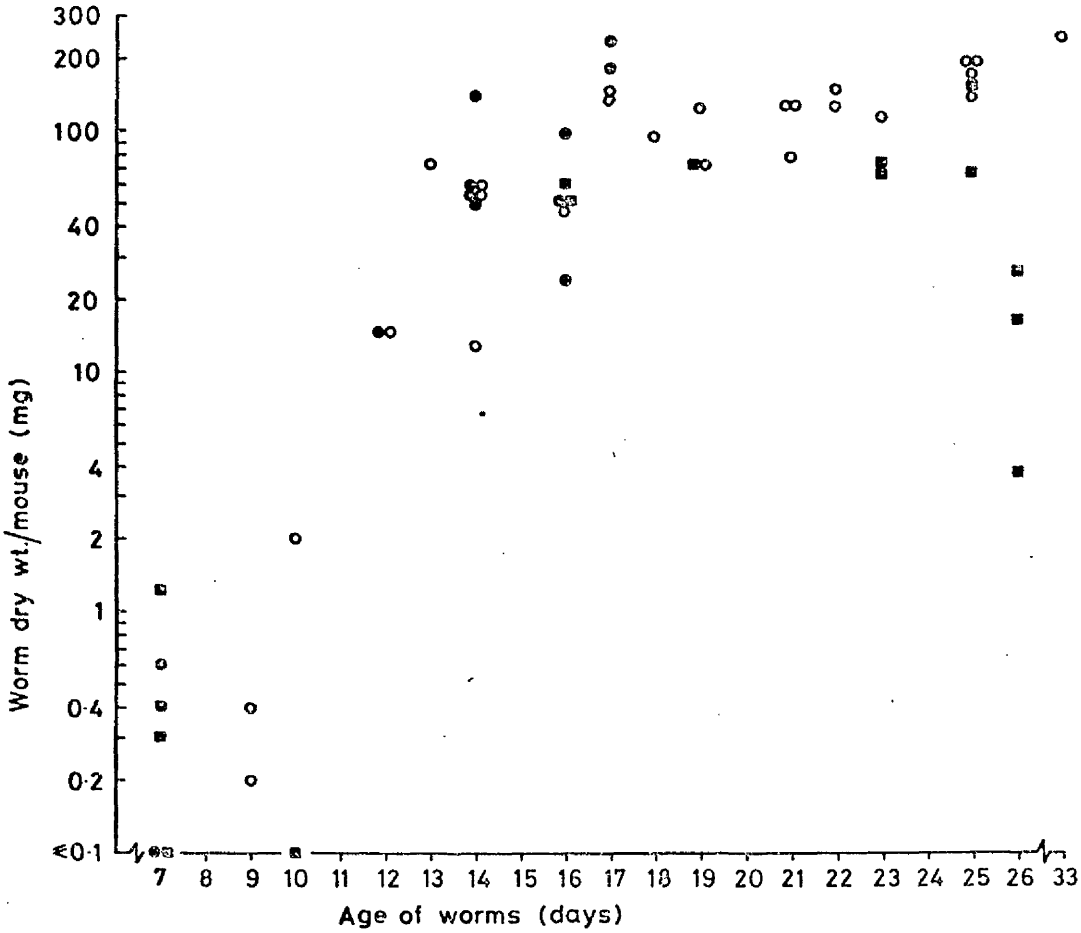
Figure 2-3

Dry weight of Hymenolepis diminuta from single cysticercoid (O), five-cysticercoid (⊖) and ten-cysticercoid (▣) infections in nude mice. Each point represents the total dry weight of worm recovered from a single mouse.


300
200
100
40
20
10
4
2
1
0·4
0·2
≤0·1
Worm dry wt./mouse (mg)
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 33
Age of worms (days)

Because of the relatively small numbers of mice available, it is only possible to discern general trends in worm growth at each level of infection in the nude mice from Fig. 2-3. It would appear that worms in the three levels of infection followed the same rate of growth over the exponential growth period. The figure suggests that the worm weight/mouse in the mice infected with 10 cysticercoids levelled off at a lower value than in the single worm infections. This observation may be an artifact produced by the small numbers of mice autopsied at this stage of the infection, but it is supported by the low worm weights obtained on day 26. Although the three mice autopsied on day 26 were infected and autopsied simultaneously, they were derived from different parents and were caged individually. It seems unlikely, then, that all three provided a poor environment for worm growth because of genetic or environmental factors. Rather, it suggests that factors which had induced a degree of worms stunting up to this point in the infection, now caused sufficient stress to induce partial destrobilation.

Several attempts were made to establish immunological competence in nude mice in order to induce a response to H. diminuta. Nude mice were grafted subcutaneously in the axillary region or beneath the kidney capsule with thymi from neonatal NLM mice. None of these operated mice survived long enough (maximum, six days) to allow subsequent infection. At autopsy, none of the thymus grafts were found to have established.

DISCUSSION

The results demonstrate that the immune response of the mouse to H. diminuta requires the participation of mature T cells. This was indicated initially by the unresponsiveness of the adult thymectomised, irradiated, BMC-reconstituted mice. Although these mice were unresponsive, doubts may be raised by the results from the mice which were sham-operated and irradiated. These mice had apparently recovered, as shown by their ability to mount a humoral response to SRBC (T cell-dependent), but they were only partially responsive to H. diminuta infection. It may be that a defect additional to T cell depletion was produced in both of these groups of mice. This could have arisen because of the non-specific effects of irradiation on local, intestinal, elements of the response (see SECTION 1). The results, however, were confirmed by the unresponsiveness of the naturally-depleted nude mice. In terms of worm recovery, the nude mice appear to have been completely unresponsive to H. diminuta at any of the levels of infection used. Thus, there was virtually no worm loss from one-, five-, or ten-cysticercoid infections.

The plot of worm growth (Fig. 2-3) indicates that the maximum weight of worms/mouse was less in ten-worm infections than in single worm infections. Thus, not only were individual worms stunted in multiple infections, which could be attributed to a physiological crowding effect, but the total weight of worms supported by multiple-infected mice was lower than in single worm infections. This seems to indicate that a threshold of antigenic stimulation exists above which immunologically-mediated worm

stunting occurs. Such a threshold has been described for H. citelli in mice (Hopkins & Stallard, 1974), although in this case worm loss accompanied stunting of growth in multiple infections. In the present investigation, total worm weight/mouse in ten-worm infections did not reach the levels found in single-worm infections so the threshold does not appear to depend upon weight of worm tissue. It seems more likely to be dependent on surface area of worm. Thus, the greater surface area of tegument presented by the ten-worm infection stimulates the small number of T cell precursors present in a nude mouse which in turn initiate worm stunting, whereas the single-worm infection fails to offer sufficient antigenic stimulus to the T cell-poor nude mouse. Alternatively, the greater antigenic stimulus in the ten-worm infection may derive from the greater number of solices present than in the single-worm infection. The origin of H. diminuta antigen(s) has not been defined.

The work presented here is supported and extended in some respects by work carried out concurrently by Isaak, Jacobson & Reed (1975). These authors report that the rejection mechanism can be induced in nude mice by administration of syngeneic thymus cells or by grafts of syngeneic neonatal thymus. They give no comparative information, however, on worm growth in their nude mice. In one of their experiments they report an apparent rejection of worms from untreated nude mice, although this fact is ignored in their discussion. A 44% recovery of worms from a 21-day old three-worm infection in these mice is reported. This latter phenomenon of worm rejection from untreated nude mice is substantiated by Andreassen, Hindsbo & Ruitenberg (1974) who

showed that rejection of five-worm infections took place from two strains of nude mouse. Complete expulsion from both strains was demonstrated between day 11 and day 20. Further studies showed that the expulsion rate was dependent on the level of infection. Accelerated expulsion was found to take place from infections of 10 or 20 worms but 1- or 2-worm infections survived for at least 136 days. This expulsion could be prevented by administration of cortisone (Andreassen, personal communication).

Why do the nudes used by these workers and those used in the present study differ in responsiveness to H. diminuta? It is possible that, as the different groups of nudes differ genetically, they possess different numbers of T cell precursors. They may differ because of variation in defects other than depletion of T cells, such as defects in cell migration and homing (see Gillette, 1975). Although differences between different populations of nude mice can occur due to variable environmental antigenic stimulation (Rygaard, 1973), this is unlikely to be the responsible factor in this case. The mice of Andreassen et al. were maintained under conventional, albeit isolated, conditions (Andreassen, personal communication), whereas those of Isaak et al. were kept isolated in an SPF environment (Jacobson, personal communication).

The question remains - why are T cell-depleted mice unresponsive to H. diminuta infection, i.e., what part do T cells play in the response? T cells have the role of amplifiers or suppressors in the production of humoral antibody (Morse, Prescott, Cross, Stashak & Baker, 1976). It is unlikely, however, that this role is important in the response to H. diminuta as humoral antibody has not been demonstrated (Befus, 1975a). However, T cells also

appear to modulate the production of local antibody (IgA, at any rate) in the gut (Guy-Grand et al., 1975). Befus (1975a), though, was able to demonstrate IgA, IgM, IgG_1, and IgG_2 by immunofluorescence on the tegument of a single worm from a nude mouse, although at lower densities than on worms from normal mice. This was an isolated observation and requires verification. It is a particularly perplexing result when it is considered that more IgA was demonstrated than IgM and yet the production of IgA has been reported on several occasions to be severely depleted in nude mice (Bloemmen et al., 1973; Luzzati et al., 1972).

Reaginic antibodies are known to be depleted under conditions of T cell depletion (Okumura & Tada, 1971; Michael & Bernstein, 1973). It is unlikely, however, that lowered serum concentrations of these antibodies affects the response to H. diminuta as specific anti-H. diminuta IgE could not be demonstrated by PCA tests (Goodall, 1973). It is not known to what extent these antibodies are required locally in the small intestine to mediate the response to H. diminuta.

The involvement of thymus-dependent cells, such as mast cells (Ishizaka, Okudaira, Mauser & Ishizaka, 1976) and eosinophils (Walls et al., 1971), in the pathogenesis of H. diminuta infection in the mouse is unknown. In the rat, Andreassen, Hindsbo & Ruitenberg (1976) followed the development of mast cells and eosinophils in control and ATS-treated rats infected with 100 H. diminuta. Numbers of both types of cell increased slightly over the period of infection and infected rats appeared to have higher counts. These results, however, are of doubtful significance because of the wide range in counts. Administration of ATS appeared to have

little effect on the counts in either group. It is probable that cell counts in infected mice would show a completely different picture because of the more aggressive response. Andreassen (personal communication) found a gradual rise in eosinophils in five-worm infected nude mice but could detect no mast cells.

Although the observations reported here answer the basic question of thymus-dependency of the response to H. diminuta, they provide no information regarding the phase of the response requiring T cells. Comparisons between the histopathogenesis of the local intestinal response to H. diminuta in control and T cell-deprived mice would be a worthwhile starting point in investigation of the kinetics of thymus-dependency of the response. At a later stage, selective reconstitution of deprived mice with relevant cell populations may then further define the T cell requirements of the response.

SUMMARY

1. Brief reviews of thymus-dependency in host-parasite relationships and of the athymic nude mouse are given.

2. It is demonstrated, using single and multiple worm infections in nude mice and adult thymectomised, lethally irradiated, bone marrow-reconstituted mice, that the immune response of the mouse to *H. diminuta* is thymus-dependent.

3. Possible points of action of T cells in the response to *H. diminuta* are discussed.

4. It is suggested that observation of the pathogenesis of the local intestinal response to *H. diminuta* in T cell-deprived mice be observed, in order that the nature of the thymus-dependency may be further defined.

CHAPTER II

THE EFFECT OF A CONCURRENT INFLAMMATORY RESPONSE: INTERACTION OF HYMENOLEPIS DIMINUTA AND TRICHINELLA SPIRALIS

INTRODUCTION

It has been established for three species of nematode (Nippostrongylus brasiliensis, Trichinella spiralis and Trichuris muris) that the immune expulsion of adult worms from the host's intestine involves two or more phases requiring the co-operation of specific antibody and sensitised lymphoid components. Both components are involved in rejection of adult T. spiralis from both rats (Love, Ogilvie & McLaren, 1976) and mice (Wakelin & Lloyd, 1976b). The ultimate effector causing worm expulsion, however, remains controversial. In the case of T. spiralis, acute inflammation of the host intestine accompanies worm expulsion and Larsh (1963) has suggested that this inflammatory response is responsible for worm expulsion. Moreover, Larsh & Race (1975) have proposed, as a general model, that helminths stimulate a cellular reaction in the host tissues resulting in local inflammation which provides an unsuitable environment for worm survival and induces worm expulsion. If this hypothesis is generally applicable, T. spiralis-induced inflammation ought to be non-specific in its action and should result in the expulsion of other helminth species from the host intestine. Ogilvie & Jones (1973, page 120) suggest that the possible toxic effect of such inflammation on other, unrelated species ought to be determined and it is to this end that the present study was carried out.

Previous studies on interaction between helminths have largely concentrated on the factors involved in the determination of parasite distribution within or between hosts. Some studies have concentrated on physiological and nutritional factors affecting distribution (e.g., Holmes, 1961, 1962a, b) or on the

evolutionary selection of distribution factors (see Schad, 1966 for review). Many interaction investigations have included observation of the host immune response (e.g., Larsh & Donaldson, 1944; Larsh & Campbell, 1952; Cox, 1952; Heynemann, 1962b; Louch 1962; Weinmann, 1964; Stahl, 1966; Courtney & Forrester, 1973; Bruce & Wakelin, 1974), usually as one such factor involved in the determination of worm distribution. Unfortunately, much of this work was carried out before information on the specific mechanisms of host resistance became available and its interpretation in the light of this subsequent knowledge is made difficult because, in many cases, controls of one or other of the concurrent infections have been omitted.

As far as is known, a local inflammatory response is not induced in the mouse by H. diminuta although worms are immunologically rejected. It is unlikely, therefore, that a non-specific inflammatory response is the normal agent causing rejection of H. diminuta from the mouse. It was decided to test the validity of the hypothesis of Larsh & Race (1975, see above) on a species which apparently does not normally induce such a response and yet is expelled. In the present study, then, the interaction between T. spiralis and H. diminuta was observed. This study, however, formed part of a more extensive investigation into the action of T. spiralis-induced inflammation on a wide range of species of intestinal helminths, carried out with other workers in this laboratory.

MATERIALS AND METHODS

1. *T. spiralis*.

Origin of the strain of *T. spiralis* used, its maintenance in the laboratory, methods of infection and methods used to recover adult worms for counting are described elsewhere (Wakelin & Lloyd, 1976a). In all experiments, between 400 and 500 larvae were administered to mice and between 2,500 and 3,000 to rats.

2. *H. diminuta*.

Methods for infection with, and recovery of, *H. diminuta* have been described in GENERAL MATERIALS AND METHODS. Infections with five cysticercoids were used in all experiments. In certain experiments worm lengths are given. Worms were allowed to relax overnight in HBSS at 4°C. They were then drawn with the aid of a camera lucida, the drawings measured using a map measurer and the original worm length calculated.

RESULTS

Figure 3-1 has been reproduced from Wakelin & Lloyd (1976a) to demonstrate the normal pattern of rejection of T. spiralis from young (6-weeks old) NIH mice infected with 450 larvae. Worm expulsion begins on day 8 and is virtually complete by day 11.5. These workers also reported that challenge infections were expelled within 24h if challenged 14 days after the primary infection and within 7 days if challenged 21 days or later after the primary infection. The experiments which are described here were designed on the basis of these reports. To a large extent, the inflammatory response associated with rejection of T. spiralis followed the kinetics of expulsion given above but the timing of expulsion varied between experiments.

Effect of a primary T. spiralis infection on subsequent infection with H. diminuta.

The experimental design is set out in Table 3-1. Fifty male, 3 star NIH mice were divided into four groups of ten mice and two groups of five mice. Two groups of ten mice and the two groups of five mice acting as controls for the T. spiralis infection were infected with T. spiralis when 40 days old. The remaining two groups of ten mice were controls for the H. diminuta infection.

Recoveries of H. diminuta and T. spiralis and lengths of H. diminuta are given in Table 3-2.

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Figure 3-1

Recovery of Trichinella spiralis from 6-8 weeks old male NIH mice infected with 450 larvae. O-O, number of worms in small intestine (mean ± S.D.); ■- - -■, number of worms in large intestine (mean only).
(From Wakelin & Lloyd (1976a), Parasitology 72, 173-182).

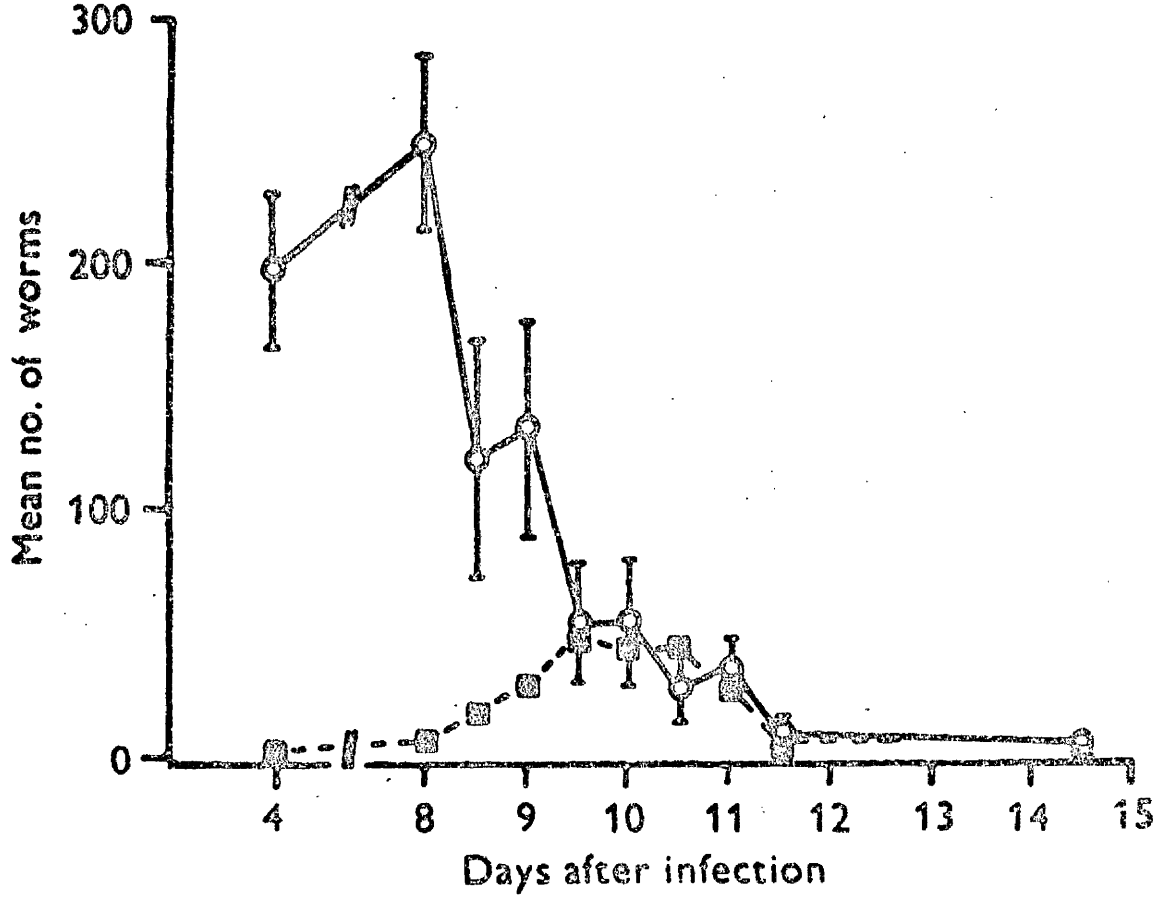
300
200
100
0
Mean no. of worms
4
8
9
10
11
12
13
14
15
Days after infection

Table 3-1

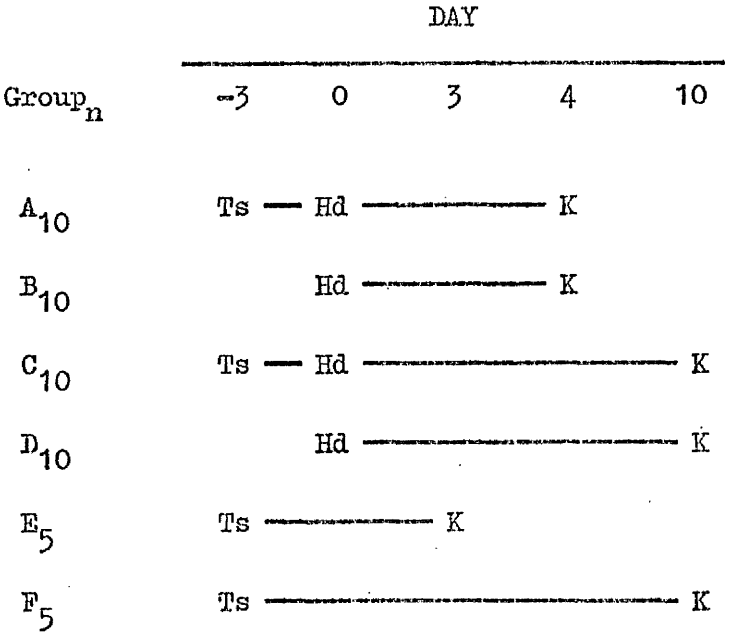

Table 3-2

Group	Mean number of *T. spiralis* recovered per mouse ± S.D.	Total *H. diminuta* recovery	*H. diminuta* mean worm lengths (mm ± S.D.)
A	250.4 ± 28.6	$\frac{0(+24)}{50}$[a]	0.5 ± 0.3
B	-	$\frac{0(+40)}{50}$	4.3 ± 1.8
C	1.9 ± 2.3	$\frac{0(+3)}{50}$	ND[b]
D	-	$\frac{43(+0)}{50}$	ND
E	242.2 ± 15.5	-	-
F	1.8 ± 2.4	-	-

a. $\frac{\text{Number of worms } >0.1\text{mg } (+ \text{ number of worms } \leqslant 0.1\text{mg})}{\text{Number of cysticercoids administered}}$

b. Not done

Although establishment of H. diminuta in control mice (group B) was high (80% recovery on day 4), recovery was low from mice with a concurrent T. spiralis infection (group A). This may indicate either reduced establishment or enhanced rejection of H. diminuta in this group, or a combination of both these factors. The marked reduction in growth rate of H. diminuta in the T. spiralis-infected mice also shows the adverse effect that established T. spiralis exerts on a subsequent H. diminuta infection. Although the inflammatory response was well advanced at this stage (day 7 of the T. spiralis infection), there was no reduction in T. spiralis worm numbers. By day 10 of the H. diminuta infection (day 13 of the T. spiralis infection), however, rejection of T. spiralis was complete and had been accompanied by rejection of H. diminuta (group C), whereas H. diminuta persisted in the control group (group D).

At least a proportion, then, of H. diminuta can establish and survive the initial response to T. spiralis, but are expelled prematurely during subsequent stages of the T. spiralis-induced response.

Can H. diminuta establish at the height of the response to T. spiralis?

Fifty-two female, 3 star NIH mice, 71 days old were divided into six groups and treated as shown in Table 3-3.

Cortisone acetate was given to mice in groups C and C' as an immunosuppressant and anti-inflammatory agent to allow those H. diminuta which had established and survived for 2 days in the T. spiralis-infected mice to survive until day 8.

Recovery of H. diminuta from all groups is given in Fig. 3-2. Recovery from both control groups (A and A') on day 8 was 100%. As

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Figure 3-2

Recovery (%) of Hymenolepis diminuta from female NIH mice given a five-cysticercoid infection for 6 days. Solid areas of bars represent worms >0.1mg dry weight; open portions represent destrobilated worms (≤0.1mg). Groups A and A', controls; groups B and B', infected with Trichinella spiralis 6 days and 9 days before infection with H. diminuta respectively; groups C and C', infected with T. spiralis 6 days and 9 days before infection with H. diminuta respectively and injected s.c. with 2.5mg cortisone acetate on alternate days beginning 2 days after infection with H. diminuta.

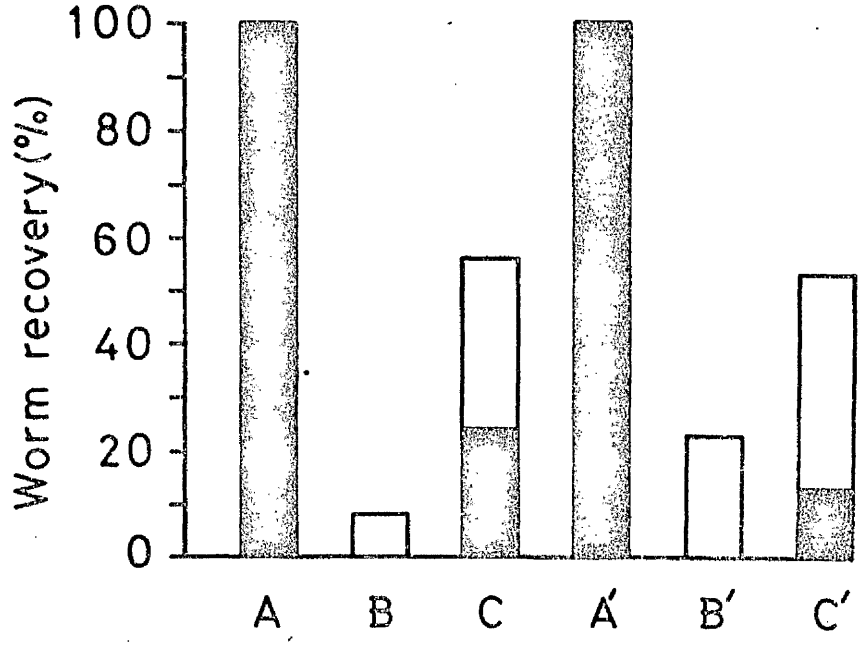
Worm recovery (%)
100
80
60
40
20
0
A
B
C
A'
B'
C'

Table 3-3

	DAY					
Group$_n$	-6	0	2	4	6	8
A_8		Hd ———	———	———	———	K
B_8	Tsp —	Hd ———	———	———	———	K
C_{10}	Tsp —	Hd ——	c ——	c ——	c ——	K

	DAY					
	-9	0	2	4	6	8
A'_8		Hd ———	———	———	———	K
B'_8	Tsp —	Hd ———	———	———	———	K
C'_{10}	Tsp —	Hd ——	c ——	c ——	c ——	K

a. 2.5mg cortisone acetate, s.c.

in the previous experiment, the response to T. spiralis prevented H. diminuta from surviving the full 8 days - very few worms, $\leq$0.1mg dry weight, were recovered from groups B and B'. Recoveries of 50% - 60% from both group C and group C', however, show that at least this percentage of worms established and survived for at least 48h in the T. spiralis-infected mice.

In this experiment rejection of T. spiralis was abnormally delayed. Large numbers of worms remained in group B autopsied 14 days post-infection. However, few worms remained in group B' autopsied on day 17.

Does cross-immunity exist between T. spiralis and H. diminuta?

The more rapid inflammatory response to, and rejection of worms from, challenge infection with T. spiralis was utilised in this experiment to allow precise timing of the inflammation in relation to the H. diminuta infection.

The experimental plan is given in Table 3-4. Male, 3 star NIH mice were 28 days old when given the T. spiralis primary infection. Groups of identical mice were included as controls for the T. spiralis primary and challenge infections.

Table 3-4

	DAY				
$Group_n$	-19	-12	0	6	9
A_{10}			Hd ———		K
B_{10}			Hd ——	Tsp ——	K
C_{10}	Tsp ———		Hd ——	Tsp ——	K
D_{10}	Tsp ———		Hd ———		K
E_4	Tsp ——	K			
F_5				Tsp ——	K
G_4	Tsp ———			Tsp ——	K

Recoveries and dry weights of H. diminuta from groups A to D are given in Fig. 3-3. Over 90% of worms administered were recovered from the control group (group A) on day 9. Worm recovery and growth from group B (infected with T. spiralis on day 6 of the H. diminuta infection) were not significantly

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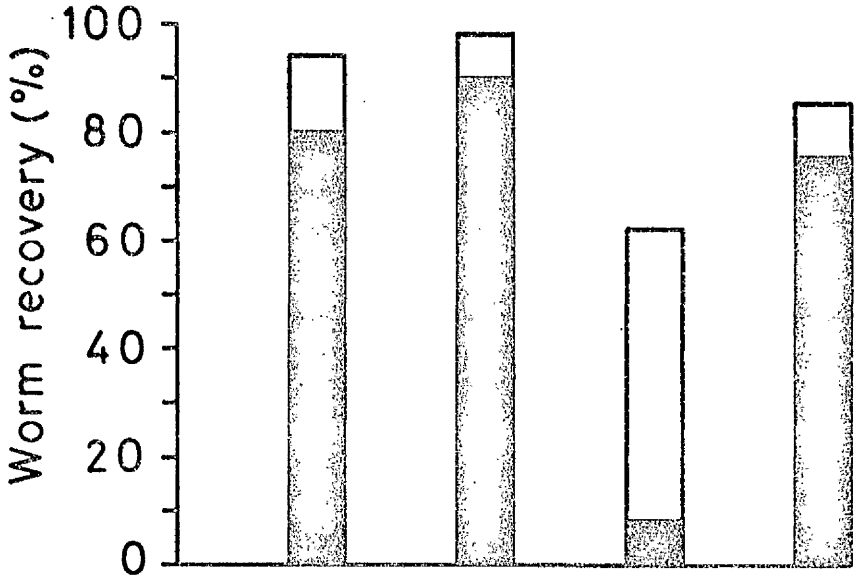
Figure 3-3

Recovery of 9-day old Hymenolepis diminuta from male NIH mice given a five-cysticercoid infection. Solid areas of bars represent worms >0.1mg dry weight; open portions represent destrobilated worms (≤0.1mg). Group A, control; group B, infected with Trichinella spiralis on day 6 of the H. diminuta infection; group C, infected with T. spiralis 19 days before H. diminuta infection and challenged with T. spiralis on day 6 of the H. diminuta infection; group D, infected with T. spiralis 19 days before H. diminuta infection only.



	A	B	C	D
Mean worm (mg) d. wt./mouse ± S.D.	4·7 ±2·4	5·3 ±2·3	0·3 ±0·1	3·7 ±2·7

different from the control group indicating that the 3 days in which T. spiralis existed alongside H. diminuta had no effect on the H. diminuta. Worm recovery and growth in group D were also not significantly different from the control group, suggesting that no cross-immunity exists between the two species. In group C, however, which was given both primary and challenge infections of T. spiralis, there was a marked reduction of strobilate worms by day 9. The majority of worms which were recovered had destrobilated. These worms were destrobilated rather than stunted because the T. spiralis-induced inflammation (which occurs within 24h following a challenge infection) did not begin until day 6 of the H. diminuta infection, by which time worms normally possess a well-developed strobila.

Although a rapid inflammatory response developed to a challenge T. spiralis infection, T. spiralis had not been rejected from mice 3 days after challenge (Group G, below). Recoveries of T. spiralis from mice given primary and/or challenge infections are given below:

Group	Numbers of T. spiralis (mean ± S.D.)
E (primary only)	198.5 ± 9.4
F (challenge only)	278.6 ± 21.0
G (primary and challenge)	298.8 ± 29.3

The development of the effect on *H. diminuta* of the response induced by *T. spiralis*.

The abrupt response of the mouse to challenge infection with *T. spiralis* was again utilised in this experiment but groups of mice were autopsied over a period of 4 days to investigate the onset of effects on the *H. diminuta* infection.

Male, 3 star NIH mice, 32 days old when given the primary *T. spiralis* infection were treated as shown in Table 3-5.

Recovery of *H. diminuta* and mean length of worms recovered from group A (given a *T. spiralis* primary infection, day -21) and group B (given primary and challenge infections of *T. spiralis*, days -21 and +5 respectively) are shown in Fig. 3-4. *H. diminuta* recovered from mice in group A increased in length with age, indicating that as in the previous experiment, a primary infection with *T. spiralis*, preceding *H. diminuta* infection by about 20 days, confers no immunity to the succeeding *H. diminuta*.

Although *H. diminuta* in group B mice established at levels similar to those in group A mice, many had destrobilated 2 days after challenge with *T. spiralis* and, henceforth, only destrobilated worms were recovered. No significant reduction in numbers of *H. diminuta* occurred in group B mice although the worms were destrobilated. The lack of rejection of *H. diminuta* from mice in group B may be associated with the delayed rejection of *T. spiralis* from the challenge infection. *T. spiralis* established in unusually low numbers in the primary infection (group C) given on day -21 (mean recovery on day 7, 123.4 $\pm$ 32.6), but in more normal numbers in the challenge infection given on day 5 of the *H. diminuta* infection. There was no reduction in these numbers until 4 days after challenge (see below), even though, as in the

Table 3-5

	DAY						
Group A	-21	0	5	6	7	8	9
10	Tsp	Hd	K				
10	Tsp	Hd		K			
10	Tsp	Hd			K		
10	Tsp	Hd				K	
10	Tsp	Hd					K
Group B							
10	Tsp	Hd	Tsp	K			
10	Tsp	Hd	Tsp		K		
10	Tsp	Hd	Tsp			K	
10	Tsp	Hd	Tsp				K
Group C							
5	Tsp	K*					
Group D							
3			Tsp	K			
4			Tsp		K		
3			Tsp			K	
4			Tsp				K
Group E							
4	Tsp		Tsp	K			
4	Tsp		Tsp		K		
4	Tsp		Tsp			K	
4	Tsp		Tsp				K

* autopsied day 7 of T. spiralis primary infection.

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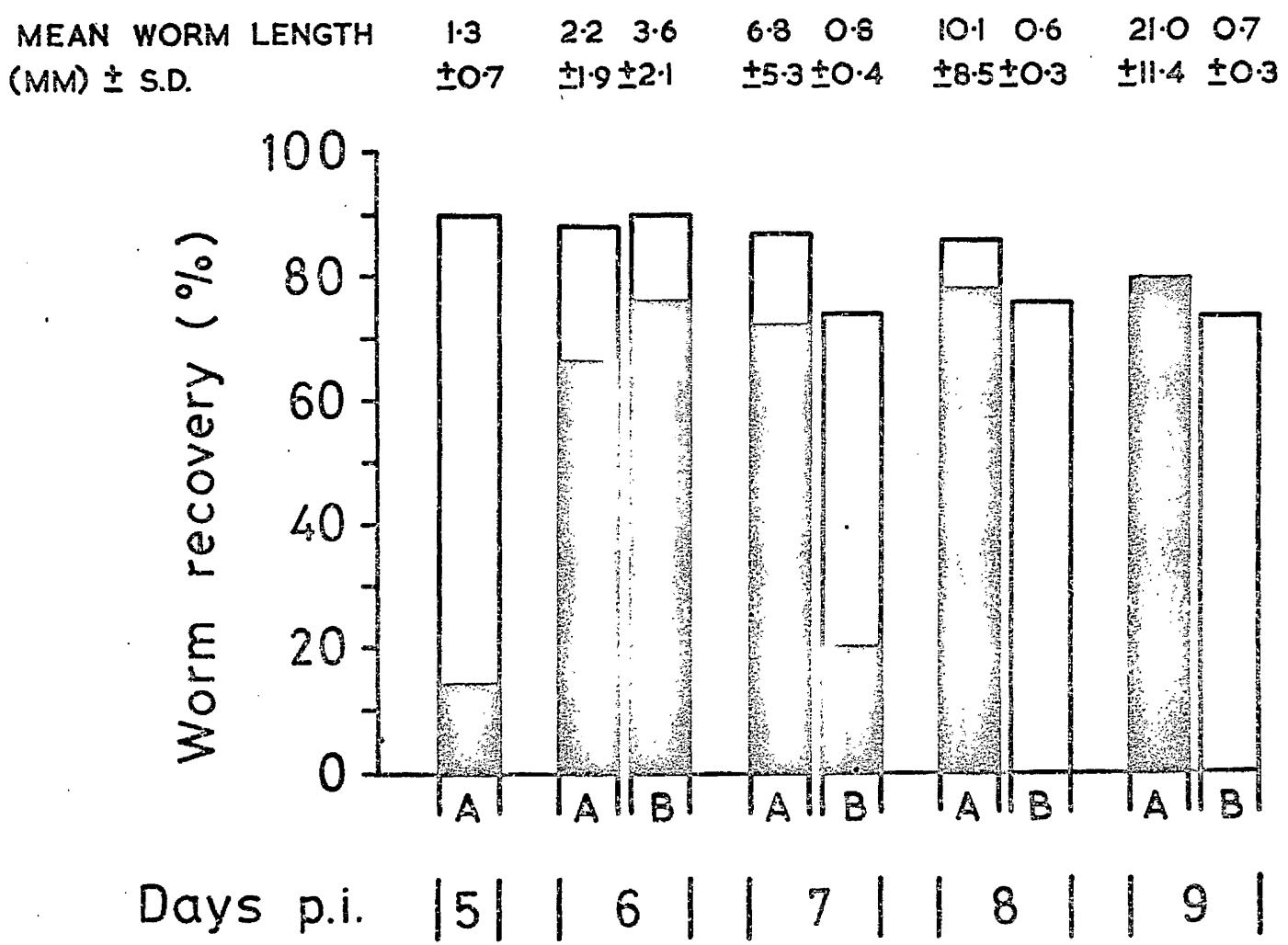
Figure 3-4

Recovery (%) of Hymenolepis diminuta from five-cysticercoid infections in male NIH mice. Solid areas of bars represent worms $>$0.1mg dry weight; open portions represent worms $<$0.1mg. Group A, infected with Trichinella spiralis 21 days before infection with H. diminuta; group B, infected with T. spiralis 21 days before infection with H. diminuta and challenged with T. spiralis on day 5 of the H. diminuta infection. Mean ($\pm$ S.D.) worm lengths are given above the appropriate group.


MEAN WORM LENGTH
(MM) ± S.D.
1·3 ±0·7
2·2 ±1·9
3·6 ±2·1
6·8 ±5·3
0·8 ±0·4
10·1 ±8·5
0·6 ±0·3
21·0 ±11·4
0·7 ±0·3
Worm recovery (%)
100
80
60
40
20
0
A
A
B
A
B
A
B
A
B
Days p.i.
5
6
7
8
9

previous experiment, the associated inflammatory response appeared to occur swiftly following the challenge infection.

Mean recovery (± S.D.) of *T. spiralis* from challenge infection

Group	Days after *T. spiralis* challenge infection 1	2	3	4
D	177.3 ± 56.7	206.8 ± 26.9	227.0 ± 17.5	250.0 ± 17.2
E	214.3 ± 38.5	221.0 ± 38.2	230.0 ± 16.5	170.0 ± 6.6

D - challenge-only control
E - primary and challenge infections

Summing up the results at this stage, it had been demonstrated that *H. diminuta* is extremely sensitive to the inflammatory response induced by *T. spiralis*. Although worms manage to establish during the height of this response they rapidly destrobilate and may or may not be eliminated. The sensitivity to this inflammation appears to be non-specific as no cross-immunity exists between *H. diminuta* and *T. spiralis*.

The following experiments were devised to determine whether *H. diminuta* is equally sensitive to *T. spiralis*-induced inflammation in the rat, a host to which *H. diminuta* is well adapted.

Can *H. diminuta* survive the response to *T. spiralis* in the rat?

Ten to eleven-weeks-old female CFHB rats, bred in the Wellcome Laboratories were treated as shown in Table 3-6.

The mean dry weight of *H. diminuta* recovered from each group and the corresponding worm recoveries are given in Fig. 3-5. Although there was no significant loss of *H. diminuta* from rats

Table 3-6

Group$_n$	DAY 0	7	8	16	18	20
A_6	Hd ———					K
B_6	Hd ———		Tsp ———			K
C_6		Hd ———				K
D_7		Hd ——	Tsp ———			K
E_9			Tsp ——	K ——	K ——	K

challenged with T. spiralis on day 8 of infection (group B), growth of H. diminuta in these rats was substantially less than worm growth in control rats (group A). In fact, the mean dry weight of the 20 day old worms recovered from rats in group B was almost identical to the mean dry weight of worms recovered from control rats infected with H. diminuta for only 13 days (group C). A large number of H. diminuta (69% of those administered) in rats challenged with T. spiralis the day following infection (group D) survived at least 13 days but these worms were severely stunted.

Recovery of T. spiralis from control rats autopsied on days 8, 10 and 12 post-infection (group E) are given below:

Days after infection	Number of T. spiralis recovered per rat (mean ± S.D.)
8	1,723.3 ± 152.9
10	947.0 ± 658.1
12	11.5 ± 4.5

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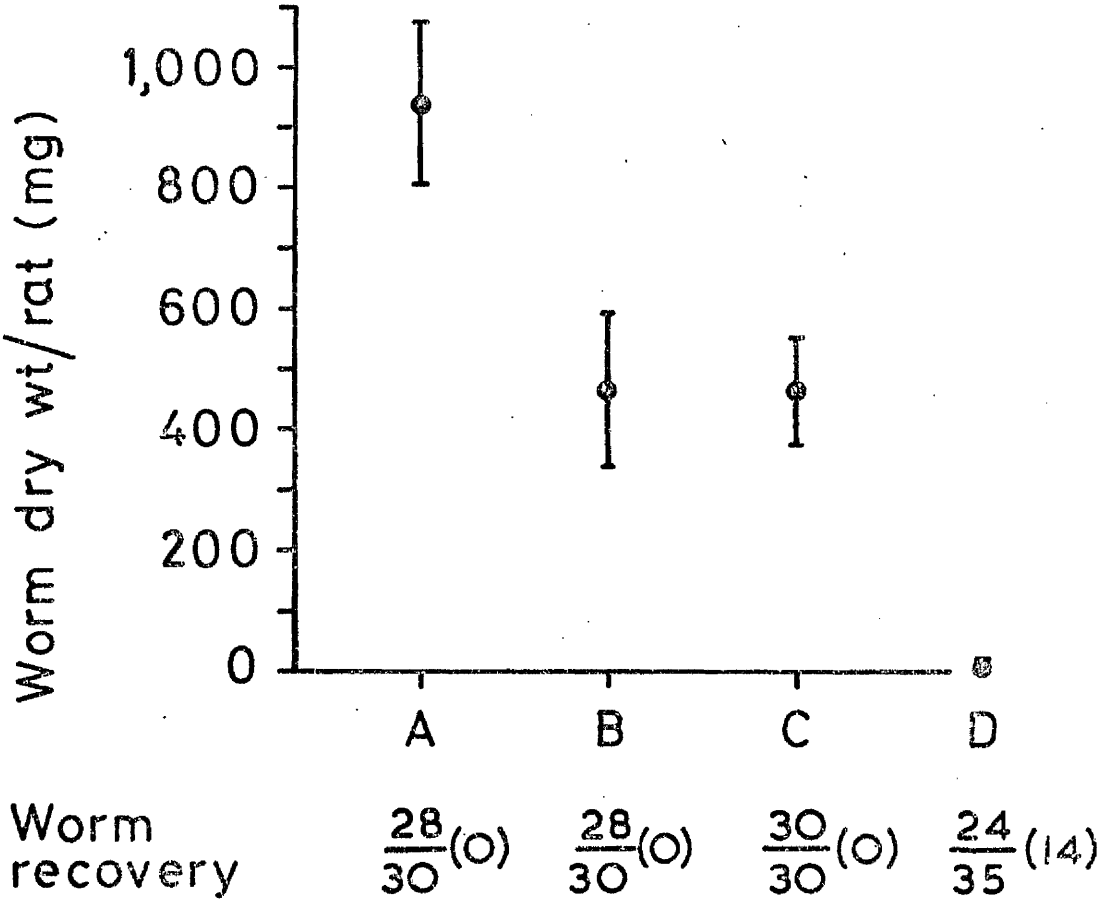
Figure 3-5

Mean (± S.D.) total dry weight per female CFHB rat of *Hymenolepis diminuta* from five-cysticercoid infections given 20 days (groups A and B) or 13 days (groups C and D) previously. Group A, control; group B, infected with *Trichinella spiralis* on day 8 of the *H. diminuta* infection; group C, control; group D, infected with *T. spiralis* on day 1 of the *H. diminuta* infection. Worm recoveries are given below the relevant group as a fraction of the number of cysticercoids administered. Numbers in parentheses are numbers of destrobilated worms ($\leq$0.1mg).


Worm dry wt/rat (mg)
1,000
800
600
400
200
0
A
B
C
D
Worm recovery
28/30 (0)
28/30 (0)
30/30 (0)
24/35 (14)

Thus, the rejection of T. spiralis over this period was abrupt and was complete by day 12, i.e., the day upon which H. diminuta-infected rats were autopsied.

It is evident, then, that H. diminuta which had been established in a rat for 8 days is not rejected when the rat is infected with T. spiralis. Worm growth, however, is halted by the inflammatory response which develops in response to the T. spiralis infection. Even if rats are challenged with T. spiralis the day following infection with H. diminuta, substantial numbers of H. diminuta survive for at least a further 12 days, although worm growth in this case is minimal.

Development of the effect of the response to T. spiralis on growth of H. diminuta.

Male, 50 days old CFHB rats, bred in the Wellcome Laboratories were treated as shown in Table 3-7.

Table 3-7

	DAY							
n	0	2	7	10	12	14	18	25
4	Hd ———		K					
4	Hd ———		———	———	———	K		
4	Hd ———		———	———	———	———	K	
4	Hd ———		———	———	———	———	———	K
4	Hd —	Tsp —	K					
4	Hd —	Tsp —	———	———	———	K		
4	Hd —	Tsp —	———	———	———	———	K	
4	Hd —	Tsp —	———	———	———	———	———	K
6		Tsp ———	K —	K —	K ———			K

The dry weight of H. diminuta recovered from control rats and from rats challenged with T. spiralis 2 days following infection with H. diminuta is shown in Fig. 3-6. On days 7, 14 and 18 post-infection, the weight of worms recovered from rats challenged with T. spiralis was significantly less than the weight of worms recovered from control rats. By day 25, however, worm growth in the challenged rats had recovered to control levels. Worm recoveries in both groups ranged from 75% to 95% of cysticercoid numbers administered, there being no significant worm loss from either group.

Rejection of T. spiralis (given below) in this experiment was less abrupt than in the previous experiment, the different age and sex of rats used possibly contributing to this.

Days after infection	Number of T. spiralis recovered per rat (mean ± S.D.)
8	1,054.5 ± 26.5
10	344.0 ± 210.5
12	539.5 ± 173.5

It appears that, in the rat, the inflammatory response to T. spiralis severely restricts the growth of established H. diminuta. The number of tapeworms which are rejected presumably depends upon the relative timing of H. diminuta and T. spiralis infections and upon the severity of the response to T. spiralis. Worm growth resumes when the inflammatory response to T. spiralis has died down.

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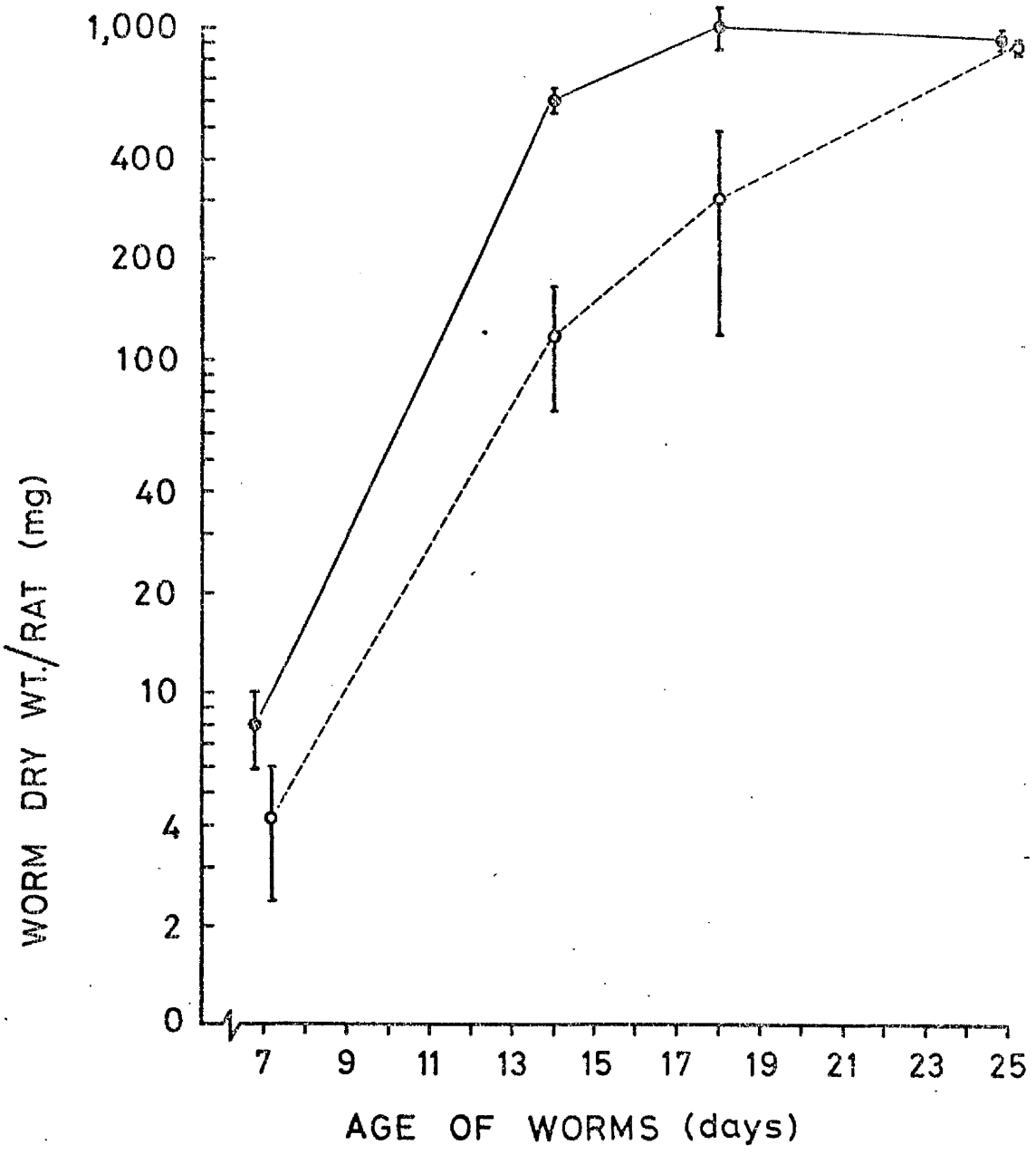
Figure 3-6

Mean (± S.D.) dry weight of Hymenolepis diminuta recovered per male CFHB rat given five cysticercoids. ● - - - ●, controls; ○ - - - ○, infected with Trichinella spiralis on day 2 of the H. diminuta infection.


1,000
400
200
100
40
20
10
4
2
0
WORM DRY WT./RAT (mg)
7
9
11
13
15
17
19
21
23
25
AGE OF WORMS (days)

DISCUSSION

Several species of nematode induce an inflammatory response during their intestinal phase. Numerous investigators have demonstrated the non-specific effects of such inflammation on concurrent infection with other species, although the interaction has not always been attributed to these effects.

Cox(1952) investigated the interaction between T. spiralis and Ancylostoma caninum in mice. The mouse is an abnormal host for A. caninum and Cox reported that maximum intestinal inflammation occurred 2 days following an oral infection, coinciding with maximum larval penetration of the gut mucosa.

> [Reduced numbers of adult T. spiralis were recovered on day 7 from mice infected with A. caninum 2 or 10 days previously, suggesting that the A. caninum-induced inflammation increased resistance to T. spiralis. Whether this resulted from reduced establishment of T. spiralis, or from enhanced rejection is unknown as no concurrently infected mice were autopsied prior to day 7. The migration of T. spiralis larvae also appeared to be adversely affected by inflammation of the lung tissues caused by A. caninum.]

Louch (1962) demonstrated an interaction between T. spiralis and the inflammatory response induced by adult Nippostrongylus brasiliensis (= N. muris) in rats.

> [Rats were given three immunising infections of N. brasiliensis and, 14 days after the last of these, they were infected with T. spiralis. Significantly fewer T. spiralis larvae developed in these rats than in rats infected with T. spiralis only.]

Louch interpreted his results as being due to the N. brasiliensis-induced intestinal inflammation causing either reduced establishment of adult T. spiralis or reduced penetration of the gut mucosa by larval T. spiralis. No cross-reaction was found between T. spiralis antigen and the serum from N. brasiliensis-infected rats, supporting the suggestion of a non-specific factor acting on T. spiralis.

No controls, however, were included to monitor the N. brasiliensis immunising infections or to demonstrate the establishment of T. spiralis adults. Although little information is available on the progression of inflammation accompanying infection with N. brasiliensis, it is likely that because large immunising infections of N. brasiliensis were given, the consequent inflammatory response was severe and that Louch's interpretation of a non-specific effect on T. spiralis is correct.

Liu & Ivey (1961) reported that infection of mice with T. spiralis had no effect on established Nematospiroides dubius (a nematode, not normally rejected from mice). Behnke (personal communication), however, has found that interaction between these two species can occur, the severity of which depends upon the relative timing of the concurrent infections.

An interaction between the inflammation induced by T. spiralis and the development of Hymenolepis nana has been reported (Weinmann, 1964).

> [Eggs of H. nana were administered to mice at varying times following infection with T. spiralis and subsequent counts were made of H. nana cysticercoids developing in the gut villi. The only significant reduction in cysticercoid counts was found in mice infected with H. nana on day 15 of the T. spiralis infection. No controls were included to monitor the response which developed to T. spiralis but Weinmann does state that the distribution of cysticercoids in all concurrently infected mice did not coincide with regions showing inflammation and that "those mice with the most extensive areas of inflammation also harboured the fewest cysticercoids".]

Stahl (1966) observed reduced establishment or enhanced expulsion of Aspiculuris tetraptera (which does not normally induce an inflammatory response) when mice were infected up to 12 days after infection with T. spiralis. This was interpreted as a

non-specific acquired resistance, probably due to T. spiralis-induced inflammation of the intestine. No effect was observed if the A. tetraptera were administered 20 days after the T. spiralis infection. No attempt was made to monitor the response induced by T. spiralis, but if the kinetics of T. spiralis expulsion were similar to those described in the present study, it seems likely that the maximum effect on A. tetraptera was produced if the infection was given during the inflammatory response to T. spiralis. Behnke & Wakelin (personal communication) have found that the most marked effect on numbers of A. tetraptera occurred when mice were infected at the time of T. spiralis rejection, when the associated inflammation is at its peak.

Bruce & Wakelin (1974) reported that a reduction in numbers of Trichuris muris occurred from CFLP mice when the mice were infected up to 18 days after infection with T. spiralis.

> [A marked reduction in T. muris numbers was observed 14 days after infection (T. muris is normally rejected from this strain of mouse after about 21 days) when T. muris were given at the same time as T. spiralis or 7 days later. A delay in rejection of both species was reported after administration of the anti-inflammatory drug, indomethacin. No cross-immunity between the species developed - T. muris developed normally in mice immune to T. spiralis.]

Most of the studies described above clearly show the necessity for adequate controls. In the present investigation, every effort was made to include such controls. The results show, however, that future investigations ought to include more careful monitoring of the species producing the non-specific inflammatory effect (T. spiralis in this case).

It was demonstrated that no cross-immunity existed between T. spiralis and H. diminuta and infection with H. diminuta appeared to have no effect on the progression of the response to an existing T. spiralis infection. The response to T. spiralis, however, had a marked effect on the development of H. diminuta depending on the relative timing of the concurrent infections and on the severity of the response to T. spiralis. Interpretation of this effect is complicated by the varying response of mice and rats to T. spiralis.

In NIH mice, if the H. diminuta infection was given just prior to the appearance of the inflammatory response to T. spiralis, or as the response reached its peak, then although some tapeworms survived for 2 days or so, almost complete expulsion followed. If, however, H. diminuta was allowed to establish and grow for 5 or 6 days before the onset of the acute phase of T. spiralis-induced inflammation, then destrobilation of H. diminuta took place but the destrobilated worms persisted in the inflamed intestine. On the other hand, it may be argued that in the two experiments in which H. diminuta was allowed to establish for several days before being subjected to an inflamed environment, rejection of T. spiralis was delayed and so the persistence of destrobilated worms may be due to a less severe inflammatory response in these instances.

A similar ambiguity arises from the experiments using CFHB rats. In the first experiment, the response to T. spiralis was strong and rejection was abrupt between days 10 and 12. Many H. diminuta administered on day 1 of this T. spiralis infection did not survive the response to T. spiralis. Those tapeworms, however, which had been established for 8 days before the

T. spiralis infection, survived the inflammatory response although their growth was reduced. This seems to mimic the situation described for the mouse, i.e., if *H. diminuta* is allowed to establish well before the response to *T. spiralis* begins to operate, then it will survive even though growth is stopped; if *H. diminuta* is allowed to establish just prior to the inflammatory response, then most worms will be lost in the face of the inflammation, others will show a marked reduction in growth rate.

The results of the second experiment using rats tend to contradict the above hypothesis. In this experiment, although *H. diminuta* was allowed to establish for only a short time before *T. spiralis* was administered, there was no loss of *H. diminuta*. A possible explanation may be that a less severe response to *T. spiralis* occurred in this experiment, shown by the incomplete rejection of adult nematodes by day 12. Whether *H. diminuta* would have been expelled from these rats had the response to *T. spiralis* been as abrupt as in the first experiment, rather than persisting and growing to control sizes once the response to *T. spiralis* had passed, is conjectural.

Thus, as it would be difficult to plan future investigations more precisely, given the inherent variability in response to both helminth species, a quantitative histological or biochemical marker of the severity of the response to *T. spiralis* should be used in order to aid correct interpretation of results.

Although the present investigation shows that *T. spiralis*-induced inflammation exerts a profound effect on *H. diminuta*, it gives no information regarding the nature of the interaction. It has been demonstrated that the acidosis associated with inflammation

may have a direct effect on survival of T. spiralis (Castro, Cotter, Ferguson & Gordon, 1973). It is probable, however, that lowering of the intestinal pH would have a less marked effect on H. diminuta as it has been shown that H. diminuta is adapted to surviving at low pH (Podesta & Mettrick, 1974). Other factors, e.g., raised phospholipase levels which have been demonstrated to occur in the T. spiralis-inflamed intestine (Larsh, Ottolenghi & Weatherly, 1974), may exert a direct effect on a concurrent H. diminuta infection. On the other hand, indirect factors may be involved. For example, the onset of inflammation undoubtedly disturbs the eating behaviour of the host and this must play a part in systems involving tapeworms which are extremely sensitive to disturbances in host diet.

In conclusion, it would appear that the hypothesis (see INTRODUCTION) of Larsh & Race (1975) may be over-simplified. Different species of helminth, and even different developmental stages of the same species, differ in their susceptibility to allergic inflammation. It seems more likely that, as Wakelin & Lloyd (1976b) suggest, the immune expulsion of intestinal helminths probably occurs as a result of several components of the immune response acting co-operatively. Inflammation undoubtedly plays a role in the expulsion of many helminths but its importance within the overall complex mechanism varies from species to species.

SUMMARY

1. It is demonstrated that cross-immunity between H. diminuta and T. spiralis does not exist.

2. H. diminuta is able to establish in the mouse at the height of the inflammatory response to T. spiralis and survives for at least 2 days. However, almost all tapeworms are subsequently expelled prematurely.

3. It is shown that, if H. diminuta is allowed to establish for 5 or 6 days before the onset of severe inflammation, destrobilation takes place but a high percentage of tapeworms survive at least until day 9 post-infection.

4. A high percentage of cysticercoids administered to rats established and the worms continued to survive in the face of subsequent inflammation induced by T. spiralis.

5. In rats infected with both T. spiralis and H. diminuta, the growth of H. diminuta recommenced and eventually returned to control levels when the inflammatory response to T. spiralis subsided.

6. Further investigation of cellular and biochemical changes taking place in the inflamed intestine, and careful monitoring of the host's dietary intake during the inflammatory response are urged so that assessment of the specific nature of the interaction may be made.

CHAPTER III

THE EFFECT OF IMMUNITY ON MEMBRANE TRANSPORT IN HYMENOLEPIS DIMINUTA

INTRODUCTION

Preceding chapters of this thesis have been concerned with the identification of the immune mechanisms involved in the response to H. diminuta. However, as Ogilvie & Jones (1973) suggest, demonstration of the effect of host immunity on the structure and metabolic functions of helminth parasites is an equally important objective. They (Ogilvie & Jones) propose that, as immune mechanisms are likely to be mediated through changes in aspects of host function upon which the parasite is dependent, observation of the changes induced will reveal the relative importance of these host functions in the host-parasite relationship.

The effect of host immunity on a parasite can be assessed in two ways: by observation of changes in parasite structure or by measurement of changes in metabolic function. H. diminuta is particularly suitable as a subject for observation of both structure and function because both facets have been studied extensively. The structure of the tegument, which represents the interface between parasite and host, and the membrane transport mechanisms which operate across the tegument have both been recently and comprehensively reviewed (Lumsden, 1975; Pappas & Read, 1975; respectively) so only brief discussion of important points is made here.

The tegument of H. diminuta (and, indeed, of all cestodes) is a metabolically active structure and is the surface over which all metabolites must pass to enter the parasite (unlike the surface of nematodes which is covered by an impermeable cuticle). It is divided into a distal, syncitial region, in

many ways analogous to the mammalian intestinal epithelium, and a proximal region containing the nuclei and connected to the distal layer by cytoplasmic bridges. The outer surface is covered by a brush border comprising microvillus-like projections, termed microtriches. The surface of the microtriches is coated with a thin, carbohydrate-rich glycocalyx (Lumsden, Oaks & Alworth, 1970; McCracken & Lumsden, 1975b). The glycocalyx is manufactured in the perinuclear cytoplasm and then transported to the tegumental surface, with a turnover time of about 6h (Oaks & Lumsden, 1971). It comprises neutral carbohydrates (McCracken & Lumsden, 1975b) and a high density of acidic glycans (Lumsden et al., 1970) which give the tegumental surface a net negative charge (Lumsden, 1972). Calcium and other inorganic cations are reportedly adsorbed to the negative charges of the glycocalyx (Lumsden, 1973) and certain of these cations have been shown to activate phosphohydrolase activity at the tapeworm surface (Lumsden & Berger, 1974). These enzymes have significance in the digestive/absorptive function of the tegument (see below). In addition to functions of digestion and absorption, the tegument has been shown to offer a degree of protection against enzyme attack by the host. Thus, in the presence of H. diminuta, trypsin and α- and β-chymotrypsin have been shown to be irreversibly inactivated (Pappas & Read, 1972a, b).

The surface phosphatases have an important digestive function, but are not themselves involved in transport mechanisms (Arme & Read, 1970). Their inhibition does not affect transport of non-phosphorylated nutrients (Phifer, 1960b; Dike & Read, 1971; Pappas & Read, 1974), but they are important in hydrolysing phosphate esters which cannot cross the membrane into derivatives

which can. Dike & Read (1971) showed that the close association of a phosphohydrolase and the corresponding specific transport locus gives the liberated base a "kinetic advantage" for subsequent absorption by the worm, i.e., it does not diffuse into the medium but is absorbed.

There is extensive literature dealing with the transport mechanisms of H. diminuta. The transport systems currently described include at least four amino acid systems; one monosaccharide system; two glycerol systems; three purine-pyrimidine systems; two fatty acid systems and two water-soluble vitamin systems (Pappas & Read, 1975). Only those under investigation in the present study will be discussed here.

Read, Rothman & Simmons (1963) postulated several different amino acid carrier systems and Harris & Read (1968) have described four distinguishable systems as follows:

(a) An aspartic-glutamic acid, or dicarboxylic acid-preferring system, possibly also transporting small amounts of neutral amino acids.

(b) An arginine-lysine, or diamino-preferring system interacting with basic amino acids.

(c) Two or more systems transporting neutral amino acids.

Methionine, the neutral amino acid used in the present study, has been shown to be transported in H. diminuta by a true active transport mechanism (Read et al., 1963; Pappas, Uglem & Read, 1974), i.e., it is accumulated against a concentration gradient. The kinetics of methionine transport and its competitive inhibition

by other amino acids have been adequately described (Read et al., 1963).

Arme & Read (1968) described a mediated (cf., active) transport system for short-chain fatty acids using acetate as substrate. They were unable to demonstrate accumulation of acetate against a concentration gradient, suggesting that transport was via a facilitated diffusion system rather than via active transport. They showed that at low substrate concentrations acetate transport was non-linear. At higher concentrations, uptake was linear suggesting a large diffusion component at these concentrations. Acetate uptake was shown to be pH-dependent. At pH 7.4 uptake was non-linear with increasing substrate concentration; at pH 6.2 acetate uptake was greater than at pH 7.4 and was linear with respect to concentration, suggesting a large diffusion component operating at the lower pH.

Glucose (D-isomer only) is transported across the tegument of H. diminuta by a process almost entirely consisting of active transport (Phifer, 1960a, b, c; Pappas et al., 1974). Transport of glucose has been shown to be linked to Na^+ fluxes occurring across the tegument (Read, Stewart & Pappas, 1974).

There are, then, a great many facets of the structure and function of H. diminuta which offer potential points of attack for the host immune system. As Ogilvie & Jones (1973) suggest, the "strong immune system" - of the mouse to H. diminuta - "seems an ideal one for examining the way in which immunity affects the structure and metabolism of adult cestodes".

With regard to the effect on structure of the tegument, recent work has demonstrated tegumental lesions on H. diminuta from mice and rats (Befus & Threadgold, 1975; Chappell & Pike, 1976). The aetiology of these lesions is unknown but examination of their frequency of occurrence and of their ultrastructure led Befus & Threadgold (1975) to propose a hypothesis of immune damage. The damaged areas consisted of regions showing increased electron density; abnormal mitochondria; reduced granular endoplasmic reticulum; Golgi complexes and discoidal secretory bodies and an accumulation of lipid droplets. This appears to be quite different to immune damage to schistosome tegument which seems to result, at least initially, in increased membrane turnover (Perez & Terry, 1973).

Befus (1974, 1975a) showed that H. diminuta in mice and rats is coated with antibody and Threadgold (personal communication) has demonstrated that this antibody is bound to the tegumental glycocalyx and does not penetrate beyond this layer. This information stimulated the present study. It seemed likely that antibody, specifically bound to one or more components of the glycocalyx must have an effect on the metabolic function of this layer (discussed above).

Accordingly, in the present study, the possible effect of the immune response on the membrane transport of worm nutrients was investigated. Three such metabolites (L-methionine, sodium acetate and D-glucose), operating via three distinct carrier systems, were used. Transport by worms from mice was compared with transport by worms of equivalent weight from immunosuppressed mice and from rats.

MATERIALS AND METHODS

1. Hosts and parasite.

Mice used in the investigation of methionine transport by H. diminuta were CD-1 strain males, six weeks old at infection. On arrival they were treated with piperazine citrate to remove probable pinworm infections originating from the animal suppliers (see GENERAL MATERIALS AND METHODS). In all other studies, male 4 star CFLP mice, 6 weeks old at infection were used. In all experiments, male rats of the outbred CFHB strain, bred in the animal house of the Wellcome Laboratories, were used and were infected with H. diminuta when 8-9 weeks old.

In some experiments, groups of mice and rats were immunosuppressed using cortisone acetate (Boots Ltd.). Mice were given 1.0mg s.c., rats 5.0mg s.c., on alternate days beginning on the day of infection. All animals (cortisone-treated and untreated) were given oxytetracycline in their drinking water (see GENERAL MATERIALS AND METHODS).

All mice and rats were infected with six cysticercoids of H. diminuta using the method described in GENERAL MATERIALS AND METHODS. Worms were recovered on various days post-infection as follows. The host was killed and the entire small intestine was carefully separated from the mesentery and removed. Using a 50ml disposable syringe fitted with a stainless steel cannula inserted into the duodenum, the intestine was perfused with HBSS at room temperature so that entire worms were recovered. Broken or obviously damaged worms were discarded.

2. Incubation, extraction and counting.

All incubations were carried out under air in 25ml beakers, covered with glass slides to minimise concentration by evaporation and held in a water bath at 37°C. Immediately following their removal from the host animal, worms were rinsed free from adhering intestinal debris, transferred to 15ml of HBSS and allowed to pre-incubate for 10 minutes to minimise water and ion fluxes in worm tissues. Normally worms were incubated individually, but occasionally very small worms were incubated in pairs to aid measurement of isotope intake.

After preincubation, worms were transferred to 4ml of incubation medium containing ^{14}C-labelled nutrient and incubated for either 10 minutes (methionine) or 2 minutes (sodium acetate and glucose). Worms were then incubated for 2 minutes in each of three changes of HBSS (15ml), rinsed briefly in 15ml HBSS, blotted gently on filter paper and extracted overnight in 5ml 70% ethanol in stoppered tubes. In all experiments, at least 10 replicate incubations were carried out, i.e., at least 10 worms were incubated from each type of host animal on each day of autopsy.

Extracted worms were transferred to small aluminium cups, dried overnight at 98°-100°C and weighed to within 0.01mg on a Stanton Unimatic Balance. The ethanolic extracts were assayed for radioactivity using duplicate 0.5ml aliquots dried on aluminium planchettes (Sigma Ltd.) and counted on an end window, gas-flow counter (Model D47, Nuclear Chicago Corp.). Two blank planchettes counting background activity and a planchette containing a standard of known concentration were also included.

^{14}C-labelled compounds (L-[methyl-^{14}C]methionine; [1-^{14}C] sodium acetate; D-[U-^{14}C]glucose) were obtained from the Radiochemical Centre, Amersham as freeze-dried solids. They were dissolved in sufficient HBSS to provide stock solutions with an activity of 10μCi/ml. Small amounts of these solutions were added to solutions of metabolites of known molarity to provide incubation media of the required specific activity. All unlabelled chemicals were of "AnalaR" grade, except L-methionine which was of the highest available grade (B.D.H. Ltd.).

RESULTS

Membrane transport is a surface area-dependent phenomenon. The growth rates of H. diminuta in mice, immunosuppressed mice, rats and immunosuppressed rats are different. As worms become larger, so the ratio of surface area to weight decreases. Worms recovered from different host types on the same day post-infection will differ in weights, their surface area to weight ratios will differ and so uptake of a given nutrient by these worms of similar age but of different host source will not be comparable. To overcome this, throughout this chapter comparison is made between worms of equivalent weight from the different host types, regardless of the day post-infection on which the worms were recovered.

1. Methionine.

In this experiment, mice and rats were autopsied over the same range of days post-infection. Because of the different growth rates of H. diminuta in different hosts, comparison of methionine uptake was only possible between worms from untreated mice and worms from cortisone-treated mice. Insufficient numbers of worms from rats were of equivalent weight to allow meaningful comparison between "mouse worms" and "rat worms".

Table 4-1 shows the percentage recovery of H. diminuta from normal and immunosuppressed mice and rats. With the method used for worm recovery, very small worms and destrobilated worms were usually not found. However, the totals give an indication of the progression of worm rejection from untreated mice. Over the range of days post-infection when animals were autopsied, untreated

Table 4-1

Recovery (%) of Hymenolepis diminuta from six-cysticercoid infections of untreated and cortisone-treated 6 week old CD-1 male mice and of untreated and cortisone-treated 8-9 week old CFHB male rats.
ND, not done.

Age of worms (days)	Untreated mice	Cortisone-treated mice	Untreated rats	Cortisone-treated rats
7	100	94	ND	ND
8	88	92	100	100
9	78	89	83	100
10	54	72	94	83
11	20	83	94	33
12	ND	ND	83	89

mice showed an increasing response to H. diminuta manifested by a gradual reduction in worm recovery from 100% on day 7 to 20% on day 11. As expected, cortisone-treated mice, untreated rats and cortisone-treated rats showed no corresponding rejection of worms. The only exception to the consistently high recovery of worms from these latter hosts was the unusually low number of worms recovered from cortisone-treated rats on day 11. That this does not indicate rejection from this group, is shown by the high recovery on the following day.

Ethanol-extracted dry weight of these worms is represented in Fig. 4-1. Rapid worm growth in cortisone-treated mice, untreated rats and cortisone-treated rats was in marked contrast to the stunted worm growth in untreated mice. Over the range of worm age investigated, cortisone treatment apparently had little effect on worm growth in rats. Over only a small range of worm age were worms from untreated mice comparable in weight to worms from cortisone-treated mice or from rats. As explained above, uptake of nutrients by H. diminuta is only comparable between worms of similar size. Fewer rats were autopsied than mice, so in this experiment it was only possible to compare uptake by worms from untreated mice and immunosuppressed mice.

The uptake of 2mM methionine from 10-minute incubations by worms of similar weights from untreated mice and cortisone-treated mice over a range of worm weights is given in Fig. 4-2. Each column represents a mean value calculated from not less than five worms. The uptake of methionine by worms from untreated mice was consistently lower than by worms of comparable weight from cortisone-treated mice over the entire range of worm weights. Taking the

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Figure 4-1

The growth of Hymenolepis diminuta in six-cysticercoid infections in untreated (●) and cortisone-treated (○) male CD-1 mice, and in untreated (■) and cortisone-treated (□) male CFHB rats. Each point represents the mean (± S.D.) ethanol-extracted dry weight determined from at least ten individual worms.

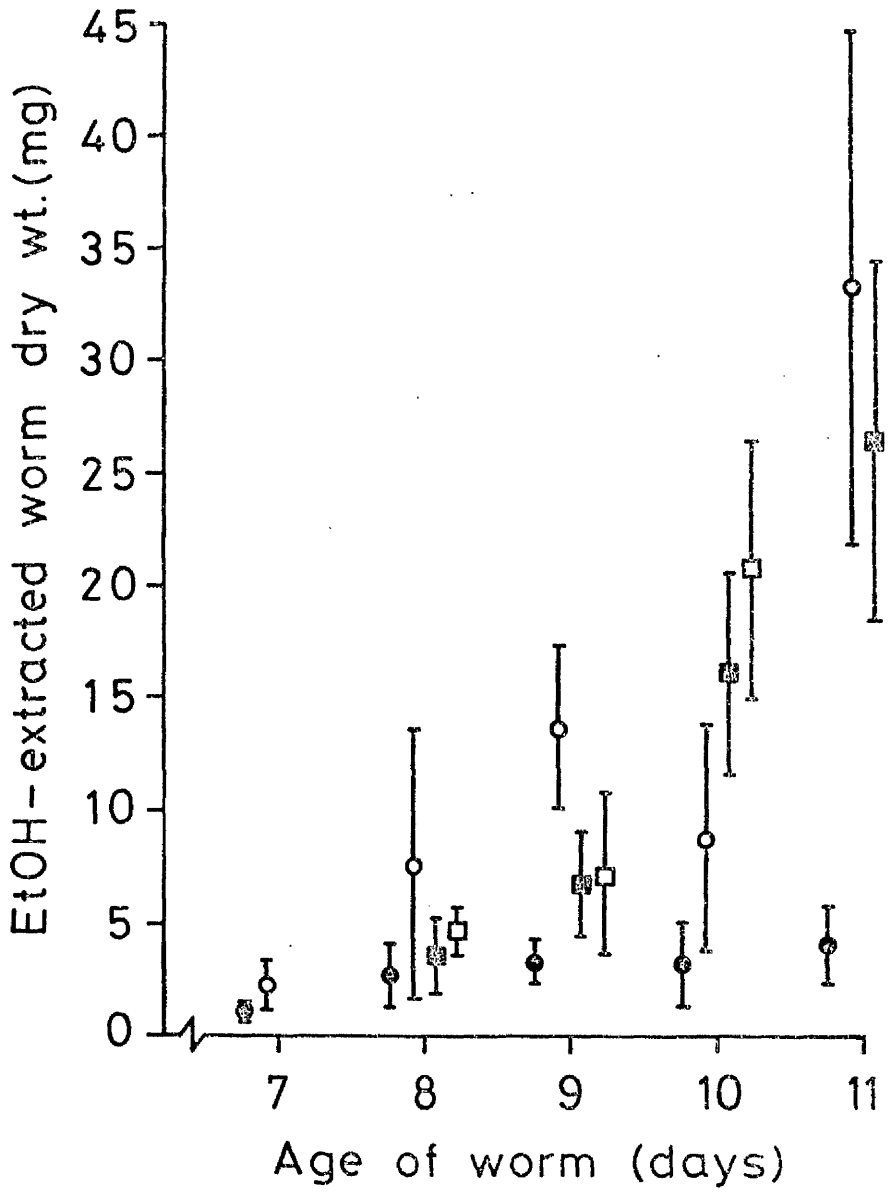
EtOH-extracted worm dry wt.(mg)
45
40
35
30
25
20
15
10
5
0
7
8
9
10
11
Age of worm (days)

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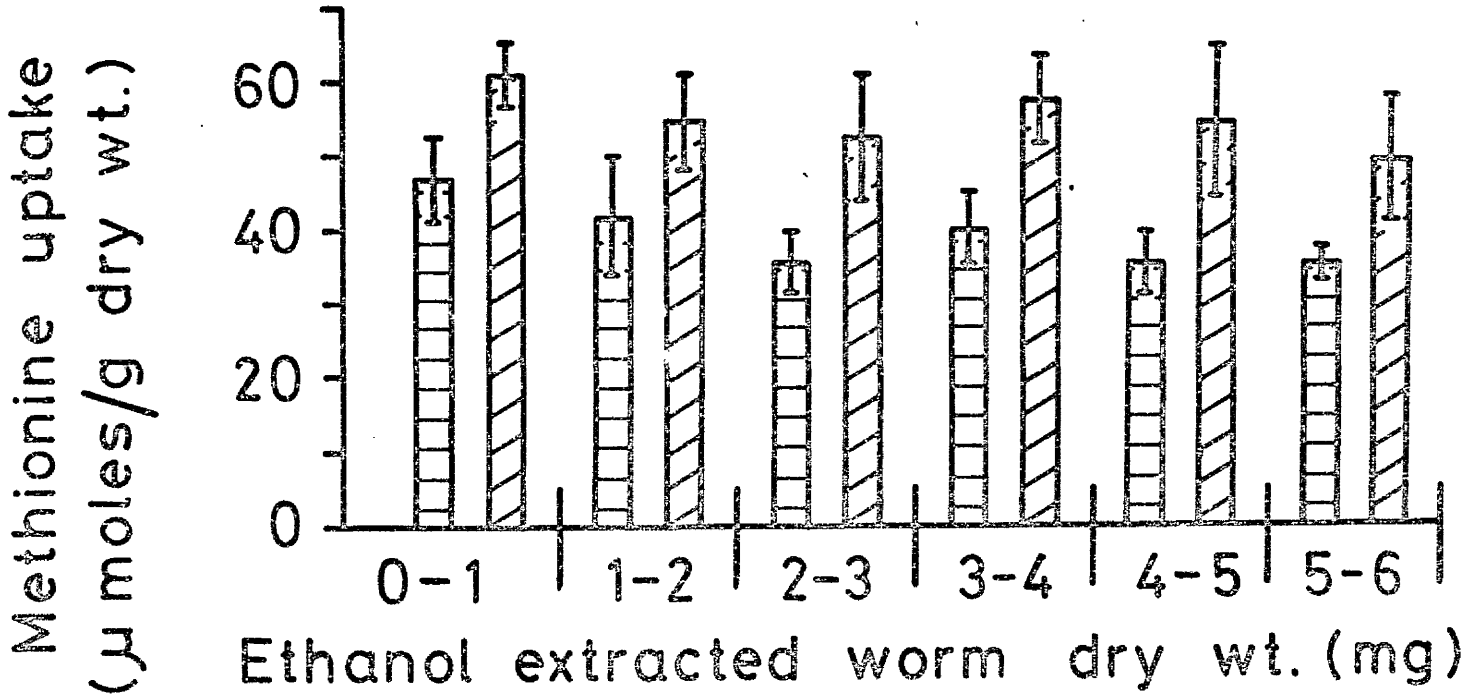
Figure 4-2

Uptake of ^{14}C-methionine as a function of ethanol-extracted worm dry weight from untreated male CD-1 mice () and from cortisone-treated male CD-1 mice (), ± S.D. Each column represents the mean of at least five determinations.


Methionine uptake
(μ moles/g dry wt.)
60
40
20
0
0-1
1-2
2-3
3-4
4-5
5-6
Ethanol extracted worm dry wt. (mg)

mean, uptake by worms from untreated mice was 71% of uptake by worms from immunosuppressed mice.

2. Sodium acetate.

In this experiment, only untreated mice and rats were used. An attempt was made, by autopsy of mice and rats over different ranges of worm age, to provide worms of equivalent weight from both host species. Thus, mice were autopsied from day 8 to day 11 post-infection, whereas rats were autopsied from day 6 to day 9. Percentage worm recoveries (Table 4-2) show that the response by mice caused abrupt worm expulsion so that only small numbers of worms were recovered on days 10 and 11. There was no significant reduction in worm numbers recovered from rats over the period of autopsy.

It can be seen (Fig. 4-3) that this type of manipulation provided considerable overlap between worm weights from mice and rats, so that the uptake by worms from both host species could be compared over almost the entire range of worm weights obtained.

Fig. 4-4 represents the uptake of 0.1mM sodium acetate from 2-minute incubations by worms from mice compared with uptake by worms of equivalent dry weight from rats. Two features may be observed. Firstly, the uptake per gram of sodium acetate by worms from both host species gradually decreased as worm weight increased. This indicates either the surface area-dependency of membrane transport (as worm weight increases, so the ratio of worm surface area to worm weight decreases and uptake per gram of worm tissue decreases proportionately), and/or the possibility that transport is greater over the more metabolically active neck region and that this effect

Table 4-2

Recovery (%) of Hymenolepis diminuta from six-cysticercoid infections of untreated 6 week old CFLP male mice and of untreated 8-9 week old CFHB male rats. ND, not done.

Age of worms (days)	Mice	Rats
6	ND	100
7	ND	92
8	87	88
9	83	100
10	21	ND
11	22	ND

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Figure 4-3

The growth of Hymenolepis diminuta in six-cysticercoid infections in male CFLP mice (●) and male CFHB rats (▩). Each point represents the mean (± S.D.) ethanol-extracted dry weight determined from at least five individual worms.

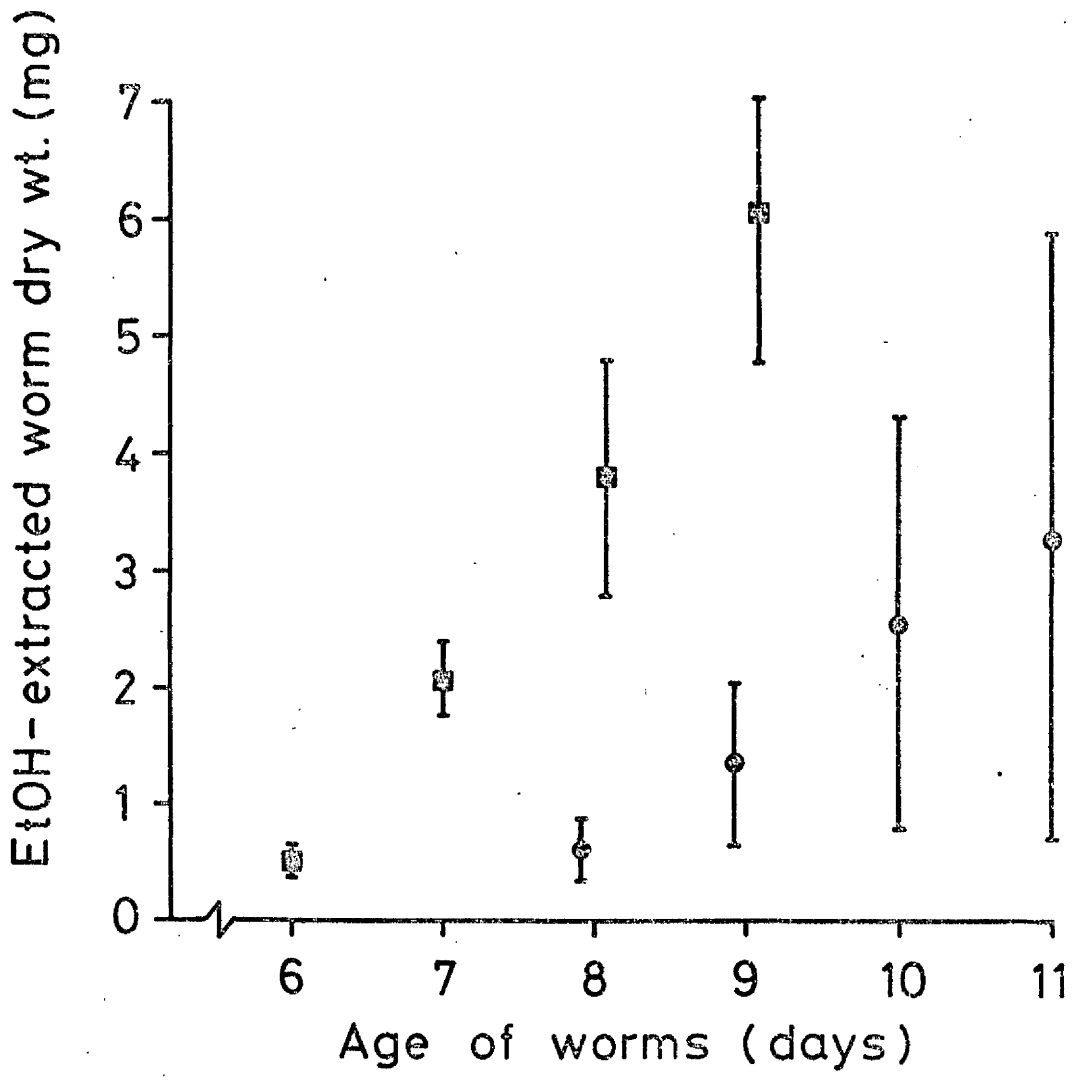
EtOH-extracted worm dry wt. (mg)
0
1
2
3
4
5
6
7
6
7
8
9
10
11
Age of worms (days)

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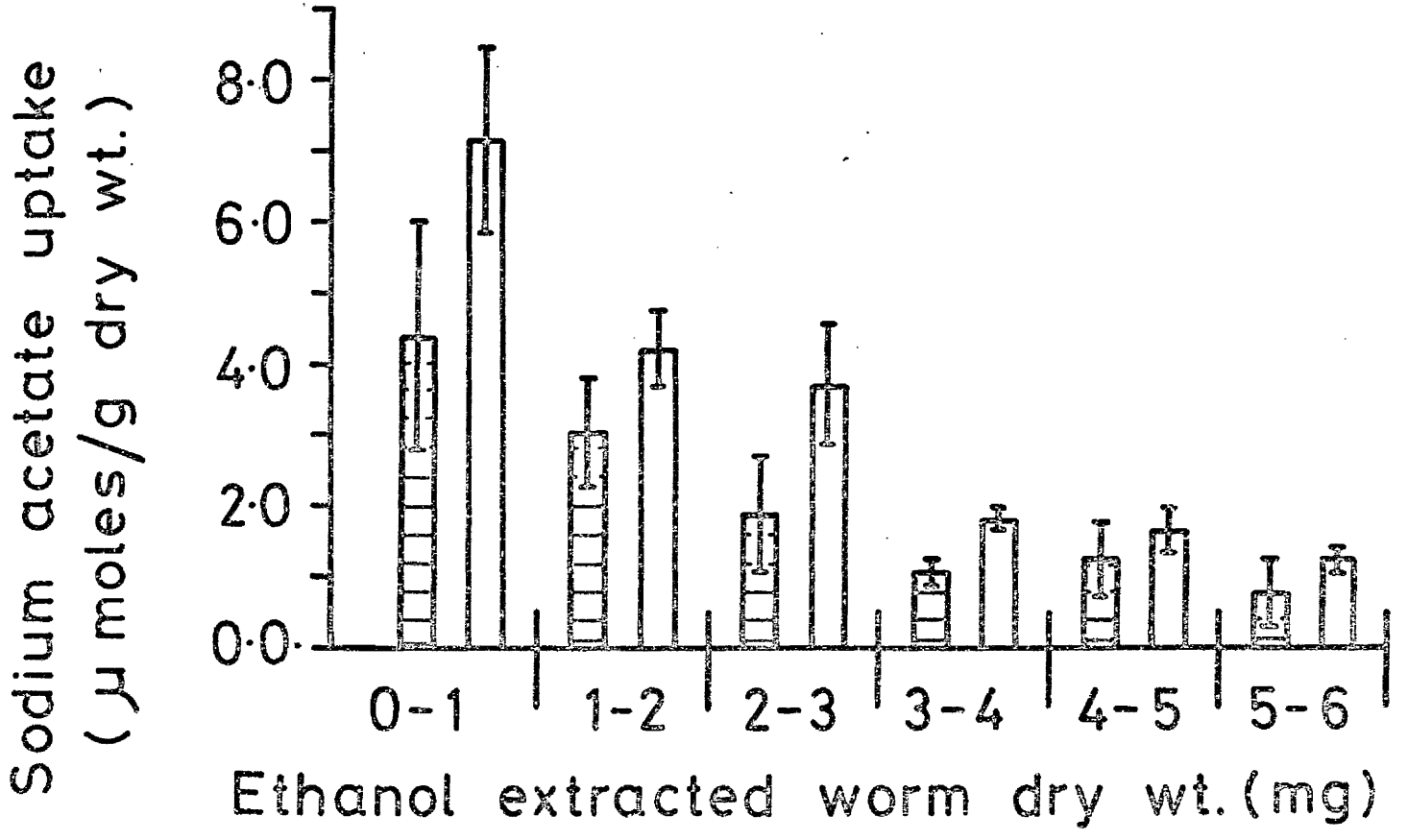
Figure 4-4

Uptake of ^{14}C-sodium acetate as a function of ethanol-extracted worm dry weight from male CFLP mice () and from male CFHB rats (), ± S.D. Each column represents the mean of at least five determinations.


Sodium acetate uptake
(μ moles/g dry wt.)
8·0
6·0
4·0
2·0
0·0
0-1
1-2
2-3
3-4
4-5
5-6
Ethanol extracted worm dry wt.(mg)

becomes diluted as the worm grows. Secondly, the uptake of sodium acetate by H. diminuta from mice was consistently depressed when compared with uptake by worms from rats. Uptake by "mouse worms" was 63% (mean value) of uptake by "rat worms" of equivalent weight.

3. Glucose.

Cortisone-treated mice and rats were included in this experiment in addition to untreated mice and rats. As in the previous experiment, the hosts were autopsied over different time periods post-infection to enable recovery of worms of comparable weights. Worm recoveries are given in Table 4-3. The low recovery on day 7 from untreated mice and on day 5 from cortisone-treated rats indicates the small size of the worms at these stages and the consequent difficulty experienced in their recovery. High worm establishment is confirmed by the 100% recoveries from these hosts on succeeding days and from the other groups on the first day of autopsy. Recovery from untreated mice dropped dramatically on day 11, demonstrating that a strong response developed in these mice. No significant reduction in recovery was experienced in any of the other groups.

Fig. 4-5 shows that in most cases worms recovered from a particular host were comparable in weight to worms recovered on the same or different days post-infection from other hosts. Once again, no significant difference was observed between the weights of worms from untreated rats or from cortisone-treated rats, indicating that at this stage of the infection there is no immunologically-mediated suppression of worm growth in rats infected with six cysticercoids.

Table 4-3

Recovery (%) of Hymenolepis diminuta from six-cysticercoid infections of untreated and cortisone-treated 6 week old CFLP male mice and of untreated and cortisone-treated 8-9 week old CFHB male rats.

ND, not done.

Age of worms (days)	Untreated mice	Cortisone-treated mice	Untreated rats	Cortisone-treated rats
5	ND	ND	ND	67
6	ND	100	100	100
7	67	100	100	100
8	100	100	100	100
9	89	83	100	ND
10	67	ND	ND	ND
11	29	ND	ND	ND
12	24	ND	ND	ND

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Figure 4-5

The growth of Hymenolepis diminuta in six-cysticercoid infections in untreated (●) and cortisone-treated (○) male CFLP mice, and in untreated (▣) and cortisone-treated (□) male CFHB rats. Each point represents the mean (± S.D.) ethanol-extracted dry weight determined from at least ten individual worms

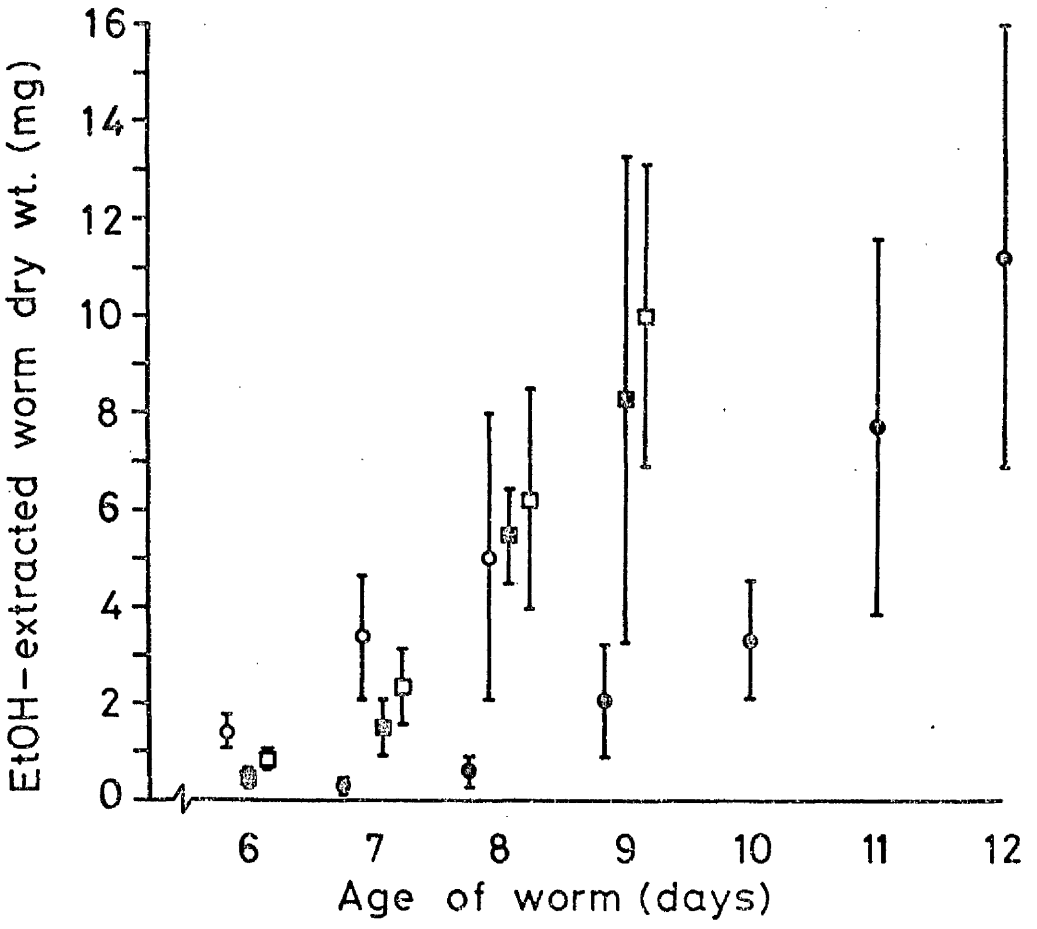
EtOH-extracted worm dry wt. (mg)
16
14
12
10
8
6
4
2
0
6
7
8
9
10
11
12
Age of worm (days)

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Figure 4-6.

The uptake of ^{14}C-glucose as a function of ethanol-extracted worm dry weight from untreated () and cortisone-treated () male CFLP mice and from untreated () and cortisone-treated () male CFHB rats, ± S.D. Each column represents the mean of at least five determinations.

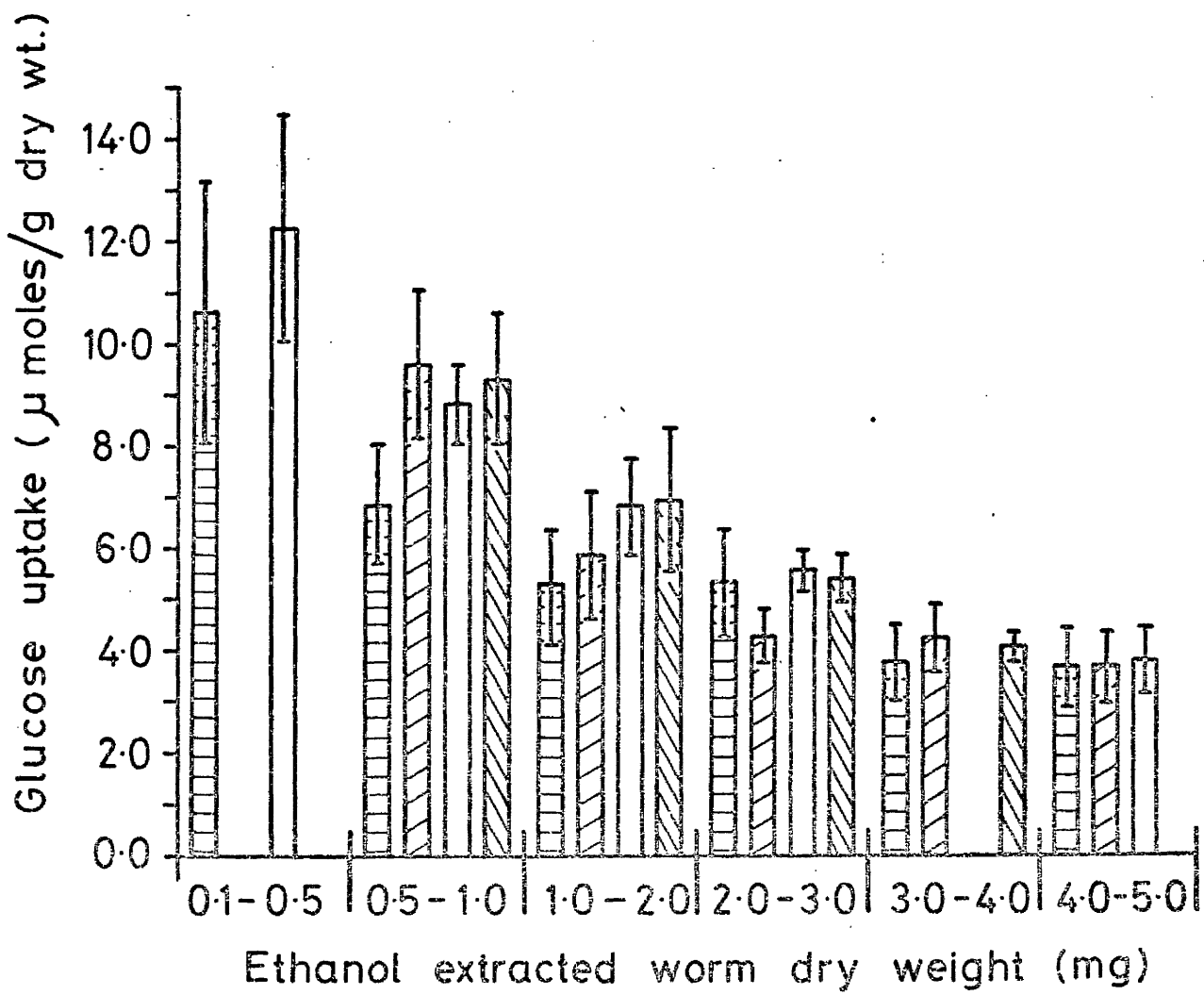
Glucose uptake (μ moles/g dry wt.)
14·0
12·0
10·0
8·0
6·0
4·0
2·0
0·0
0·1-0·5
0·5-1·0
1·0-2·0
2·0-3·0
3·0-4·0
4·0-5·0
Ethanol extracted worm dry weight (mg)

Fig. 4-6 demonstrates that uptake per gram of worm of 0.1mM glucose from 2-minute incubations by untreated or cortisone-treated mice and rats gradually decreased as worm weight increased. There was no significant difference, however, between uptake by equivalent worms from the different hosts.

DISCUSSION

The results indicate that transport of methionine and acetate by *H. diminuta* from mice was depressed by the immune response of the mouse to the worm. Transport of glucose, on the other hand, remained unaffected. The extent to which methionine and acetate transport were lowered did not depend upon the stage reached in the immune response, but remained stable as the response progressed.

Befus (1975a) showed that anti-*H. diminuta* antibody on the surface of the worm increased in intensity as the infection progressed. If the depression of membrane transport is mediated through the binding of antibody to specific elements of the glycocalyx concerned with transport, then it is difficult to explain why the reduction of transport does not increase as the production of antibody increases. Befus also described antibody coats on "rat worms" and on worms from cortisone-treated mice, similar to those found on "mouse worms". It is unlikely, therefore, that interference with transport of nutrients by "mouse worms" can be explained in terms of antibody binding to transport loci. Blocking of certain, specific transport loci, however, does occur but the blocking must be carried out by some immune mediator other than (although possibly in conjunction with) antibody.

The blocking mechanism is specific - only the well-defined carrier systems transporting methionine and acetate were affected. The separate system transporting monosaccharides remained unaffected by immunity. Methionine transport was depressed, but glucose transport was not, and yet both substrates are transported via energy-dependent active transport systems. On the other hand, acetate

transport is equally depressed even though it takes place via an energy-independent facilitated diffusion pathway. The immune mechanism, therefore, is not acting against energy metabolism of the worm or, non-specifically, against some energy-requiring process, producing a drain on energy and in turn affecting transport.

Interpretation of the results is complicated by the use of different substrate molarities (2mM methionine, 0.1mM sodium acetate, 0.1mM glucose). This may have a bearing on the kinetics of transport of the different substrates because the diffusion component of active transport systems increases with increasing substrate molarity. Thus, the diffusion component of glucose uptake at 0.1mM will be negligible compared to the diffusion component of methionine uptake at 2mM.

Interference with specific membrane transport systems in H. diminuta has been demonstrated after binding of the plant lectin, Concanavalin A (Con A), to the tegumental glycocalyx (McCracken & Lumsden, 1975b). Binding of Con A reduced subsequent glucose uptake by one-third. Transport of galactose, lysine, uridine and uracil were also inhibited by different amounts. Uptake of glycerol and methionine remained unaffected. Thus, adsorption of Con A did not exert a general transport barrier but affected only specific transport loci. The transport constant (Kt) of glucose uptake was not affected, but the maximum uptake velocity (Vmax) was reduced. The authors postulate that this indicates an effect on carrier mobility rather than on carrier-solute affinity.

In the present study, comparisons were made throughout between transport per unit tissue weight of worm in "mouse worms" and in worms from other host sources. Such treatment assumes that

worms of equivalent weight, but of different age (because they were grown in different hosts allowing different worm growth rates), are comparable, i.e., that Vmax is a function of the number of transport loci per unit tissue weight and that as this number changes as the worm ages, then Vmax will change. Kt should remain unchanged as the worm ages. Read et al. (1963) present evidence which, they claim, shows that the decreasing uptake per unit weight of worm (from rats) with increasing age of worm is not simply due to a decrease in the number of qualitatively identical uptake loci per unit of tissue weight. However, the figures presented by these workers for a range of worm age are extremely variable and their evidence is, at best, equivocal. Measurement of kinetic parameters, however, must be made in future studies of transport in worms progressively damaged by immunity.

Changes in the metabolism of other helminth species due to the action of the host immune response have been reported. For example, Henney, MacLean & Mulligan (1973) showed that uptake of ^{75}Se-labelled methionine and ^{32}P-labelled sodium dihydrogen orthophosphate by N. brasiliensis showed marked reduction by day 8 post-infection. This is presumably linked to the damage which has been shown to take place in gut cells of this nematode (Lee, 1969; Ogilvie & Hockley, 1968) and which is known to be due to antibody (Jones & Ogilvie, 1971). Changes in the iso-enzyme patterns of this worm due to the immune response of the rat host have also been shown to take place (Edwards, Burt & Ogilvie, 1971).

In conclusion, the present study provides evidence that an unidentified mediator of the mouse's immune response acts to specifically depress the transport of methionine and acetate

across the tegument of H. diminuta. Further studies are required to determine the nature of the interaction. Studies with specific inhibitors of the affected transport loci (see McCracken & Lumsden, 1975a, b) may show whether blocking of carrier recognition or of carrier mobility are involved. Continued study of the interaction of immunity with transport mechanisms of H. diminuta should include parallel investigations into possible effects on the many other important functions of the worm tegument outlined in the INTRODUCTION to this chapter.

SUMMARY

1. The structure and function of the tegument of H. diminuta are briefly discussed with respect to possible sites of attack by host immunity.

2. Transport of ^{14}C-labelled L-methionine and sodium acetate was shown to be less in H. diminuta from mice than in H. diminuta of equivalent weight from immunosuppressed mice or rats respectively. Uptake of D-glucose by H. diminuta was independent of host type.

3. The results are interpreted as indicating the specific depression of methionine and sodium acetate transport by an immune mediator in mice acting on tegumental transport loci of H. diminuta.

4. It is suggested that future studies investigate further the kinetics of membrane transport inhibition demonstrated in this study and that observation of possible immune damage to other tegumental functions be included.

GENERAL DISCUSSION

Before the investigations described above were begun it had been demonstrated that Hymenolepis diminuta is rejected from the mouse by an immunological mechanism and the characteristics of this rejection had been described for certain strains of mouse (Hopkins et al., 1972a, b). However, except for the demonstration of suppression of the response by administration of ALS (Hopkins et al., 1972b), suggesting a degree of thymus-dependency, nothing was known of specific immunological components involved in the response or of the effects exerted on the worm by these mechanisms. On the other hand, from work carried out in this laboratory and elsewhere, a picture was emerging of the mechanisms involved in the immune expulsion of certain nematode species from their hosts. It seemed natural, in order to provide a basis for studying immunological aspects of the mouse-H. diminuta relationship, to adopt certain methods and approaches used by workers investigating the nematode systems for use with the tapeworm model. In retrospect, it appears naive to assume that similar host responses are elicited by different species of helminth parasite merely because they inhabit similar environments and, indeed, over the period of investigation important differences emerged between host responses in the two types of model. Moreover, it became evident that, notwithstanding the difficulties involved in working with immunity to adult cestodes, the mouse-H. diminuta system offers great potential, not only in the study of immunity to helminths, but also in the study of many aspects of intestinal immunity.

The difficulties mentioned above largely stem from the inherent variability of the mouse-H. diminuta relationship. This variability in worm growth rate and timing of worm rejection can, however, be

minimised in several ways. Variation in worm growth and rejection is most pronounced in single worm infections, as may be seen from the results of early experiments described above. This prompted the use of five- or six-worm infections in all following experiments as rejection had been shown to be more abrupt and less variable in multiple-worm infections (Befus, 1975b). This was shown to reduce variation, but it may be advisable to use even higher levels of infection (eg., 10 worms) in future investigations to further reduce variability. The abrupt rejection occurring at high levels of infection would ease interpretation of experiments in which time of worm rejection is used as the parameter measuring the response. However, crowding effects will occur as available nutrient becomes limiting at these high levels of infection and this will necessitate care in interpretation of experiments in which worm growth is to be measured.

The age of mice at infection has been shown to be an important factor in determining immunological responsiveness. Befus & Featherston (1974) showed that mice aged 2-4 weeks at infection rejected H. diminuta more slowly than mice aged 5-7 weeks at infection. An attempt was made throughout the present study to infect mice with H. diminuta at 6 weeks of age. It may be that certain individual mice within a group reach immunological maturity at a later age than this. Raising the age at infection to about 8 weeks may, therefore, help to reduce intra-group variation. Against this suggestion must be weighed possible effects on mouse quality produced by this delay. Commercial animal suppliers will rarely supply mice over 6 weeks of age and it is unlikely that high grade SPF mice would remain SPF if they were held in a conventional environment for several weeks before infection.

The effect of mouse strain on variability of the system must also be taken into account. Wakelin (1975b) demonstrated that random-bred mice showed greater variation in responsiveness (to Trichuris muris) than inbred strains. The variability of response to H. diminuta in the random-bred CFLP strain was demonstrated in early experiments in the present study. Wakelin (1975b) also showed that the timing of expulsion of T. muris differed markedly between different inbred strains, NIH strain mice being the most rapid responders. The NIH strain was used in the present study primarily because large numbers of high quality mice of this strain could be purchased at reasonable cost and because the kinetics of their response to H. diminuta had been resolved by other workers in this laboratory. Extensive tests have not been carried out to determine the responsiveness of other inbred strains of mouse to H. diminuta, but it has been shown (Bland, unpublished) that inbred CBA mice rejected a five-cysticercoid infection more rapidly than NIH mice of identical age and quality. Clearly, further experiments are required to identify the most suitable strain for use in the H. diminuta system.

Perhaps the most important hypothesis suggested by the results given in CHAPTER I is that the response to H. diminuta is locally mediated. This hypothesis complements the work of Befus (1975a) who demonstrated the presence of a local antibody response to H. diminuta and the apparent absence of circulating anti-H. diminuta antibodies.

The results of CHAPTER I, SECTION 1 emphasise that the effects of whole-body X-irradiation on local immune responses in the intestine are still, to a large extent, unknown. For example,

the fate of lymphoid cell function and antibody production in the parasitised intestine following irradiation must be established. Events occurring at these levels could then be correlated with the observations of worm recovery and growth reported above.

The results describing worm growth following X-irradiation at different times during the response indicate exciting possibilities for future work. Simple extension of the experiments described by following worm growth over the complete post-irradiation period would yield valuable information regarding recovery of the mechanism involved in the stunting of worm growth. This would help to define the relationship between recovery of this mechanism and the mechanism inducing worm expulsion.

As discussed in CHAPTER I, there are several possible explanations for the failure to reconstitute the irradiation-depleted response with the lymphoid cell populations used. The period following 8 days post-infection is obviously a critical point in the response with respect to radiation sensitivity. It is interesting that this coincides with the period when antibody can be demonstrated on the surface of H. diminuta (Befus, 1975a). If this antibody is marking the worm for subsequent attack by cellular mediators, and if it is removed by irradiation depleting the plasma cells of the lamina propria, then cells which are subsequently transferred will be rendered ineffectual. In this respect, it is vital that observation is made of local antibody responses to H. diminuta in the X-irradiated mouse. It is equally possible that defects were induced in the gut-associated lymphoid tissue (GALT) by irradiation producing, for example, a reduced capacity for ecotaxis of the transferred cells. Until more is

known of cell function in GALT following irradiation, the usefulness of further cell transfers into irradiated hosts to investigate the mechanism of the response to H. diminuta must be doubted.

In CHAPTER I, SECTION 2 it was shown that the response to H. diminuta requires the participation of fully-differentiated T cells. The role of these cells and their point of action in the response, however, requires clarification by selective reconstitution of T cell-deprived mice at varying times post-infection. It is unlikely that the outbred nude mice used in the present study could be made responsive in this way by transfer of allogeneic T cells. Although the adult thymectomised, irradiated, BMC-reconstituted mice showed unresponsiveness to H. diminuta, the lack of full recovery of the response in the sham-operated mice suggests an additional defect in this model. Adoptive reconstitution of the T cell-dependent arm of the response in these mice, therefore, does not seem likely. The use of nude mice backcrossed onto an inbred line to provide a model for syngeneic cell transfer would seem to offer the best approach.

The results of experiments involving infection of nude mice with different numbers of worms suggested the presence of an immunological threshold, possibly dependent on worm surface area rather than on weight. This in turn suggests that the tapeworm tegument, or some component of it, represents the worm antigen. The assumption is often made that, because the tegument is a metabolically active layer with a high rate of replacement, it is antigenic. Although this is highly likely, as antibody has been demonstrated by electron microscopy to be bound to the tegumental glycocalyx (Threadgold, personal communication), it requires

verification by direct immunological methods. Previous studies have used crude whole-worm homogenates as antigen (Goodall, 1973; Harris & Turton, 1973; Befus, 1975a) although it is theoretically possible to isolate the distal tegument (Oaks & Knowles, 1974) for use in antigen-antibody tests. Studies on antigenicity would aid comparison of results from single worm and multiple worm infections in which, frequently, assumptions are made regarding the nature of the antigenic stimulus.

In CHAPTER II it was demonstrated that inflammation of the intestine induced specifically in response to Trichinella spiralis had a marked adverse non-specific effect on a concurrent H. diminuta infection. The destrobilation and reduced growth rate of H. diminuta observed is, perhaps, not surprising as these appear to be mechanisms used by cestodes to permit survival in adverse environments offering reduced nutrient levels (Read, 1959) or an immune response (e.g., Gray, 1973; this study). The effect on H. diminuta, however, was differential, depending on the stage of growth which was reached before inflammation became intense. The specific nature of the interaction remains undefined. Further investigation must take several approaches. There is an obvious need for careful histopathological and biochemical monitoring of the changes occurring in the inflamed intestine. Future studies must include close observation of the host's dietary intake. This study also emphasises the need for an investigation into the cellular changes taking place in the mouse intestine during a normal response to H. diminuta.

The demonstration in CHAPTER III of the interference of the immune response in specific nutrient transport sites of H. diminuta

paves the way for more detailed investigation into the specific nature of the effect. Study of the kinetic parameters of the transport systems studied above may reveal the point at which the mechanism is disturbed. There are countless other possible physiological and structural sites for attack by immunological mediators and the mouse-*H. diminuta* system provides an unequalled model for the study of the effects of host immunity on intestinal helminths.

In conclusion, the work described in this thesis has demonstrated the advantages and drawbacks of applying methods used in other host-parasite systems to the investigation of immunity to adult cestodes. Above all, it has shown that there are a great many basic questions still to be answered about the mouse-*H. diminuta* system, that there are a number of approaches which may be used to answer these questions, and that observations made in any of the directions suggested in this discussion would add to current concepts of local immunity in the mammalian intestine.

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