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INTESTINAL IMMUNITY TO TAPEWORMS:

THE REJECTION OF *HYMENOLEPIS CITELLI*

BY MICE AND RATS

THESIS

for the

Degree of Doctor of Philosophy

by

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ABBREVIATIONS

α	alpha (chain)
B	Thymus independent; antibody producing
c	cysticercoid
CA	Cortisone acetate
C_3	Complement component three
Fig.	Figure
FITC	Fluorescein isothiocyanate
GAD	Goat anti-dog
GAM	Goat anti-mouse
GI	Gastrointestinal
HBSS	Modified Hanks' balanced salt solution
Hc-	*Hymenolepis citelli*
Hd	*Hymenolepis diminuta*
Hm	*Hymenolepis microstoma*
Ig	Immunoglobulin
J	Joining (chain)
K	Autopsy
n	Number in a group
ND	Not done
Nd	*Nematospiroides dubius*
N.S.	Not significant
P	Probability
Py	Pyrantel embonate
PBS	Phosphate buffered saline

PCA	Passive cutaneous anaphylaxis
p.i.	post infection
RAD	Rabbit anti-dog
RAG	Rabbit anti-goat
SAD	Sheep anti-dog
sIgA	Secretory IgA
T	Thymus dependent
TGE	Transmissible Gastroenteritis
TRTM	Treatment with cortisone acetate
VCU	Villus crypt unit
Z	'Zanil' (anthelmintic)
Zd7	'Zanil' day 7
Zd21	'Zanil' day 21
1°	Primary infection
2°	Secondary infection

SUMMARY

The work described in this thesis was undertaken to provide evidence that *Hymenolepis citelli* is rejected by an immunologically-mediated mechanism and that acquired immunity to homologous challenge infections is present in the absence of the primary infection.

The growth and survival of the parasite was characterised in CFLP mice: it was shown that over 80% of *H. citelli* worms became established and grew, thereafter survival depended on the intensity of the primary infection. Immunity to homologous challenge infections was unequivocally demonstrated in the absence of the primary worms in mice. Immunity was manifested mainly as stunting/destrobilation of secondary worms; the severity of stunting was related to the intensity and duration of the primary infections. The effectiveness of the protective response waned with time in the absence of continuing antigenic stimulation. Rejection was completely suppressed in cortisone-treated mice and furthermore, growth of worms was much enhanced.

The *in vivo* interactions between *H. citelli*, *H. diminuta* and *H. microstoma* were investigated: cross-protection exists between the species. An interaction between *Nematospiroides dubius* and *H. citelli* was also studied; the survival of *H. citelli* was enhanced, but its

growth depressed in concurrent primary infections with the nematode. Immunity against a homologous challenge infection with H. citelli was not ablated by a concurrent N. dubius infection.

The rat was also used as a model for studying immunity to H. citelli. Growth and survival of worms in primary infections of varying intensities were described in CFHB rats. Acquired immunity to challenge infections was demonstrated; the effectiveness of the protective response was related to the intensity of both the primary and secondary infections. Immunity diminished with time in the absence of the primary worms. Cross-protective responses between H. citelli and H. diminuta were also demonstrated.

The proliferation of IgA, IgM and IgG_1-positive immunocytes in the intestinal lamina propria of uninfected, primary and secondary infected mice was studied: there was no evidence for the involvement of plasma cells in the response to H. citelli and H. diminuta, although with H. microstoma infections there was some evidence for the involvement of IgA and IgM immunocytes. The occurrence of immunoglobulins on worm surfaces was also investigated.

The results presented in the thesis are discussed in relation to current concepts of immunity to tapeworms. Future lines of research are suggested.

GENERAL INTRODUCTION

Immunoparasitology is in a dynamic state and there is a constant interchange of ideas among biologists working in "basic" immunology and those engaged in "applied" immunological research. Over the last decade, a wealth of information from research in immunology has resulted in an entirely new view of the immune system which is having an impact upon parasitology (see commentary by Waksman, 1979). Studies on the understanding of the role of the immune system in the evolution of host-parasite relationships are proliferating (Smyth, 1969a Mims, 1977, Dineen, 1978).

Parasites and hosts are in a state of evolution (Smyth, 1966) and one can therefore expect extreme complexities in the immunological aspects of parasitism. An equilibrium which favours the survival of large numbers of susceptible hosts with anti-parasitic immune responses, has considerable restraints on the residence, proliferation and invasion of parasites. Inevitably, the understanding of the immune response in the regulation of parasite populations will provide a "stepping-stone" in the eradication, but more pragmatically the control, of diseases of major economic and public health significance in the developing world (W.H.O. 1976; Wright, 1972).

Experimental host-parasite systems are continually being utilised in attempts to characterise the

immune responses elicited by parasitic organisms (reviewed Ogilvie and Jones, 1973, Wakelin, 1978a). Immune elicitation does not invariably lead to complete protection of the host; although many host-parasite systems exhibit "spontaneous-cure" (Wakelin, loc. cit.). Extrapolation from studies in "abnormal" host-parasite laboratory models may or may not be useful in predicting disease outcome, the assessment is nevertheless relevant, although generalisations should be avoided. Mitchell (1979) suggests that such studies could highlight "tools" in the mechanisms involved in the pathogenesis of diseases. Attempts at the analyses of the mechanisms involved in immune expulsion indicate that two or more components, involving antibodies and thymus-dependent lymphocytes are required; acting sequentially to bring about expulsion of parasitic worms in adult animals (Ogilvie and Parrott, 1977, Cohen, 1976). Failure of young hosts to exhibit "spontaneous-cure" has been attributed to some deficiency in the lymphocyte-mediated components of expulsion (Dineen and Kelly, 1973, Love and Ogilvie, 1975, Befus, 1975b). This immunological immaturity in young animals poses probable stagnation in vaccination studies. Vaccines are potentially invaluable in controlling diseases e.g. Dictyocaulus viviparus (Jarret and Craig-Sharp, 1963). For a comprehensive review of prospects for the development of dead vaccines against helminths, the reader is referred to Clegg and Smith (1978) and Urquhart (1980). An essential requirement

in studies on the immune response is the identification of "functional" antigens (see Cox, 1978). Isolation of these immunogenic molecules would enhance vaccination studies. The production of an ideal vaccine provided it is relatively cheap, would no doubt be welcomed in the medical and veterinary services of poor countries, where it probably would help alleviate the financial and health problems in these mostly tropical regions plagued with a "hotch-potch" of endemic diseases. Whether there is an immediate need for vaccines in endemic areas of concurrent malarial and trypanosomiasis infections which exert profound generalised depressive effects upon immunological responses (Freeman et al., 1973, Greenwood et al., 1971 and Phillips et al., 1974) is equivocal. The author however believes that in diseases where chemoprophylaxis is possible, there is little immediate need for a vaccine.

Tapeworms are important to man and his domestic animals, although adult forms of these parasites are not very pathogenic (reviewed by Rees, 1967). Host-protective immune responses in parasitic infections eventually lead to parasites evolving evasive strategies which might include reduced antigenic disparity between parasite and hosts and the induction by the parasite of selective immunological unresponsiveness in the host. These mechanisms aid parasites to escape the immunological effector molecules of the hosts's responses (Ogilvie and

and Wilson, 1976). The complexities of evasive strategies are diverse and no doubt tapeworms may employ some of these mechanisms.

Tolerance by the mammalian hosts and lack of pathogenicity by the adult tapeworm (Rees, 1967) has been interpreted as a probable reason for the long association of cestodes with vertebrates (Smyth, 1969b). Host-specificity in cestode parasitism exhibits a dichotomy in being physiological (Read, 1959, Roberts, 1966) and immunological (Hopkins _et al_., 1972a and b; Befus, 1975a and b; Andreassen _et al_., 1978a and Chappell and Pike, 1976a and b). Ecological factors are no less important between hosts and parasites. Differences between normal and "abnormal" hosts in physico-chemical characteristics e.g. pH, bile acids and morphological factors (Smyth, 1969b) also influence the development of adult cestodes in different animals. Size differences of adult _H. diminuta_ and _H. citelli_ are probably in a general way related to the size of the host species (interspecifically) in which they developed (Voge, 1956, Read and Voge, 1954).

There is now ample evidence that demonstrates unequivocally, the immunogenicity of adult tapeworms (reviewed Williams, 1979) and features of the underlying mechanisms are gradually being elucidated. Comprehensive reviews refuting earlier suggestions that unless there is considerable mucosal damage to the host,

adult tapeworms are not immunogenic (Heyneman, 1962 and 1963; Read, 1955) have been published in the last two decades (Weinmann, 1966 and 1970; Gemell and Johnstone, 1977; Williams, 1979 and Hopkins, 1980). There is abundant evidence that intestinal mucosal damage is unnecessary for macromolecular uptake (Hemmings, 1978). The intestinal mucosal surface provides an efficient barrier to ingested antigens, although immunoreactive proteins have been shown to be absorbed by mammals (Hemmings, 1978) and that the elicitation and induction of different immune responses is a reflection of the nature, amounts and duration of the immunogenic stimulus. The location and mechanism of uptake is still equivocal, although evidence suggests that macromolecular uptake occurs over the general epithelium (Walker and Isselbacher, 1972). The amount of uptake following oral presentation of immunogens is uncertain, but in the range of 0.01% to 0.2% (Ciba symposium 1977 p. 356). Hemmings (1978) mentions about 2% of bovine serum albumin uptake by adult rats.

At this juncture a short review of the *H. diminuta* and *H. microstoma* host-parasite relationships, in particular acquired immunity to these parasites is necessary as throughout this thesis analogies between these systems and that of *H. citelli* will be continually referred to. Indeed the similarities between *H. diminuta* (Rudolphi, 1819) and *H. citelli* (McLeod, 1933) adults have

been noted by various workers. Rausch and Tiner (1948) even suggested that they are synomymous, although Voge (1956) after studies on specimens of H. citelli from Citellus beecheyi (ground squirrel) and H. diminuta from Norway rats decided that they remain as separate entities.

Studies on immunity to H. diminuta were initiated by the late Asa C. Chandler, who in 1939 characterised the growth and establishment of secondary infections in rats. Read and Voge (1954) described infections with H. diminuta in "albino mice". Weinmann (1966) observed 81% establishment in mice treated with morphine and these results were subsequently confirmed by Turton (1968 and 1971) who in addition described destrobilation and concluded that "further research is required to elucidate possible causes". Hopkins et al. (1972a) confirmed Turton's result and characterised the kinetics of worm growth and expulsion in Porton and CFLP mice and more importantly proposed that destrobilation and loss of H. diminuta from mice is immunologically-mediated. Hopkins et al. (1972b) conclusively demonstrated the immunological basis of the phenomenon using the immunosuppressants cortisone acetate, sodium methotrexate and horse anti-mouse lymphocyte serum. Since then various workers have presented evidence for the involvement of an immune response to H. diminuta (Goodall, 1973; Befus, 1975b, Andreassen et al., 1978a and

Christie, 1979). Involvement of a humoral response has been shown by Coleman et al. (1968), Harris and Turton (1973), Befus (1975b) and Choromanski (1978). Attempts at transferring resistance against H. diminuta in mice, even with hyperimmune serum, have met with little success (Hopkins, 1980; Isaak, 1976 and Andreassen et al., 1978a). The thymus-dependency of the response to H. diminuta has also been shown (Bland, 1976a; Andreassen et al., 1978a; Hopkins et al., 1972b and Christie, 1979). Acquired immunity to H. microstoma has also been demonstrated in mice (Tan and Jones, 1968 and Howard, 1976a). Detectable (but low) levels of specific serum antibodies to this parasite in mice have been observed by Moss (1971) and Goodall (1973). Adult cestodes in hosts which do not reject them are nevertheless immunogenic. H. diminuta has been shown to induce a humoral response in the rat (Harris and Turton, 1973) although so well adapted that the parasite supersedes the life span of the host in low level infections (at least 14 years) when sequentially transplanted from rat to rat (Read, 1967). Studies on acquired immunity in the rat promises to be rewarding and already work on this model has begun (Andreassen et al., 1974 and 1978b, Chappell and Pike, 1976b, Andreassen and Hopkins, 1980).

The objectives of the work described in this thesis was to determine the course of development (growth and survival) of H. citelli in mice and rats, and to

highlight areas of similarities/dissimilarities with the *H. diminuta* and *H. microstoma* models already investigated at the Wellcome Laboratories.

The thesis is divided into 4 chapters:-

Chapter 1 - consists of an Introduction to the system and a) establishes representative patterns of growth and survival in primary infections of various intensities, b) characterises worm growth and survival in homologous challenge infections following primary infections of various regimes and duration and c) employs a chemical immunosuppressant as a tool to study the effects of immunosuppression on primary and secondary infections.

Chapter 2 - described the *in vivo* interactions of the *H. citelli* system with two other generically related tapeworms i.e. *H. diminuta* and *H. microstoma*, and with a nematode *Nematospiroides dubius* in mice.

Chapter 3 is a description of the immunogenicity of *H. citelli* in rats with particular reference to acquired immunity to homologous challenge. An interaction study with *H. diminuta* is also described.

Chapter 4 is a study of the effect of primary and secondary infections on immunoglobulin-positive cell numbers (plasma cells) in the small intestine of mice infected with *H. citelli*, *H. diminuta* and *H. microstoma*.

Specific objectives are stated in more detail in each section, and the thesis concludes with a general discussion with suggestions for future work.

GENERAL MATERIALS AND METHODS

General materials and methods employed are described here. Sectional materials and methods will be referred to in the appropriate sections.

1. Animals

a) Mice and rats

Male and female mice were used (mostly males). The majority of experimental mice and rats were purchased from commercial suppliers, however, some experiments utilised "home-bred" (at Wellcome Laboratories) rats. Purchase of animals from different suppliers was necessary as it was difficult to obtain animals over a long period of time from any one supplier. The majority of animals were purchased from Anglia Laboratories (Huntingdon) and where a different supplier was used will be indicated in the appropriate section. Mice were of the highest grade available, usually categorised 3/4 star (see Register of Accredited Breeders and Recognised Suppliers September 1978, Medical Research Council, Laboratory Animal Centre). Most of the mice used were 4 star outbred CFLP strain, free of all intestinal protozoa and helminth infections, although on some occasions 3 star mice with *Aspiculuris tetraptera* or *Syphacia obvelata* were utilised. These mice were treated with Piperazine (see anthelmintic section) for a couple of days before commencement of experiments.

The age, sex and source of mice and rats will be given at the beginning of each section of the experiments. Mice were caged in groups of five in polypropylene cages 45 x 15 x 13 cm. (North Kent Plastic Cages Ltd.). Wood shavings or sawdust were used as bedding and changed twice weekly. The animal rooms were maintained at 20-22°C and lighting maintained automatically on a 12 hour cycle in winter, but followed day length in summer. Rats were caged usually in groups of eight in propylene cages 56 x 38 x 18 cm. (North Kent Plastic Cages Ltd.) and when sawdust was used as bedding, this was changed twice weekly. Mice and rats were provided *ad libitum* with tap-water, (normally without additives) and diet (Standard Rat and Mouse Breeding Diet, Grain Harvesters Ltd.).

b) Parasites

The strains of *Hymenolepis spp.* used were obtained as follows:-

H. citelli - originated from Rice University, Houston, Texas and was obtained in 1970 from Dr. Austin McInnis, University of California, Los Angeles. Since 1970 it has been serially maintained in hamsters. *H. diminuta* and *H. microstoma* were obtained from Rice University in 1963 and 1964 respectively and maintained at Wellcome Laboratories by repeated passage through rats and mice respectively. The intermediate host used in maintenance of all three parasites was the flour beetle (*Tribolium*

confusum) which were kept in stock jars containing a piece of tissue paper, in unlit incubators at 25°-28°C and fed wholewheat flour.

Infection of beetles - Beetles were infected with the appropriate tapeworm eggs after recovering adult worms from the intestines of hamsters, rats or mice. Gravid proglottids (maturity assessed by appearance of eggs with fully formed oncospheres from punctured proglottids) were cut from the worm, blotted dry on absorbent filter paper to remove excess HBSS (see later) and fed to beetles that have been starved previously for 3-4 days. After 24 hours most of the proglottids containing the eggs had been eaten by the beetles, which were then fed wholemeal flour. This usually gave 15-20 cysticercoid (*H. diminuta*), 10-13 cysticercoid (*H. citelli*) and 20-30 cysticercoid (*H. microstoma*) infections in beetles. Although cysticercoids of all 3 species of cestodes are usually mature by 14-16 days after infecting beetles, cysticercoids used throughout this work were between 21-60 days old. Beetle larvae were removed monthly from infected stock to prevent dilution of the infected population.

2. Procedure for infection of mice and rats

Oral infection - Cysticercoids were usually dissected from infected beetles in HBSS with mounted needles and fine forceps. However, when large numbers of cysticercoids (>100) were required, beetles were disrupted

(Ridley and McInnes, 1968) by putting the appropriate number of beetles in HBSS in a blending jar and homogenising in an MSE blender (Measuring Scientific Equipment Ltd., Crawley, Sussex) at room temperature for 30 seconds at the 180° speed setting (uncalibrated). The homogenate was poured into a 10 cm. Petri dish, the contents of the dish were swirled continuously until the cysts gravitated towards the centre. Infectivity of cysticercoids following this method of collection was invariably between 95-100%. Cysticercoids were used within an hour to infect ether-anaesthetised mice or rats by stomach tube. The stomach tube apparatus consisted of a 2.5 ml syringe connected to a 30-40 cm. length of polythene tubing (Portex Ltd.) via a hypodermic needle of appropriate gauge. (O.D. of tubing for rats was 1.27 mm. and 1.00 mm. for mice). After sucking cysts into the lower part of the tubing, the tube was then inserted orally into the stomach of the animal. About 0.2 ml of HBSS was evacuated from the syringe and flushed into the stomach (together with the cysticercoids). The tube was then gently removed and the mouse/rat put in its cage. Day of infection is always regarded as day 0.

3. Anthelmintic

Mice found to be harbouring *A. tetraptera* or *S. obvelata* were treated with piperazine citrate (Citrazine, Loveridge Ltd.) in the drinking water at a

concentration of 3 g/litre. To chemically terminate cestode infections the anthelmintic oxyclozanide (Zanil, I.C.I. Ltd.) supplied as a 3.4% suspension was administered to mice at 250 mg/kg (Hopkins *et al.*, 1973) by stomach tube after determining the mean weight of mice in any group. 'Zanil' was diluted with distilled water accordingly so that each mouse received 0.5 ml.

4. Immunosuppression and Antibiotics

The immunosuppressive drug cortisone acetate (Cortistab, Boots Ltd.) available as 25 mg/ml suspension was used at a dosage of 1.0-1.25 mg per mouse. It was administered subcutaneously by injecting 0.04-0.05 ml. Dosage was commenced on day 0 (unless otherwise stated) and given every 48 hours. To prevent opportunistic bacterial infections, cortisone-treated animals and corresponding control groups were put on antibiotics. Oxytetracycline HCl (Terramycin - Pfizer Ltd.) was given at a concentration of 3 g/litre Terramycin in the drinking water. No effect on the survival and growth of *H. citelli* was observed in control groups on terramycin. Thong and Ferrante (1979) have, however, reported that oxytetracyclines are immunosuppressive *in vitro*; no specific experiments were performed in this work to confirm the above report.

5. Recovery of Worms

Mice or rats were killed by cervical dislocation, having been anaesthetised in ether. Worms were

recovered by removing the entire small intestine (from the pyloric end to the ileo-caecal junction) and flushing the contents into a crystallising dish with up to 50 ml HBSS, by the aid of a wide blunt cannula inserted into the anterior end. If worm recovery was less than 100% of the initial inoculum, the intestine was slit longitudinally, the intestine agitated under HBSS and examined under a dissecting binocular microscope (X6 and X12 magnification) using transmitted light. To find very small worms that might still be attached to the intestinal mucosa, the intestine was cut transversely into equal parts and these incubated separately in HBSS at 37°C in small Petri dishes and thereafter examined at hourly intervals for detached worms. Worms over 3-4 mm. long (i.e. over 0.1-0.2 mg) were collected. Worms over 1 cm. long were blotted dry on filter paper to remove adhering debris and excess HBSS, placed in aluminium foil cups and dried at 90°-100°C for a minimum of 24 hours. The worm dry weight (to the nearest 0.1 mg) was then recorded after being weighed on a Stanton Unimatic balance (Model C.L.1).

6. Statistical treatment of results

Differences in worm weights between groups (including worms $<$0.2 mg, unless otherwise stated) were compared using the Wilcoxon test for unpaired samples (Snedecor and Cochran, 1967). Values of P equal to or less than 0.05 were considered significant and are indicated on graphs showing weights.

7. Presentation of results

a) Worm recovery

Destrobilation is a feature of rejection and rejection is defined here as having two components i) destrobilation and ii) subsequent worm loss.

Destrobilation:

Destrobilated worms have been described as being 0.43-1.73 mm. long (Turton, 1971), 1-2 mm. long and weighing less than 0.1 mg dry weight (Hopkins *et al.*, 1972a). A worm $\leqslant$0.1 mg recovered after day 8, in a primary *H. diminuta* infection, is commonly taken as a destrobilated worm. It is not always possible to be certain whether a worm is destrobilated or just stunted especially in challenge infections. In *H. citelli* and *H. diminuta* infections, destrobilation normally occurs in the neck region and is an abrupt loss of the strobila with the posterior tip of the remaining worm having a "darkened area" (Befus and Threadgold, 1975). These worms are restrained from regrowing by the immune response of the mouse, although regrowth is possible if they are transplanted into naive hosts (Hopkins *et al.*, 1972a) or if the host is treated with cortisone acetate. The recovery of destrobilated/stunted worms is very tedious, difficult and unreliable and the time of expulsion is very variable. Destrobilation observed during the course of this study does not invariably precede worm expulsion as "intact"

worms have been recovered from the caecum of mice. Throughout this thesis a destrobilated worm will be defined as a worm measuring between 0.5-4 mm. in length, estimated to weigh less than 0.2 mg dry weight and more importantly and critically recovered only after day 14 post infection in naive (previously uninfected) mice. Graphically, the percentage recovery of strobilate worms is plotted against the age of the infection.

b) Worm growth

Worm survival and growth are variable (see discussion in Chapter 1) and affected by host immunity (Hopkins *et al.*, 1972a and b, Befus, 1975b, Howard, 1976b and Christie, 1979). Biomass a parameter that reflects both worm numbers and size (Hopkins, 1980) is used to express the dynamics of infection. Total biomass per group is the weight of all the worm material recovered from a group of mice or rats. It is unsatisfactory, however, as it gives no indication of the intragroup variation. Mean worm weight (i.e. total biomass divided by the number of worms $>$0.2 mg recovered) is also an unsatisfactory parameter because it often gives a mean value well-distant from many of the actual results obtained, and takes no account of the contribution of the destrobes in calculating the mean. Many results in the thesis are plotted as total dry weight of worm tissue per mouse or rat. Differences in intragroup variations are thus easily discernible. Throughout the thesis worm weight will be that of worms greater than 0.2 mg dry weight, unless otherwise stated. Where destrobilated worms are recovered, these will be indicated (i.e. their numbers) in the "total

recovery" of worms i.e. all worms including destrobilated worms recovered from a group of mice or rats on a particular day. Symbols below the horizontal scale in graphs showing growth represent mice from which no weighable worms were recovered, but from which destrobilated or stunted worms were recovered.

8. Hanks' Balanced Salt Solution (HBSS)

Modified Hanks' balanced salt solution has been described by Hopkins and Stallard (1974). It was modified by excluding glucose and $NaHCO_3$ and increasing the remaining salts pro-rata to an osmotic pressure of 300 m-osmole.

Solution 1	NaCl	168 g
	KCl	8 g
	KH_2PO_4	2 g
	Na_2HPO_4	4 g
	0.2% phenol red	200 ml
	made up to 2 litres with deionised water	
Solution 11	$CaCl_2.2H_2O$	3.92 g
	$MgCl_2.6H_2O$	2.00 g
	made up to 2 litres with deionised water	

105 ml each of solutions 1 and 11 were mixed and made up to 1 litre with deionised water, giving a final pH of 7.2.

CHAPTER 1

SECTION 1

Introduction to the Hymenolepis citelli system

Hymenolepis citelli was first described by McLeod (1933) from three species of *Citellus*. Since then Voge (1956) has described naturally occurring infections in deer-mice (*Peromyscus maniculatus*), ground squirrels (*Citellus beecheyi* and *Citellus leucurus*) and pocket gophers (*Thomomys unbrinus* and *T. talpoides*). Grundmann and Frandsen (1960) have recorded *H. citelli* in two species of chipmunks (*Eutamia sp*). Voge (1956) reported the maturation of this parasite in laboratory rats and mice and quoted Rothman as establishing *H. citelli* in golden hamsters (*Mesocrisetus auriculatus*) which is apparently now the usual laboratory host.

The longevity of *H. citelli* has been recorded by several investigators. Read (1959) states that *H. citelli* (in unspecified levels of infection) lives for 70-90 days in hamsters before growth stops, senility commences and the worm literally becomes smaller and smaller as segements are shed; Ford (1972) interpreted this as indicating a longevity of 70-90 days. Wassom, Guss and Grundmann (1973) recorded the longevity of *H. citelli* in deer-mice and showed that in a 5 cysts infection, 70% of the animals lost all their worms between days 14-28. The author during the course of

this study observed the longevity of H. citelli (2 worm infection) in CFHB male rats to be up to 120 days (as long as studied) and a 5 worm infection in hamsters to be about 7 months.

Read and Phifer (1959) using a single cysticercoid infection of H. citelli and H. diminuta showed that both parasites, when maintained separately in hamsters on a high carbohydrate diet, differed from worms maintained on normal diet. However, when carbohydrate intake of the host was limited (lower than normal), H. citelli was affected proportionally more than H. diminuta. When single H. citelli and H. diminuta were maintained together in hamsters on a high carbohydrate diet, individuals of both species were reduced in size; H. citelli being most affected. Under carbohydrate deprivation, the size of H. citelli was not affected by the presence of H. diminuta, whereas H. diminuta was further reduced in size under these conditions. The possibility that mixed infection led to poorer growth because of an immune-mediated response was not apparently considered in their studies. It was not until Weinmann (1966) observed that H. citelli worms were reduced in numbers and stunted when given to mice two months after a primary infection, that the possibility of an immune response in H. citelli infections was considered. Using a 10 cyst primary infection followed by a 10 cyst challenge infection, Weinmann (loc. cit.)

found that only 17% of the worms were present in the secondary infections, compared with 53% in the primary controls. He also noted that although the difference in mean worm lengths was not statistically significant, worms in most of the secondary infections were smaller. In another experiment in which the time between chemically terminating a 10 cyst primary infection and an 8 cyst challenge was delayed for six months, there was no indication of acquired resistance to homologous challenge. He tentatively suggested that the poor growth of challenge worms could be due to a weak immune response. Wassom, Guss and Grundmann (1973) attempted to evaluate host resistance as a factor in controlling parasite population equilibrium in a natural host/ parasite system involving H. citelli and the white-footed deer-mouse (Peromyscus maniculatus). They demonstrated that laboratory-reared deer-mice were 100% susceptible to initial infection with H. citelli, but stated that most deer-mice developed resistance, resulting in the elimination of the worms before proglottids were mature. Deer-mice challenged at a later time showed a marked resistance to reinfection, but a few were incapable of this response and retained both their primary and secondary infections, Increasing the worm burdens (10-50) elicited a stronger and more pronounced resistance. Wassom et al. (loc. cit.) suggested that light infections are encountered in nature because larger infections are eliminated entirely and this could

play a major role in regulating the population equilibrium of this parasite in nature. To determine whether the response was mediated by specific immune mechanisms or by non-specific factors and to evaluate the genetic aspects of this host resistance, Wassom et al. (1974) further conducted a study on acquired resistance to H. citelli in Peromyscus maniculatus. They demonstrated that the ability to develop resistance was controlled by a single autosomal dominant gene. They also, importantly, demonstrated that acquired resistance could be transferred to uninfected hosts with "immune" lymphoid cells (from the thymus, spleen and lymph nodes of infected animals) harvested from (10 cysts) infected hosts on days 7, 14 and 20 post infection. Recipient animals (given 10^8 cells) were resistant and eliminated their infections. Treatment with heterologous antilymphocyte serum (Rabbit anti-Mus musculus) depressed the ability of competent hosts to resist infections. Pooled "immune" serum obtained from resistant animals on days 21 and 28 post infection did not transfer resistance. Their study indicated that resistance maybe a function of T-dependent lymphocytes. Specific mechanisms, however, remain to be elucidated to justify the importance of the immune response in maintaining the equilibrium between the H. citelli/ P. maniculatus system.

Hopkins and Stallard (1974) showed that approximately 90% of 1, 3 and 6 H. citelli cysticercoids

administered to CFLP male mice became established. They described growth and survival rates and discussed the results with reference to H. diminuta and postulated the existence of an antigenic threshold in mice. They concluded that 1 worm infections survived without loss till day 30, after which a small loss occurred in mice (50% by day 80). Worms in 3 worm infections were rejected between days 17-30, but loss varied (between 50-80%) in replicate experiments during this period. With 6 worm infections rejection was faster (days 17-22), more uniform and more complete. Over 75% of worms were lost in 6 worm infections, but in both 3 and 6 worm infections a residual population usually of a single worm persisted in 20-50% of the mice. Hopkins and Stallard (loc. cit.) went on to test the hypothesis of an immunologically-mediated rejection of H. citelli in mice by using an immunosuppressant (cortisone acetate). Loss of worms was prevented in 3 and 6 worm infections and the biomass from day 12 onwards (until about day 25) in cortisone treated mice tended to be greater than in controls. This they attributed either to the suppression of an immune response which slows growth or to cortisone affecting the exocrino-enteric circulation of mice, thereby stimulating growth. They concluded that "although these results are not definitive evidence that H. citelli elicits an immune response by the mouse, they make it virtually certain when considered together with the results of Weinmann (1966)."

Precipitating antibodies have been shown to be present in the serum of mice infected with *H. microstoma* and of rats infected with *H. diminuta* (Goodall, 1973). However, Goodall (loc.cit.) was unable to detect antibodies in the serum of mice or rats infected with *H. citelli* which suggests that the immunological response by mice and rats to *H. citelli* is different from that to *H. diminuta* and *H. microstoma*. Studies will be presented later in this thesis on the *in vivo* interactions between *H. citelli*, *H. diminuta* and *H. microstoma* in mice.

The cumulative information reviewed above supports the contention that adult tapeworms living entirely in the lumen of the small intestine, evoke an immunological response. However, the extent to which the *H. citelli*/mouse model is a useful system for studying this response needs further clarification.

The objective of this section of the thesis was not only to confirm Hopkins and Stallard's (1974) results, obtained using mice from a now defunct company (Carworth, Europe), but to describe the dynamics of primary infections of *H. citelli* of varying intensities in mice. Characteristics of establishment, recovery, growth and time of rejection will be discussed and the variability of the system highlighted. This is essential before the kinetics of secondary infections can be investigated.

Materials and Methods

4-star CFLP male mice were purchased when 5 weeks ±2 days old from Anglia Laboratories and infected when 42 ±2 days old. Infection and autopsy procedures were as previously described in the General materials and methods. Autopsy of respective categories was from day 9 post infection and thereafter at intervals, as indicated in Figures 1-1, 1-2, 1-3 and 1-4.

Single worm infections

Recovery:

The percentage recovery of worms >0.2 mg is shown in Fig. 1-1(A). The results show that on day 10, 100% of the cysts administered had become established and thereafter the majority of worms grew. However, worms weighing <0.2 mg were recovered on days 12, 15 and 19 (Fig. 1-2). The reason for the occurrence of these worms at this time is unknown, but may reflect variability in worm growth inherent in the system. Worms survived without loss up to day 36 post infection, and thereafter, it is possible that loss was beginning to occur.

Growth:

The dry weight of worms obtained per mouse and the variation within a group are shown in Fig. 1-2. The worm weights on day 10 varied between 0.2 mg-2.5 mg. Between days 12-19, 20% of the administered cysts were recovered as worms <0.2 mg. On day 45, 30% of the cysts administered were recovered as worms <0.2 mg. Whether these small worms had grown poorly, or had grown normally, destrobilated and started growing again is equivocal, as the characteristic "darkened" terminal protrusion of destrobes was not invariably present. The mean biomass (excluding worms <0.2 mg) increased from day 10-23 post infection, followed by a plateau up to day 45 (as long as studied).

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Figure 1-1

Percentage recovery of Hymenolepis citelli worms (> 0.2 mg) from CFLP male mice given a single, six, twelve and twenty-four cysticercoid infections.

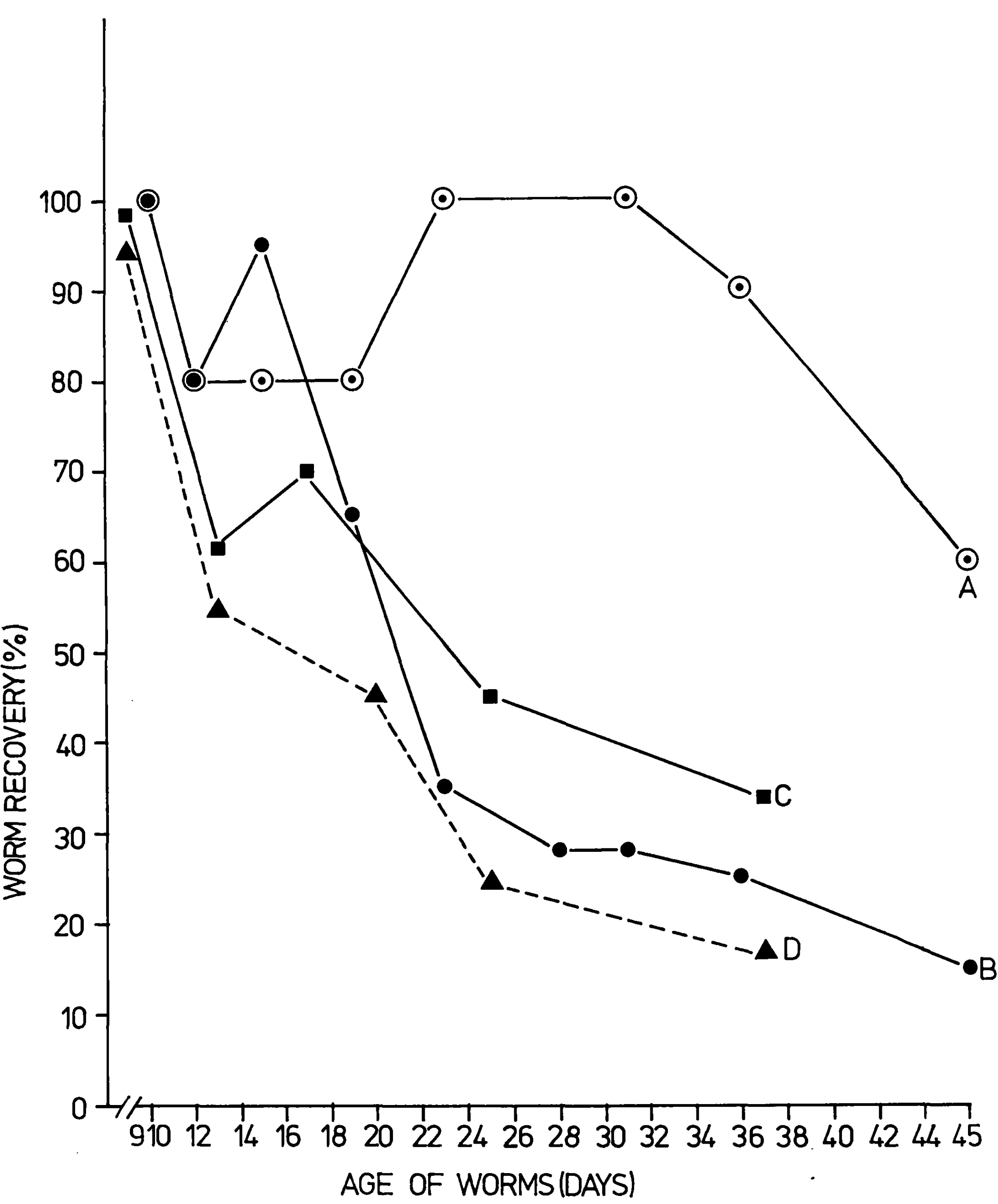
A = single cyst

B = six cysts

C = twelve cysts

D = twenty-four cysts

n = 9-10 mice/group



WORM RECOVERY (%)
100
90
80
70
60
50
40
30
20
10
0
9 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 45
AGE OF WORMS (DAYS)
A
B
C
D

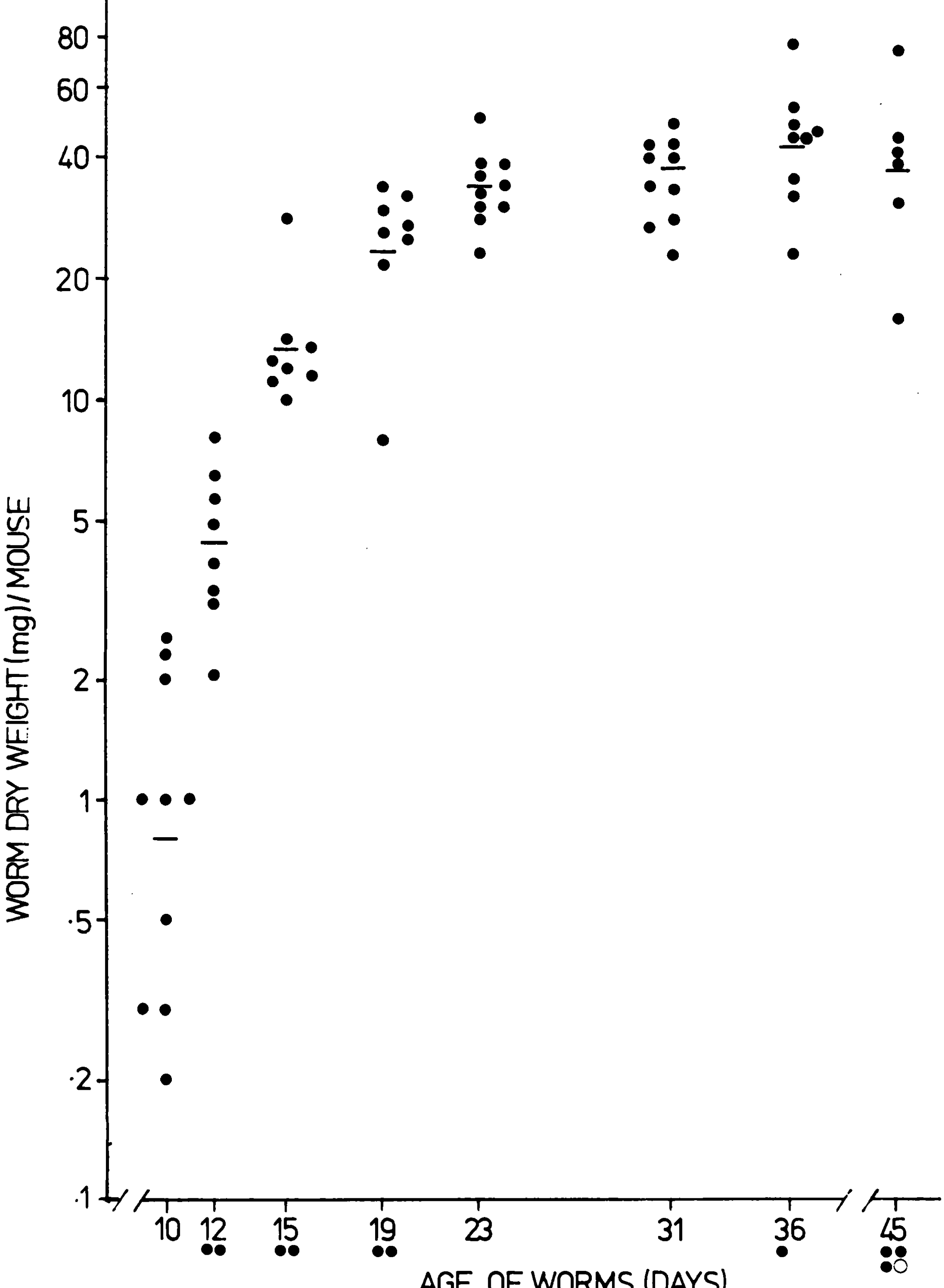
Facing page 28

Figure 1-2

Biomass of H. citelli from single cysticercoid infections of CFLP male mice. The mean biomass per group is indicated by a horizontal bar (excluding worms < 0.2 mg).

Points below horizontal scale indicate mice harbouring worms < 0.2 mg (◌), or mouse from which no worms were recovered (○)

$n = 10$ mice/group


80
60
40
20
10
5
2
1
·5
·2
·1
WORM DRY WEIGHT (mg) / MOUSE
10
12
15
19
23
31
36
45
AGE OF WORMS (DAYS)

Six worm infections

Recovery:

The percentage recovery of worms >0.2 mg is shown in Fig. 1-1(B). The results show that on day 10 post infection, all of the cysts administered were recovered as segmented worms, indicating that the establishment of six cysticercoid infections in a mouse was comparable to that of single cyst infections. By day 23, 65% of the worms had been lost, but by day 45, 15% of the total initial inoculum still remained in 60% of the mice.

Growth:

The dry weight of worms recovered per mouse is shown in Fig. 1-3. The mean biomass per group increased sharply to a maximum of 61.5 mg on day 15, and thereafter decreased to approximately 50% by day 23. Although the difference in biomass between days 15 and 23 was not statistically significant ($p > 0.05$), nevertheless 6 of the 7 mice infected with worms >0.2 mg on day 23 had a biomass less than the day 15 mean value. The mean biomass (excluding worms <0.2 mg) fluctuated between days 23 and 45, but the differences between days during this time were not statistically significant.

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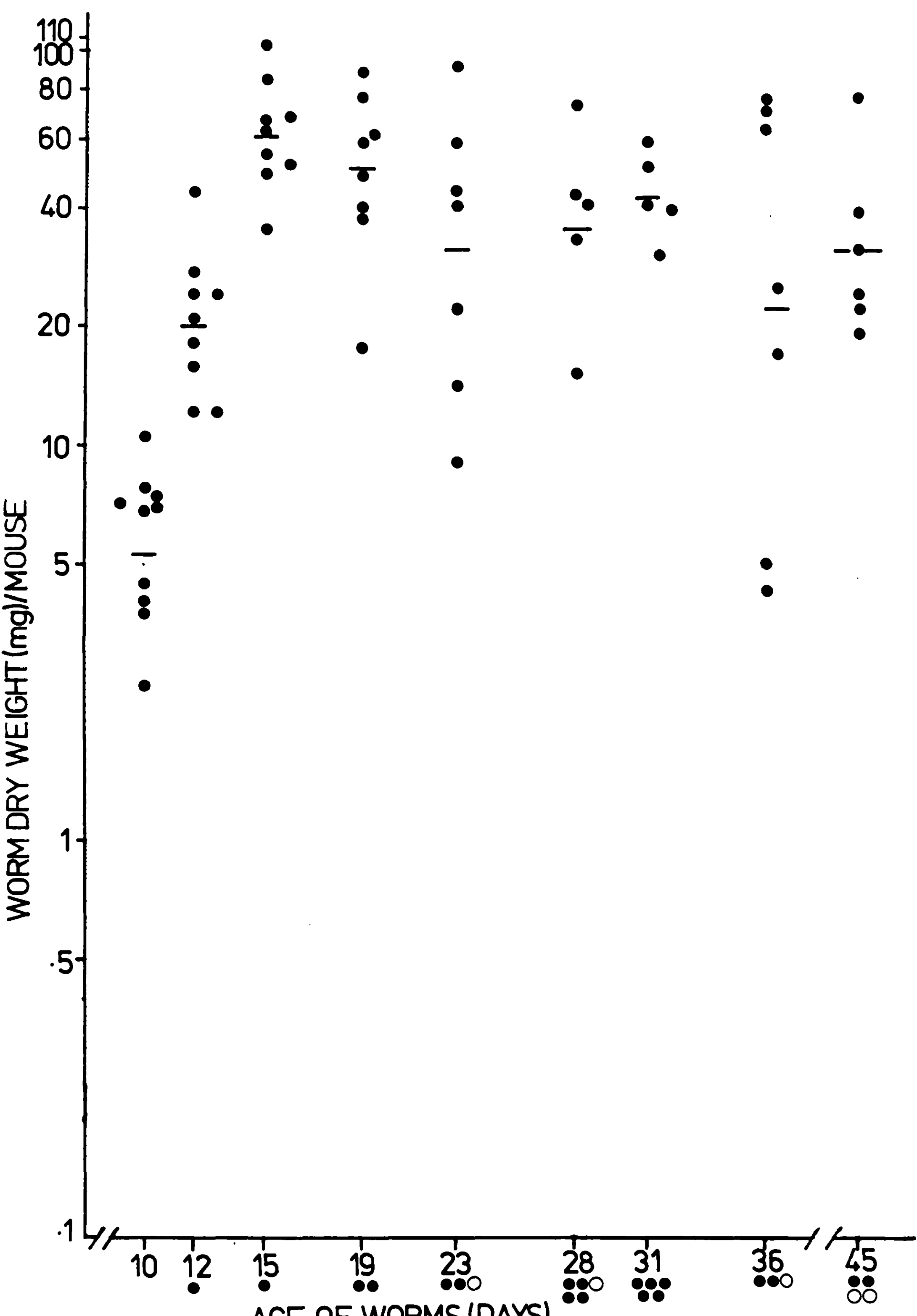
Figure 1-3

Biomass of H. citelli from six cysticercoid infections of CFLP male mice. Each point shows the total dry weight of worm tissue per mouse. The mean biomass per group is indicated by a horizontal bar (excluding worms <0.2 mg).

Points below the abscissa represent mice harbouring stunted/destrobilated worms (●) <0.2 mg or no worms (○)

n = 10 mice/group



110
100
80
60
40
20
10
5
1
·5
·1
WORM DRY WEIGHT (mg)/MOUSE
10
12
15
19
23
28
31
36
45
AGE OF WORMS (DAYS)

Twelve and Twenty-four cyst infections

Recovery:

Over 90% of both 12 and 24 cysticercoids administered to CFLP mice became established (Fig. 1-1, C and D). Worm loss commenced between days 9 and 13. The recovery on day 13 from the 12 worm infection may have been per chance unusually low, as suggested by the higher recovery on day 17. In general, however, worm loss was slower in the 12 worm infection than in the 24 worm infection. After day 19, a greater proportion of worms was lost in the 6 cysts infection than in the 12 cysts group. The reason for this is unknown and could be due to chance, particularly as the data for the latter group is based on only 2 kills after day 17, compared with 5 kills in the former. This interpretation may be supported by the fact that there was a similar loss of worms between days 20 and 37 in the 6 cysts and 24 cysts infection groups (Fig. 1-1, B and D).

Growth:

The dry weights of worms recovered per mouse and the variations within a group from both the 12 and 24 cysticercoid infections are shown in Fig. 1-4. Maximum mean biomass was reached on day 25 in the 12 worm infection. When maximum mean biomass per group was reached in the 24 cysts infection is more difficult to assess, as the results obtained on day 25 were lower than those on days 20 and 37, when approximately equivalent mean values were recorded.

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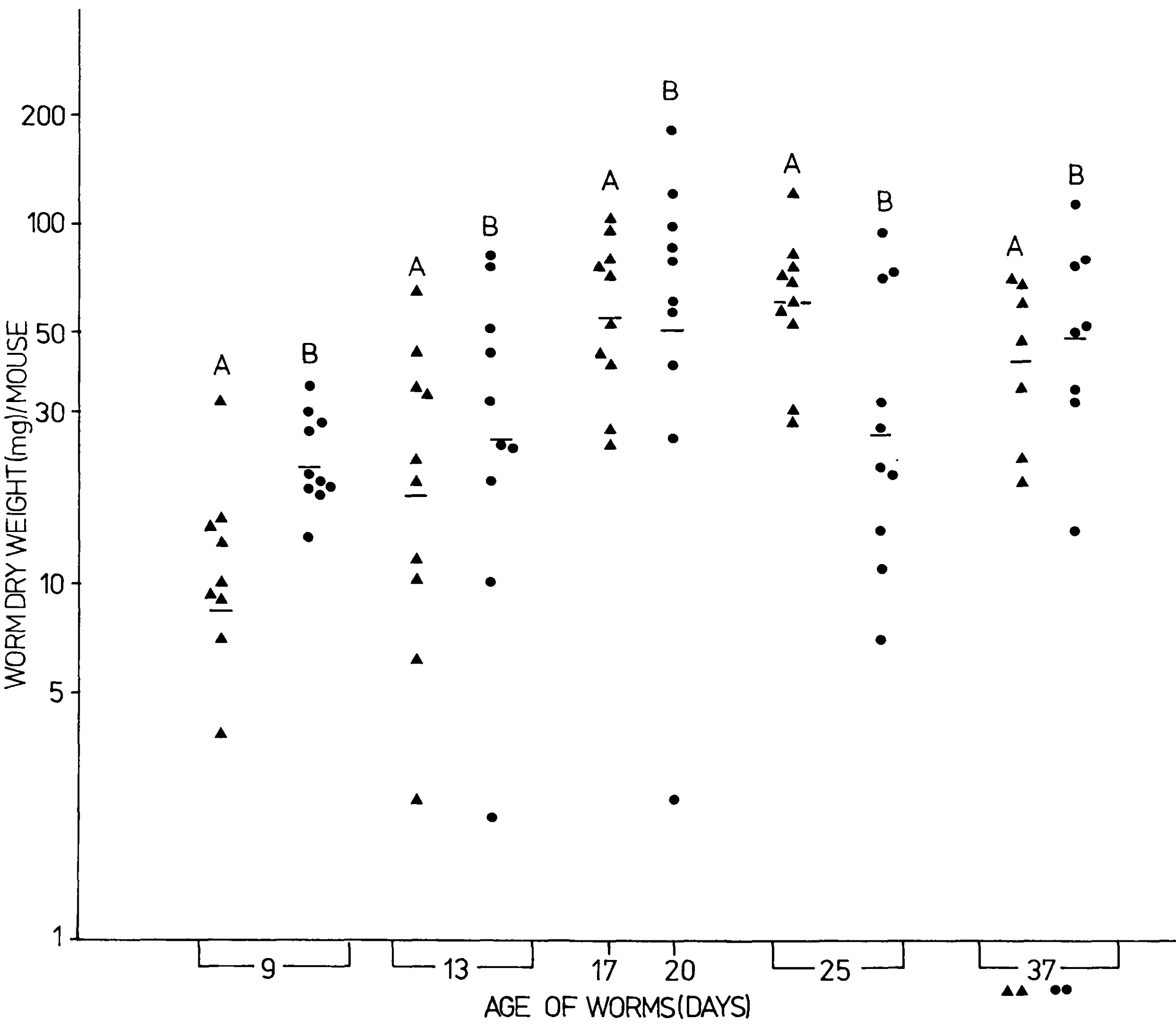
Figure 1-4

Biomass of <u>H. citelli</u> from twelve (△) and twenty-four (○) cysticercoid infections of CFLP male mice. Each point represents the total dry weight of worm tissue per mouse. The mean biomass per group is indicated by a horizontal bar.
Points below abscissa represent mice harbouring worms <0.2 mg.
A = twelve cysts
B = twenty-four cysts
n = 10 mice/group; except for D9 and D37 p.i. in the twelve cysticercoid infection.


WORM DRY WEIGHT (mg)/MOUSE
200
100
50
30
10
5
1
A
B
9
13
17
20
25
37
AGE OF WORMS (DAYS)

Facing page 33

Figure 1-5

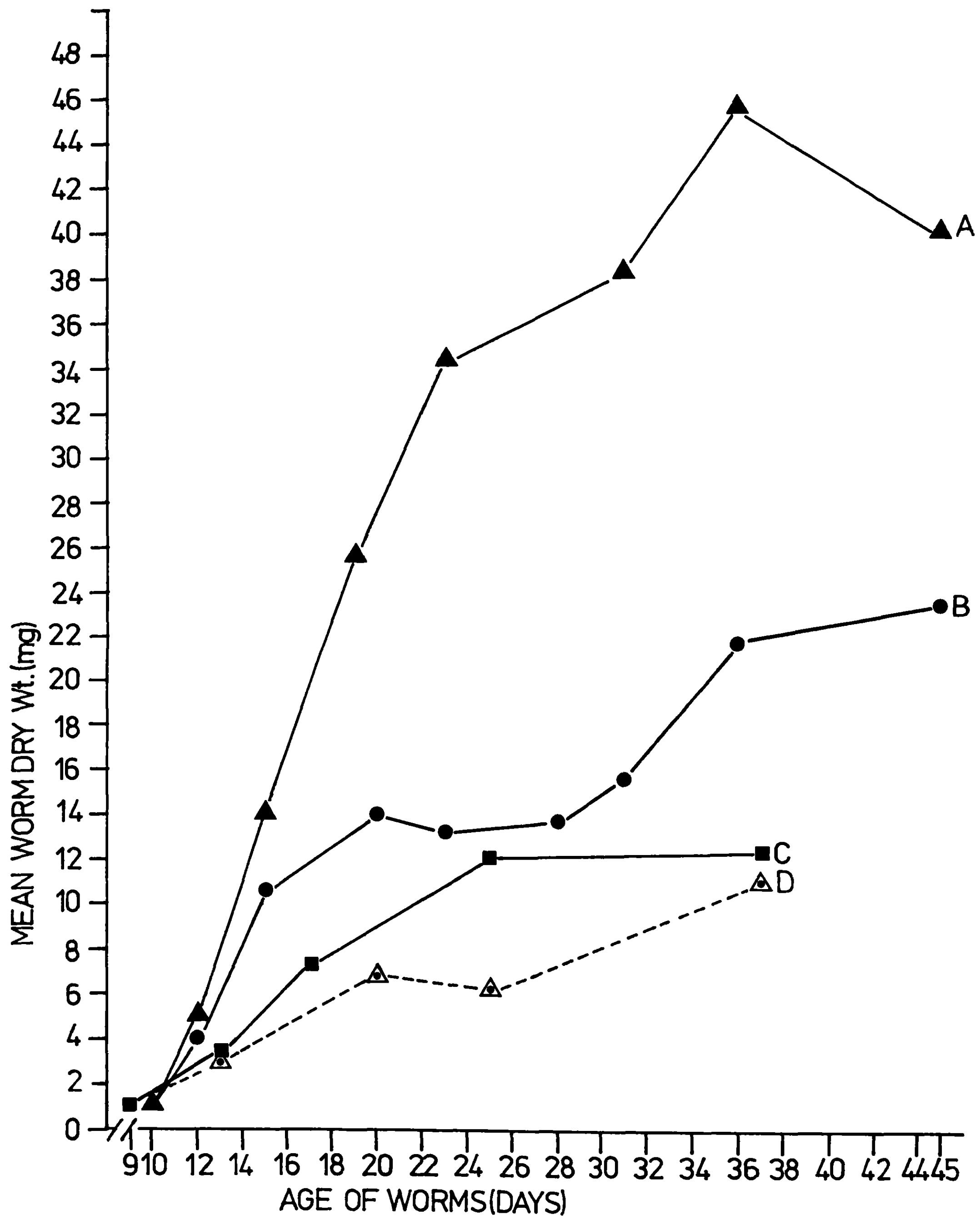
Mean worm dry weights of <u>H. citelli</u> from single, six, twelve and twenty-four cysticercoid primary infections from CFLP male mice

A = single cyst

B = six cysts

C = twelve cysts

D = twenty-four cysts



MEAN WORM DRY Wt. (mg)
AGE OF WORMS (DAYS)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
9
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
45
A
B
C
D

DISCUSSION

The results from the single worm infections show that by day 45, 40% of the mice had either lost their worms or harboured destrobilated worms. Whether this loss is because the mice were getting older and older mice reject a single worm infection faster than younger mice, as has been reported for H. diminuta (Befus and Featherston, 1974), is unknown. No further kills were done after day 45, but the level of recovery at previous kills was always in excess of 80% and it is therefore considered unlikely that the day 45 value was due purely to chance. If it is assumed that the apparent loss of worms (destrobilation being considered equivalent to early stages of rejection - Befus, 1975b) represented an immune response, it is interesting that it occurred in only 40% of the mice. It is possible that there is some variability between the mice in their sensitivity to the antigenic stimulus presented by the 1 worm infection. Some mice, with a lower threshold, appear to have been able to mount an effective response by day 45 whereas in others the threshold necessary for the response might not have been exceeded. A similar explanation of variation in the response of mice to 1 worm infections with H. citelli was proposed by Hopkins and Stallard (1974). The concept of an immune threshold response in helminth infections is supported by work with Nippostrongylus brasiliensis in the rat (reviewed by

Ogilvie and Jones, 1971) and with *Trichuris muris* in the mouse (Wakelin, 1973). To effectively clarify the importance of age on variation in the ability of mice to respond to a single worm infection, it would be necessary to carry out a series of experiments utilising mice of different ages and extending autopsies up to and probably beyond day 90 post infection.

The results described in the present study highlight the inherent variability in the growth of worms typical of the system. Worm size was variable within a group of mice of the same age, sex and strain. Worm growth variations may be related to differences in individual worm tegumental absorptive efficiency, i.e. differences in the rate of nutrient absorption. The relative susceptibility of individual worms to the primary immune response mounted by the host, may also play a part in worm growth variations, especially in multiple worm infections where the severity of the immune response could be related to the antigenic load. Befus (1975b) has discussed the inherent variability present in *H. diminuta* infections in mice and concluded that the variability seen in single worm infections could be reduced by using multiple worm infections. Comparable worm growth variations occurred in mice infected with 1, 6, 12 and 24 cysts in this study (see Figs. 1-2, 1-3, 1-4).

The results from the mean worm weights (Fig. 1-5) indicate that growth of worms was inversely related to the

population density. The close agreement among the values obtained for mean worm weights in all the categories between days 9-10, suggests that before day 10 there was little effect of population density on growth. Thereafter, the manifestation of the effect of population density upon growth in a mouse as shown by the results, is a decrease in the mean worm size as the population density increases. This relationship has been referred to in cestode infections as "crowding" (Read, 1959). Read (loc. cit.) concluded that the competition in "crowding" was for utilisable carbohydrate and that it was this substrate which was the limiting factor involved in determining the size of individual tapeworms in H. diminuta infections of varying intensities. Read's interpretation could be contentious in that it is equally plausible that other unknown factors, possibly toxicity due to excretory/secretory products produced by the worms, may hinder nutrient uptake and thereby affect growth. In addition, an indirect or direct physical and/ or chemical interaction between worms coupled with an immune response mounted by the host may otherwise explain the crowding effect. Whatever these factors are, they might directly/indirectly affect the neck region (germinative zone) which is the area of highest mitotic activity and proglottid formation (Roberts, 1961; Loehr and Mead, 1979). Caution is needed in interpreting the population consequences of the crowding effect, and whether the reduction in growth in H. citelli infections,

as the population density increases in the mouse might also reflect a reduction in egg production (probably as a consequence of a decrease in proglottid volume), as has been reported for H. diminuta infections in the rat (Hesselberg and Andreassen, 1975), remains to be determined. However, the relationship between increasing parasite densities and fecundity is not a simple one and indeed may be complex.

In conclusion, the results show that over 90% of 1, 6, 12 and 24 cysticercoids administered to CFLP mice became established, grew and thereafter survival depended on the intensity of the worm burdens. The results described here are similar to those of Hopkins and Stallard (1974), who also examined growth and survival of H. citelli in CFLP mice. However, it was necessary to re-establish these parameters as the CFLP strain had been re-derived in the intervening 3½ years before the commencement of this work and in recent years much evidence has accrued to indicate that strain variation in mice affects their response to parasitic infections (Wakelin, 1978b).

SECTION 2

Secondary infections

Introduction

The demonstration of acquired immunity to homologous challenge infections is pivotal to further analyses of the immune response in the H. citelli-mouse system. Evidence of acquired immunity to other Hymenolepis spp. has been reviewed by Williams (1979). These studies suggest that the immunological effector mechanisms (response manifested mainly as stunting or destrobilation of secondary worms - Hopkins et al., 1972a Befus, 1975b and Howard, 1976b) play a role in limiting the success of overwhelming natural infections.

Very little work, with the exception of Weinmann's (1966) has been done in the laboratory mouse to effectively demonstrate that mice are immunised against homologous challenge infections in the H. citelli-mouse model. Following on from the establishment of basic parameters in assessing the development of primary infections (see Section 1), it was decided to determine the dynamics of secondary infections in mice, prior to the undertaking of further studies on cellular or humoral factors that might be involved in the response. If there is evidence of acquired immunity in the mouse, how is it manifested? Should the parameters used in evaluating the response be worm growth (stunting/

destrobilation) or worm survival? The experiments were designed to investigate:

a) Evidence of acquired immunity in the absence of the primary worms

b) The effect of various intensities of primary infections on challenge infections

c) The effect of the duration of the primary infection on homologous challenge infections

d) How long after chemically removing the primary worms, is the response against challenge infections effective - does acquired immunity wane with time?

The characterisation of single and multiple secondary worm infections were carried out with particular observations on the establishment, recovery and growth of worms.

Results

2a One cyst challenge infections

The questions posed were: 1. Does one cyst primary infection stimulate a protective response, and 2. Is the strength of the response related to the intensity of the primary infection?

Experimental Protocol:

To observe the effect of 1 and 6 cysticercoid primary infections on the growth and survival of a 1 cyst homologous challenge.

Group	Day 0	D12	D21	D31	D43	D50	D54
a)	-	-	Z	1c	K	K	K
b)	1c	K	Z	1c	K	K	K
c)	6c	K	Z	1c	K	K	K

c = cysticercoid

Z = "Zanil" - 250 mg/kg/mouse

K = Autopsy of 10 mice

Worm recovery:

10/10 (100%) worms and 60/60 worms were recovered on day 12, from the mice infected with 1 and 6 cysts respectively, indicating 100% establishment of the "immunizing" infection.

Secondary infections:

Worm recovery:

The percentage recovery of worms from the secondary infection is shown in Fig. 2-1. On day 12 post infection (p.i.), 90%, 80% and 40% of >0.2 mg worms were recovered from the control, 1c primary and 6c primary infected groups respectively. The recoveries of >0.2 mg worms from the "immunised" groups fell to 50% on day 19, and to 50% and 30% on day 23, whereas from the control groups recoveries remained over 90% throughout the experiment.

Growth:

The dry weight of worms recovered is shown in Fig. 2-2. The results show that of the total initial inoculum (i.e. inclusive of days 12, 19 and 23) in each category, over 90% of the control worms were >0.2 mg, whereas 60% and 40% from the 1 cyst and 6 cysts "immunised" groups respectively, were recovered as worms >0.2 mg. From the control, 1 cyst and 6 cysts "immunised" groups, 3%, 20% and 30% of the worms recovered were <0.2 mg respectively. The data indicate that following a 6c primary infection, the growth of challenge worms was significantly depressed ($p < 0.01$), although to a less extent following a 1c primary infection ($p < 0.05$ on days 19 and 23).

2b Six cysticercoid challenge infections

The purpose of this experiment was to determine whether the strength of the response against a challenge infection increases with the intensity of the challenge, i.e. 6c rather than 1c, as was used in the previous experiment.

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Figure 2-1

Percentage recovery of <u>H. citelli</u> from single cysticercoid challenge infections of CFLP male mice given single (1c) or six (6c) cysticercoid primary infections (1°).

= Control (uninfected)

= 1°x1c

= 1°x6c

= stunted/destrobilated worms <0.2 mg

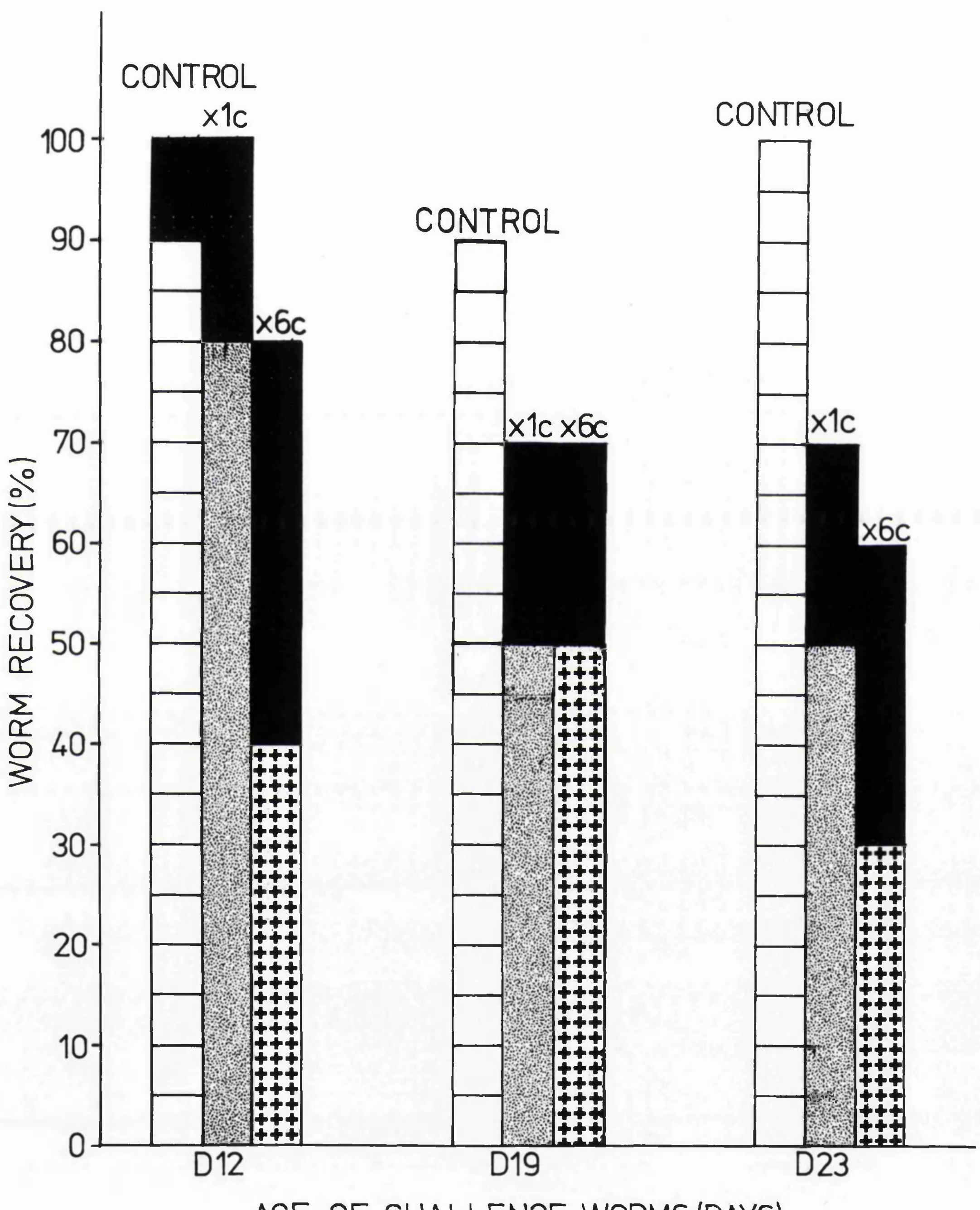
CONTROL
x1c
x6c
CONTROL
x1c x6c
CONTROL
x1c
x6c
WORM RECOVERY (%)
100
90
80
70
60
50
40
30
20
10
0
D12
D19
D23
AGE OF CHALLENGE WORMS (DAYS)

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Figure 2-2

Dry weight of H. citelli from single cysticercoid challenge infections of CFLP male mice given one (1c) or six (6c) cysticercoid primary infections

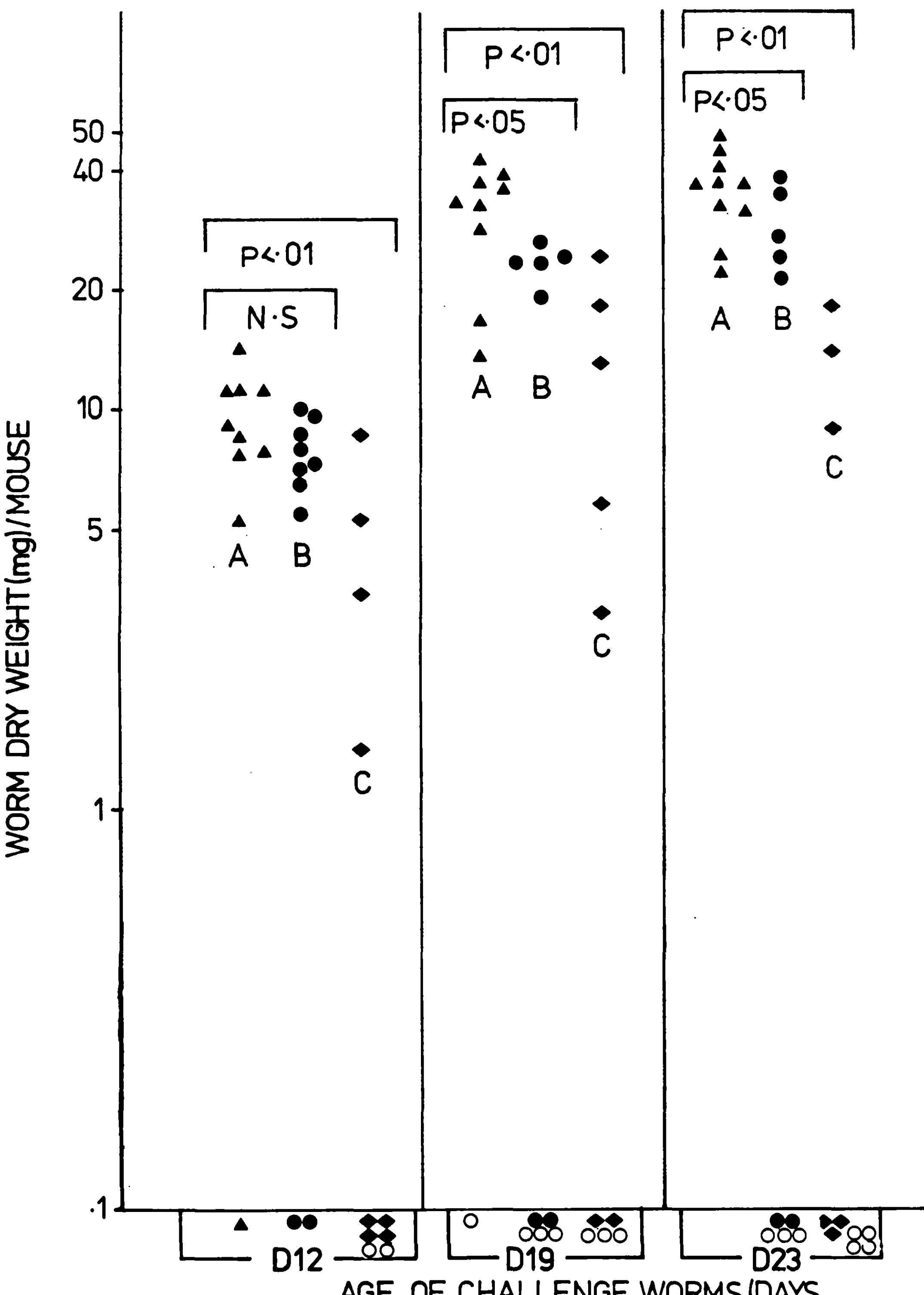
A = control (naive)

B = 1^ox1c

C = 1^ox6c

Mice from which destrobilated/stunted worms $<$0.2 mg were recovered are indicated below the abscissa (◉)◇)

(◯) Mice from which no worms were recovered.



P<·01
N·S
P<·01
P<·05
P<·01
P<·05
50
40
20
10
5
1
·1
A
B
C
WORM DRY WEIGHT (mg)/MOUSE
D12
D19
D23
AGE OF CHALLENGE WORMS (DAYS

Protocol:

Group	Day 0	D12	D21	D31	D41	D43	D45	D47
a)	-	-	Z	6c	K	K	K	K
b)	6c	K	Z	6c	K	K	K	K

K = Autopsy of 9-12 mice

Worm recovery:

56/60 worms with a mean worm dry weight of 3.4 mg were recovered from 10 mice killed on day 12, to check on the establishment of the primary (immunizing) infection. The number of worms recovered from the control and "immunised" mice on days 10, 12, 14 and 16 p.i. is shown in Fig. 2-3. There was no major change during the period between days 10-16 p.i. in the control groups, although the recoveries from the "immunised" groups are indicative of partial worm loss (see discussion later). Recovery of worms >0.2 mg was 87% in the controls and 39% in the "immunised" groups over the experimental period.

Growth:

The total dry weight of worms per mouse and the variation between mice in a group are shown in Fig. 2-4. The results demonstrate considerable reduction in the biomass of worms from mice previously infected, in comparison with the controls (primary infections). In percentage terms, the reduction in total

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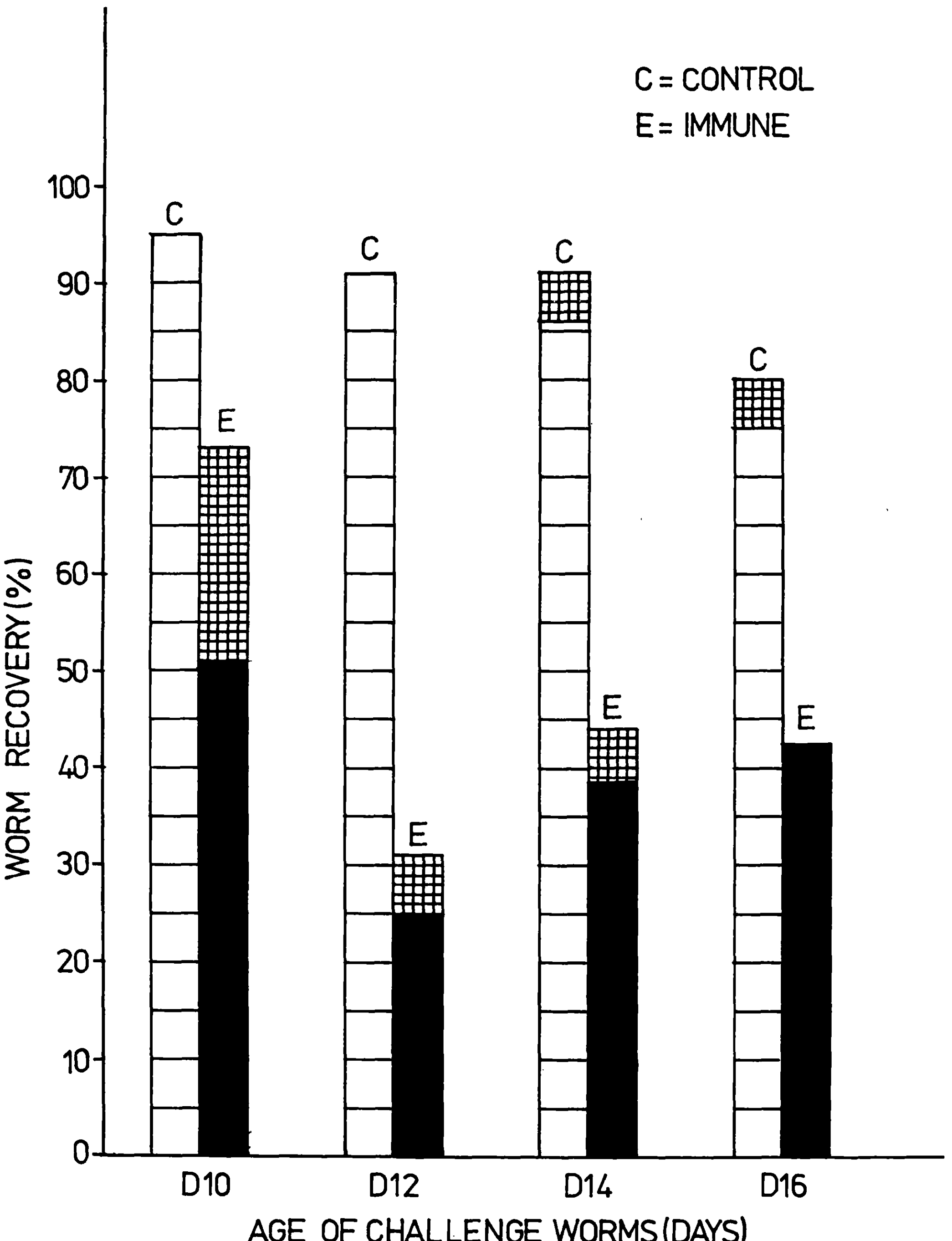
Figure 2-3

Percentage recovery of <u>H. citelli</u> from six cysticercoid challenge infections of CFLP male mice given a six (6c) cysticercoid primary infection (1°) and controls.

[horizontally divided box] = control (naive)

[blank box] = infected (1^ox6c)

[grid box] = destrobilated/stunted worms $<$0.2 mg.


C = CONTROL
E = IMMUNE
WORM RECOVERY (%)
100
90
80
70
60
50
40
30
20
10
0
C
E
D10
D12
D14
D16
AGE OF CHALLENGE WORMS (DAYS)

biomass (i.e. protective response) in the"immunised" groups was 75%, 77%, 54% and 55% on days 10, 12, 14 and 16 respectively, when compared with the biomass from controls. The mean worm weights of worms >0.2 mg in the control groups were 1.73 mg, 3.50 mg, 7.0 mg and 10.5 mg on days 10, 12, 14 and 16 whereas from the "immunised" groups it was 0.79 mg, 3.0 mg, 7.31 mg and 8.32 mg on the respective days.

2c The immunizing effect of small immature and large mature worms

In the previous experiments, immunizing infections had been allowed to grow to maturity (about day 17) before termination of the infection on day 21 p.i. To determine whether immature and/or large worms were necessary to induce a response, an experiment was designed and carried out in which infections were terminated after 7 days and 21 days p.i. To help compensate for the fact that large worms were at least three times as long as smaller (immature) worms, a second experimental group using 24 worms for 7 days was also set up. As this introduced another variable (number of scoleces), a further group was added of a 24 cysticercoid primary infection for 21 days.

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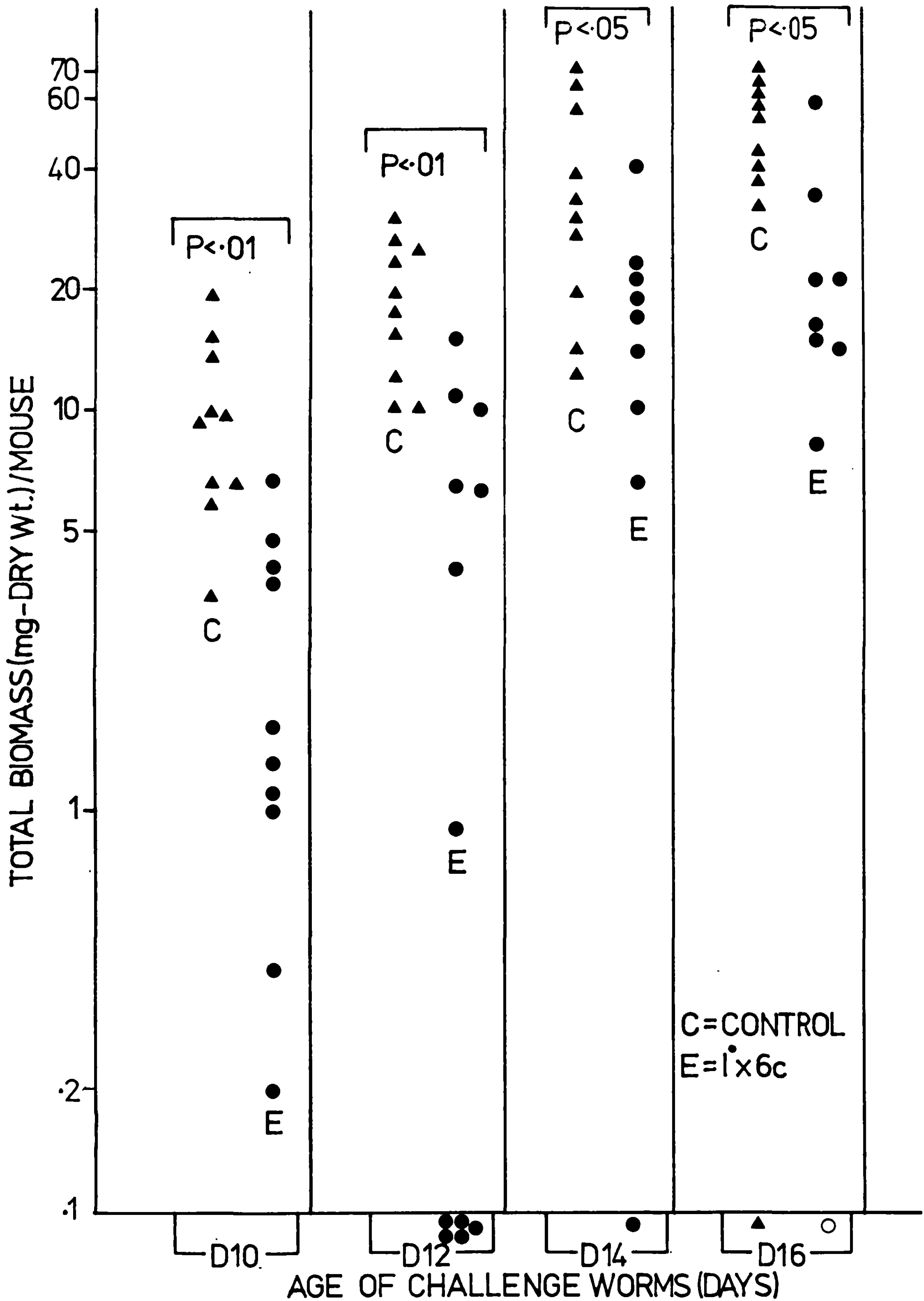
Figure 2-4

Dry weight of <u>H. citelli</u> from six cysticercoid challenge infections of CFLP male mice given a six (6c) cysticercoid primary infection (●) and controls (▲).

Mice from which stunted/destrobilated worms weighing in total <0.2 mg were recovered are indicated below the abscissa.

○ = Mouse from which no worms were recovered.



TOTAL BIOMASS (mg-DRY Wt.)/MOUSE
70
60
40
20
10
5
1
·2
·1
P<·01
P<·01
P<·05
P<·05
C
E
C
E
C
E
C
E
C=CONTROL
E=1°x6c
D10
D12
D14
D16
AGE OF CHALLENGE WORMS (DAYS)

Protocol:

Group	Day 0	D7	D17	D21	D29	D31	D43
	-	Z	6c	-	K	-	-
A	6c	Z	6c	-	K	-	-
	24c	Z	6c	-	K	-	-
	-	-	-	Z	-	6c	K
B	6c	-	-	Z	-	6c	K
	24c	-	-	Z	-	6c	K

A = immature worms (7 days old)

B = mature worms (21 days old)

K = Autopsy of 10 mice

Z = 'Zanil' (250 mg/kg/mouse)

c = cysticercoid

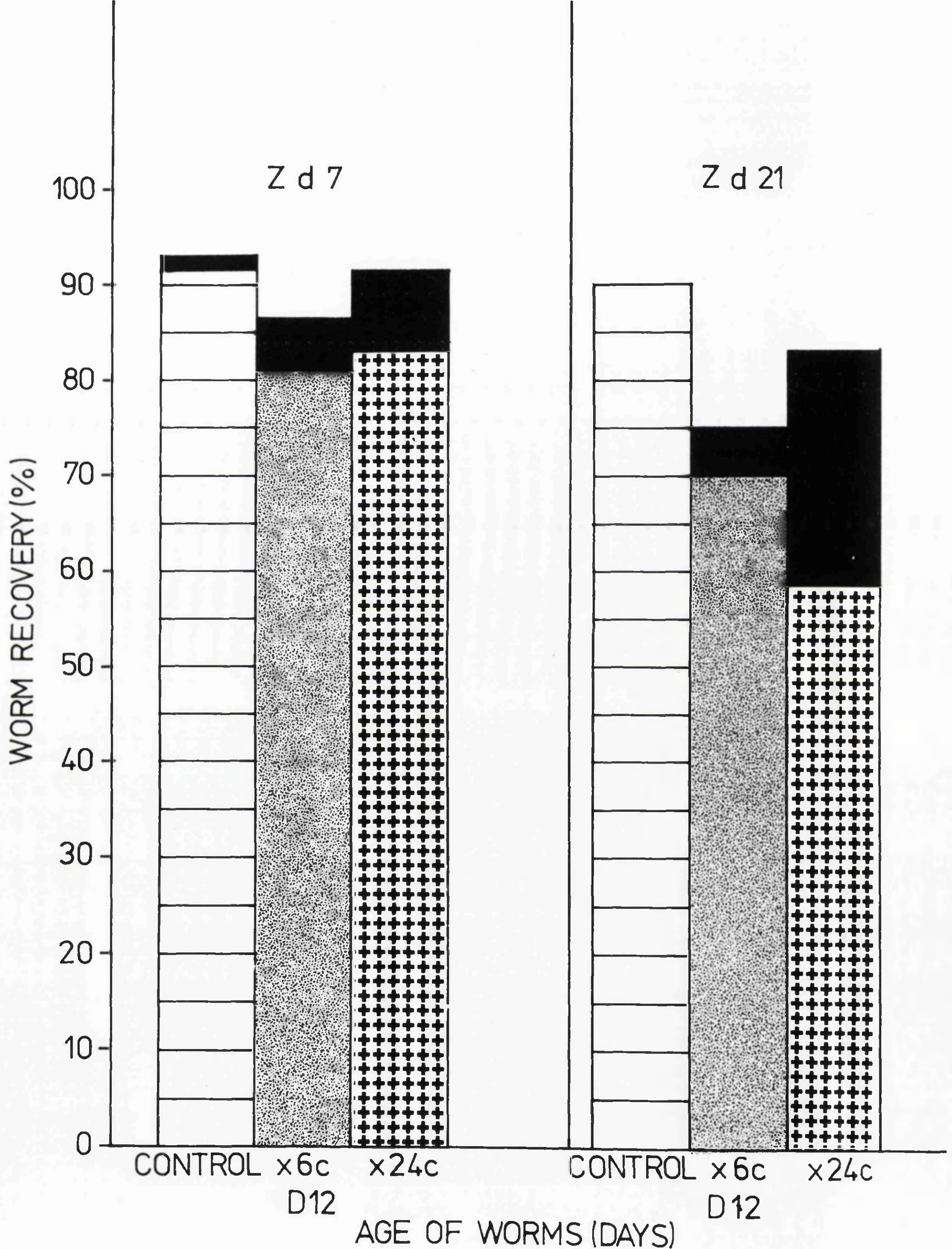
Worm recovery:

The percentage recovery of >0.2 mg worms/group and of destrobilated/stunted worms are shown on the histograms (Fig. 2.5) for the immature (Zd7 - 7 days old worms) and mature (Zd 21 -21 days old worms) immunisations. The results indicate that there was no significant worm loss from the immature immunisation category, in comparison with the control. In the Zd21 category, there was a 20% and 32% difference in the recovery of >0,2 mg worms in the 6 cysts and 24 cysts immunisations in comparison with controls. Although total worm recovery in mice immunised with 6 cysts for 21 days was higher in this experiment than in the previous experiment (cf. Fig. 2-3 and 2-5, day 12 values), it is difficult to draw a meaningful comparison between these experiments because the responses of outbred mice are inherently variable, even when the mice are obtained from the same supplier.

Figure 2-5

Percentage recovery of <u>H. citelli</u> from six cysticercoid challenge infections of CFLP male mice given a six (6c) or twenty-four (24c) cysticercoid primary infection for 7 days (Zd7) or 21 days (Zd21).

☐ = destrobilated/stunted worms <0.2 mg.


Z d 7
Z d 21
WORM RECOVERY (%)
100
90
80
70
60
50
40
30
20
10
0
CONTROL
x 6c
D12
x24c
CONTROL
x 6c
D 12
x24c
AGE OF WORMS (DAYS)

Growth:

The dry weight of worms per mouse and the variations within a group are shown in Fig. 2-6. There was no statistically significant difference between the control and immunised groups after a primary infection of 7 days. After 21 days, as was expected (see Fig. 2-4) mice immunised with 6 cysts had significantly ($p < 0.01$) lighter worms than the control mice had. Increasing the immunizing infection to 24 cysts led to slightly smaller worms when compared with the 6 cysts immunisation, although the difference was not sufficient to be statistically significant.

2d Duration of "memory" following termination of primary infection

It could be argued that the protective effect observed in the previous experiments was due to non-specific changes (physiological?) in the intestine rather than immunological memory. In an attempt to resolve this doubt, an experiment was set up in which challenge was delayed until 6 weeks after removing the primary worms. As a primary infection of 24 cysts may have immunised mice more strongly (see Fig. 2-6) than 6 cysts, both 6 cysts and 24 cysts immunised groups were set up. Groups of mice immunised for only 7 days were also included to verify the results previously obtained which, as will be discussed later,

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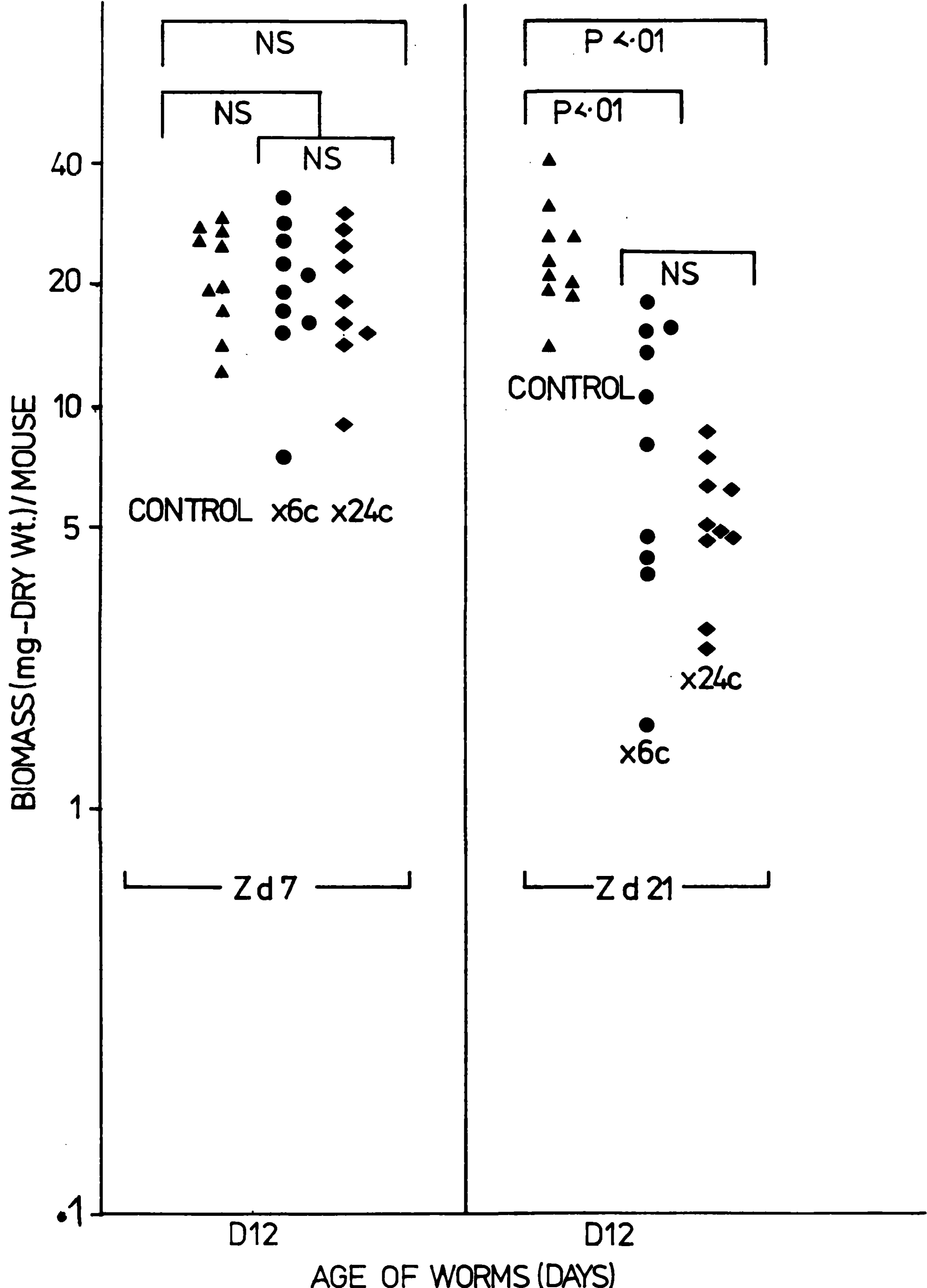
Figure 2-6

Dry weight of H. citelli from six cysticercoid challenge infections of CFLP male mice given six (6c) or twenty-four (24c) cysticercoid primary infections for 7 days (Zd7) or 21 days (Zd21).

n = 10 mice/group



NS
NS
NS
P <·01
P <·01
NS
40
20
10
5
1
·1
BIOMASS (mg-DRY Wt.) / MOUSE
CONTROL
x6c
x24c
CONTROL
x24c
x6c
Z d 7
Z d 21
D12
D12
AGE OF WORMS (DAYS)

were surprising when compared with data obtained using H. diminuta infections in mice.

Protocol:

Group	Day 0	D7	D21	D49	D63	D61	D75
	-	Z	-	6c	-	K	-
A	6c	Z	-	6c	-	K	-
	24c	Z	-	6c	-	K	-
	-	-	Z	-	6c	-	K
B	6c	-	Z	-	6c	-	K
	24c	-	Z	-	6c	-	K

*Key as page 48

Worm recovery:

Total worm recovery was over 90% in all groups (Fig. 2-7). The number of worms <0.2 mg was greater in the mice immunised with 24 cysts for 21 days, but even in this group, 83% of the worms were over 0.2 mg in weight.

Growth:

The results of dry weight of worms per mouse in a group and the intragroup variations are shown in Fig. 2-8. In comparison with the earlier results (Fig. 2-6), the total weight of worms in a group confirmed earlier results which demonstrated that 7 days immunisation with 6 cysts or even 24 cysts did not confer any resistance to mice. The data from the 21 days immunisation confirmed previous results that mice immunised for

21 days were significantly protected against homologous challenge. However, the degree of protection waned with time in both immunised categories, relative to controls. The protective response (i.e. reduction in the biomass of challenge worms relative to controls) in the 6c and 24c immunised groups in the "10 days challenge category" was 64% and 78% respectively; in the "42 days challenge category" the protection recorded was 40% and 65% for the respective immunised groups.

DISCUSSION

The results show that even a single worm primary infection stimulates a protective response against a single homologous challenge (Fig. 2-2). It is very doubtful whether the establishment of secondary worms was affected in the experiments described above (Figs. 2-1, 2-3, 2-5), especially as the problem of finding very small worms became more difficult in secondary infections. Thus the smaller number of worms recovered from the immunised groups could be partly due to not finding some of the severely stunted/ destrobilated worms. Slight reductions (10-20%) in the recovery of secondary worms have also been reported by Hopkins _et al_. (1972a) and Befus (1975a) in _H. diminuta_ infections in mice.

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Figure 2-7

Percentage recovery of <u>H. citelli</u> from six cysticercoid challenge infections of CFLP male mice given a six (6c) or twenty-four (24c) cysticercoid primary infection for 7 days (Zd7) or 21 days (Zd21) when challenged 42 days after 'Zanil' treatment

□ destrobilated/stunted worms <0.2 mg

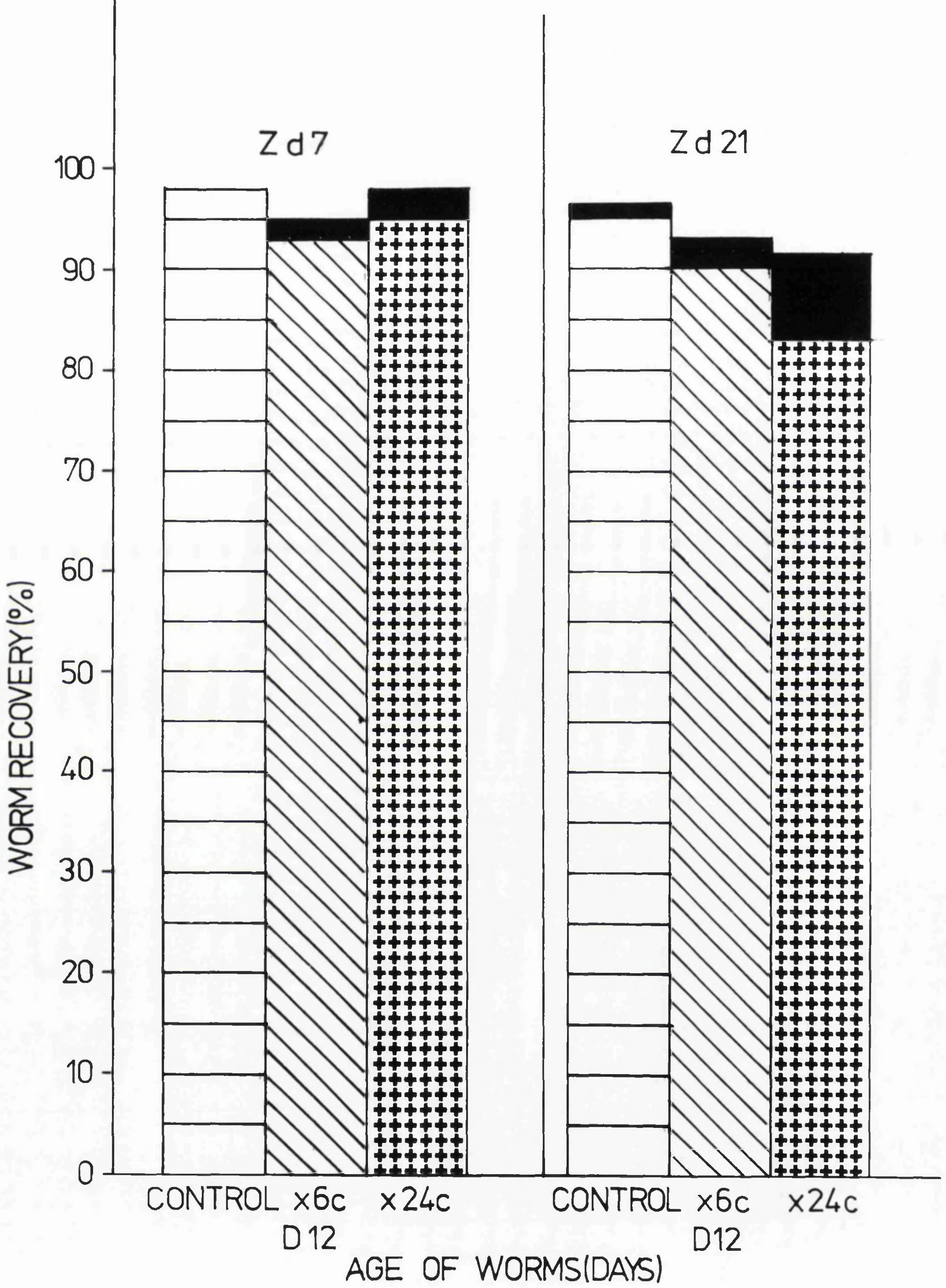
Zd7
Zd21
100
90
80
70
60
50
40
30
20
10
0
WORM RECOVERY (%)
CONTROL
x6c
D12
x24c
CONTROL
x6c
D12
x24c
AGE OF WORMS (DAYS)

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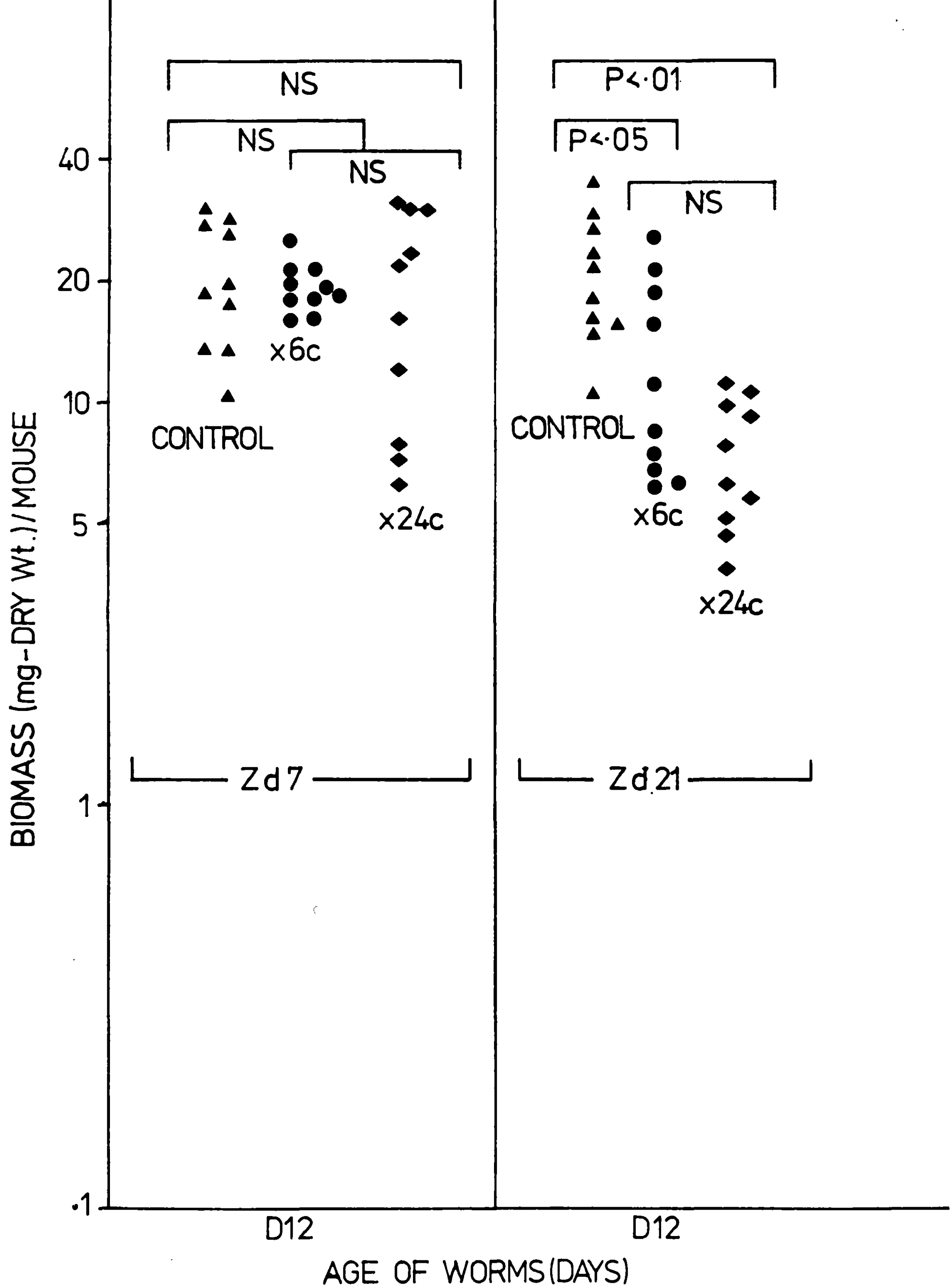
Figure 2-8

Dry weight of H. citelli from six cysticercoid challenge infections of CFLP male mice given a six (6c) or twenty-four (24c) cysticercoid primary infection for 7 days (Zd7) or 21 days (Zd21) when challenged 42 days after 'Zanil' treatment

n = 10 mice/group


NS
NS
NS
P<·01
P<·05
NS
40
20
10
5
1
·1
CONTROL
x6c
x24c
CONTROL
x6c
x24c
Zd7
Zd21
BIOMASS (mg-DRY Wt.)/MOUSE
D12
D12
AGE OF WORMS (DAYS)

Increasing the number of worms in the immunizing infections (i.e. 1, 6, 24 cysts) decreases the biomass of challenge worms vis-a-vis the controls (Fig. 2-2, 2-4, 2-6). This probably reflects an increase in the numbers of immunocompetent cells, as it is established that the level of antigen administered influences the degree of the immune response in a mouse (Black and Inchley, 1974). Such a quantitative relationship between the intensity of the immunizing infection and the manifestation of acquired immunity have also been described in Trichuris muris (Wakelin, 1969), H. diminuta (Befus, 1975b), H. microstoma (Howard, 1976a) and Trichinella spiralis (Despommier, 1977) infections in mice.

A comparison of the mean worm weights from mice immunised with 6 cysts with those from controls (results Section 2b), indicates that the difference in weight between the surviving worms in the challenge and primary infections decreased with the duration of the infection (see page 46). This "catching up" might suggest that worms in the challenge infection are affected by the immune respone and hence grow more slowly than those in the primary infection only during the early stages. It may be that this retardation in growth of worms in immunised mice was effective from the onset of challenge, but as no mice were killed before day 10, it is not possible to be certain. The better growth of secondary worms as an infection ages, could be due to "adaptation" of the worms to the

immune response, as has been shown in _H. microstoma_ infections in mice (Howard, 1976b) and in _H. diminuta_ infections in the rat (Hopkins - personal communications).

The differential immunisation produced by primary infections of varying intensity is not easily quantitatively explained. The problem is, what part of the worm is responsible for evoking a response? Is it the scolex, the germinative zone (neck) or the strobila? The origin(s) of the immunogens and the elucidation of the nature and mechanism(s) of action of these "functional antigens" intrigues workers studying immunity to _Hymenolepis sp_. There could be a direct relationship between worm numbers and amount of antigen produced, if the protective antigens originate exclusively from the scolex. However, if they are strobilar in origin, then antigen production might be expected to be dependent on total worm weight or total worm surface area per mouse (Andreassen _et al_., 1978a). Christie (1979) concluded that the protective antigens in _H. diminuta_ infections arose from the tegument, and suggested that they were produced in greater quantity per unit weight by the anterior end of the worms; he however did not exclude the immunogenic potential of the scolex. Elowni (1980) in a further attempt to locate the origin of the protective

antigens, suggested that (i) the induction of functional immunity against H. diminuta in mice is independent of the presence of strobilar antigens, (ii) the number of scoleces and/or the neck regions and the duration of antigenic stimulation from these regions determine the magnitude of the protective secondary response. There is room for studies in locating the protective antigens in H. citelli, on the line of the above works.

Elowni (1980) has shown that 6 and 30 cyst H. diminuta infections in mice for 3 days, were strongly immunogenic, resulting in a statistically significant reduction in the growth of a 1 worm challenge. Hopkins (personal communications) has shown that a primary infection of H. diminuta for 3 days in mice conferred no protective immunity to a secondary infection when given 5 days later, although when the challenge was delayed until 18 days later, a significant difference was obtained. The results obtained in this study (Fig. 2-8) did not reflect a protective response from a 7 day immunisation even when the challenge was delayed for 42 days. The results when compared in the light of those obtained for H. diminuta above, was surprising. Whether the non-immunogenicity of 6c and 24c for 7 days implies that the "functional" antigens in H. citelli are not produced within 7 days,

or produced in quantities too small for the recognition by the mouse is unknown. It may be that the immunogenicity is "stage-specific" i.e. the origins of the antigenic targets are associated with temporal changes. Thus it may be that as the parasite develops, different antigen(s) produced with time elicit different responses which might operate in concert (i.e. "juvenile and adult" antigens) against a challenge infection. An interaction between the responses to "adult and juvenile" antigens may be necessary between days 7-21 for a measurable response to be mounted. Such "stage-specific" immunogenicity has been postulated to operate in *T. spiralis* infections (Wakelin, personal communications, Bell *et al*., 1979) in the rat.

The protective response against homologous *H. citelli* challenge infections wanes with time in the absence of the immunizing infection. This observation is in line with the loss of acquired immunity to homologous *H. citelli* infections reported by Weinmann (1966), in which he showed no protective response in immunised mice when challenge was delayed until 6 months after the primary infection. A similar decline of the protective response against *H. diminuta* challenge infections in the rat has also been observed by Hopkins (1980), in contrast to the long-lasting "memory" to homologous *H. diminuta* challenge in mice (Hopkins, loc. cit.).

The results from the present study suggest that a state of "hyper-responsiveness" occurs in the intestine to homologous challenge (after mice have experienced a sensitising infection) which effectively limits overwhelming infections. The waning of the protective response could be attributed to either loss of functional capacity of death of immunocompetent cells (Feldbush, 1973).

The results discussed above unequivocally demonstrate that following the experience of a primary infection, mice exhibit acquired immunity to homologous _H. citelli_ infections. It is herein suggested that growth of secondary worms, rather than worm survival (due to the difficulty in recovering stunted/destrobilated worms) is a better parameter in evaluating the protective response against homologous _H. citelli_ challenge infections in mice.

SECTION 3

Immunosuppression

Introduction

It has been demonstrated that H. citelli establishes, grows and is subsequently rejected by one of its hosts - Peromyscus maniculatus (Wassom et al., 1973), and by CFLP male mice (Hopkins and Stallard, 1974; and Section 1, above). Hopkins and Stallard (loc. cit.) postulated that the rejection of a primary infection of H. citelli, like that of H. diminuta was immunologically-mediated. Evidence to support the involvement of an immune response in the rejection of H. diminuta and H. microstoma has been given by Hopkins et al. (1972b), and Moss (1972) and Howard (1976b) respectively. A useful technique adopted, has been the use of immunosuppressants to modify immune responsiveness and to assess the effects upon worm growth and survival in tapeworm infections. Using the immunosuppressant cortisone acetate, in the H. diminuta-mouse system, Hopkins and Stallard (1976) showed that worms matured by days 16-18 and continued to grow until day 45. Methotrexate and horse-anti mouse thymocytic serum also suppressed the rejection of H. diminuta by SPF CFLP male mice (Hopkins et al., 1972b). Moss (1972) demonstrated that in mice treated with cortisone acetate, H. microstoma grew larger than in control, untreated mice.

Corticosteroids have been widely used in a number of studies on resistance to helminthic infections - Nippostrongylus brasiliensis (Ogilvie, 1965), Taenia taeniaeformis (Oliver, 1962), Trichuris muris (Wakelin, 1970), Aspiculuris tetraptera (Behnke, 1975) and Strongyloides ratti (Moqbel, 1976). These studies were concerned with the immunosuppression of primary and subsequent infections, in host-parasite relationships characterised by a strong immune response.

The objective of the work herein, was to investigate whether, a) the rejection of a 6 cysts primary H. citelli infection and b) stunting of secondary worms in homologous challenge infections in CFLP mice were immunologically-mediated. Cortisone acetate was the drug of choice, because of the relatively good tolerance of CFLP mice to its effects (Hopkins and Stallard, 1976).

Materials and Methods

4-star CFLP male mice purchased from Anglia Laboratories were used, and infected at 6 weeks ± 2 days old. Six cysticercoids were administered to each mouse by stomach tube (see General methods). Cortisone acetate (Cortistab, Boots Ltd.) was administered every second day (commencing on days as indicated in the design of the experiments) by subcutaneous injections at a dosage of 1.25 mg (0.05 ml of a 25 mg/ml solution). To prevent opportunistic bacterial infections, both cortisone-treated mice and control, untreated mice were given the antibiotic - Terramycin (see General methods). Terramycin does not affect the growth of hymenolepid cestodes in mice (Hopkins, pers communication) and thus it was not considered necessary to include untreated controls in the experimental protocol.

Cortisone treated and untreated control mice were killed on specified days as indicated in the protocols below, and worm recovery was as in the General methods.

Results

Experimental Protocols

a) To show the effect of cortisone acetate on the growth and survival of a 6 cysticercoid primary infection.

Group	Day 0	D11	D15	D18	D21	D24
a)	6c	K	K	K	K	K
b)	6c	K	K	K	K	K
c)	6c	ND	K	K	K	K

a) Untreated control group

b) Cortisone treatment commenced on Day-2

c) Cortisone treatment commenced on D+11

K = Autopsy of 10-12 mice

ND = Not done

b) To investigate whether cortisone acetate could affect the growth and survival of secondary worms by suppressing the expression of acquired immunity to a homologous challenge infection.

Protocol

Group	Day 0	D12	D21	D31	D33	D41	D44	D47	D51
a)	-	-	Z	6c	NCA	K	K	K	K
b)	-	-	Z	6c	CA	K	K	K	K
c)	6c	-	Z	6c	NCA	K	K	K	K
d)	6c	-	Z	6c	CA	K	K	K	K

a) Control (Naive) - Untreated

b) Control (Naive) - Treated with CA

c) Secondary infection - Untreated

d) Secondary infection - Treated with CA

NCA = Not treated with cortisone

CA = Cortisone treated from D33-D51

K = Autopsy of 7-12 mice

a) Effect of cortisone on the growth and survival of a primary 6 cysticercoid infection.

The results of the dry weight of worms recovered per mouse and the intragroup variations, and the worm recovery(%) per group are shown in Fig. 3-1. The data show that cortisone treated mice consistently supported a higher total biomass per group (except for D15 in group C, where treatment was started on D11) than controls. The survival of worms was protracted in the cortisone treated groups.

b) Effect of cortisone on the growth and survival of a 6 cysticercoid homologous challenge:

The total biomass per group and worm recovery (%) per group are shown in Fig. 3-2. The results

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Figure 3-1

Dry weight of <u>H. citelli</u> worms per mouse per group from six cysticercoid primary infections of mice treated with cortisone acetate (CA).

A(▲) = untreated control

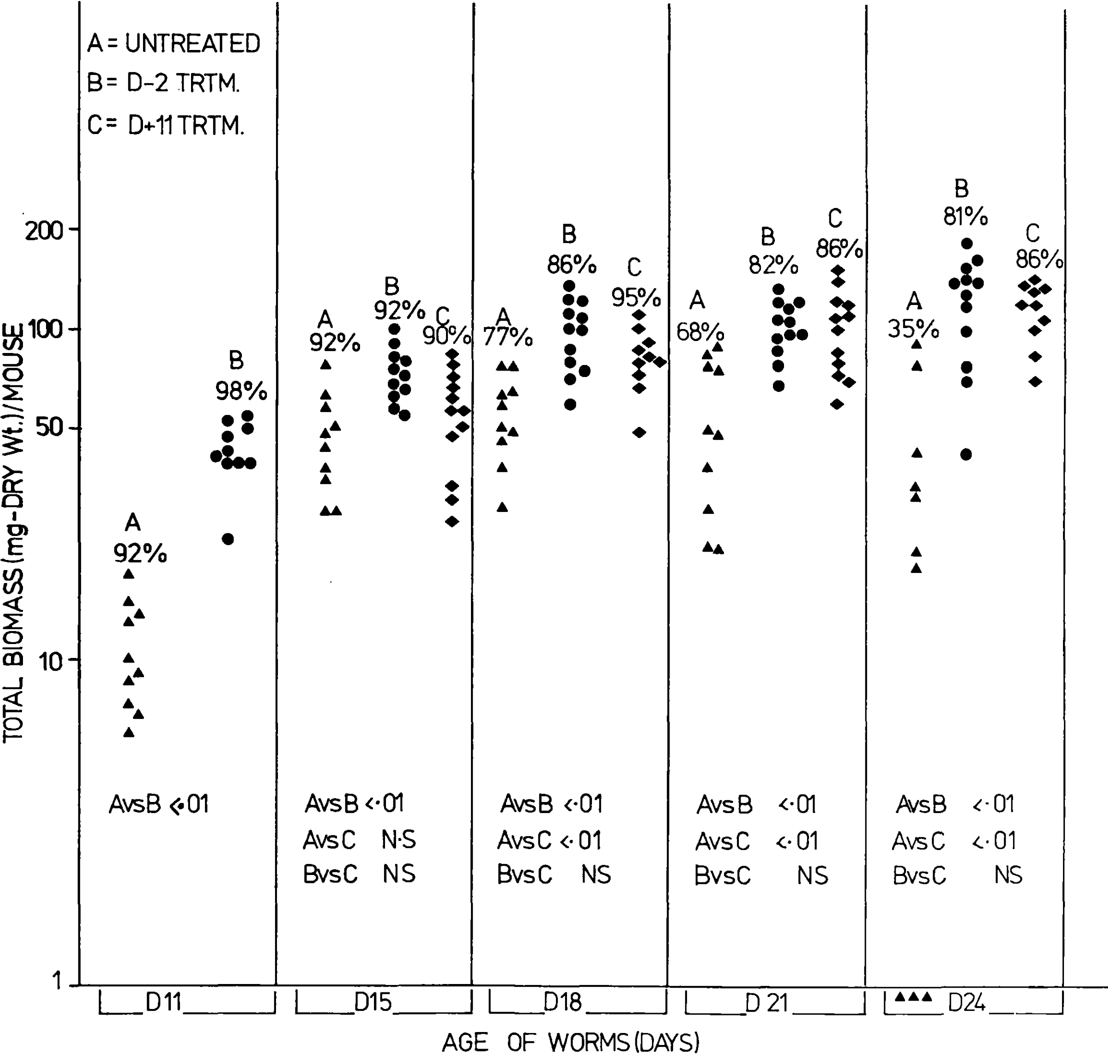
B(○) = treatment with CA from D-2 p.i.

C(◇) = treatment with CA from D+11 p.i.

Points (△) below abscissa represent mice from which stunted/destrobilated worms <0.2 mg were recovered.

Index indicates percentage recovery of >0.2 mg worms per group.

Values of P are indicated on graph.



A = UNTREATED
B = D-2 TRTM.
C = D+11 TRTM.
TOTAL BIOMASS (mg-DRY Wt.)/MOUSE
200
100
50
10
1
A 92%
B 98%
AvsB <·01
A 92%
B 92%
C 90%
AvsB <·01
AvsC N·S
BvsC NS
A 77%
B 86%
C 95%
AvsB <·01
AvsC <·01
BvsC NS
A 68%
B 82%
C 86%
AvsB <·01
AvsC <·01
BvsC NS
A 35%
B 81%
C 86%
AvsB <·01
AvsC <·01
BvsC NS
D11
D15
D18
D 21
D24
AGE OF WORMS (DAYS)

Facing page 67

Figure 3-2

Biomass of H. citelli worms per group of CFLP male mice treated with cortisone acetate (CA)

◇ - Primary infection (1°) treated with CA

□ - Secondary infection (2°) treated with CA

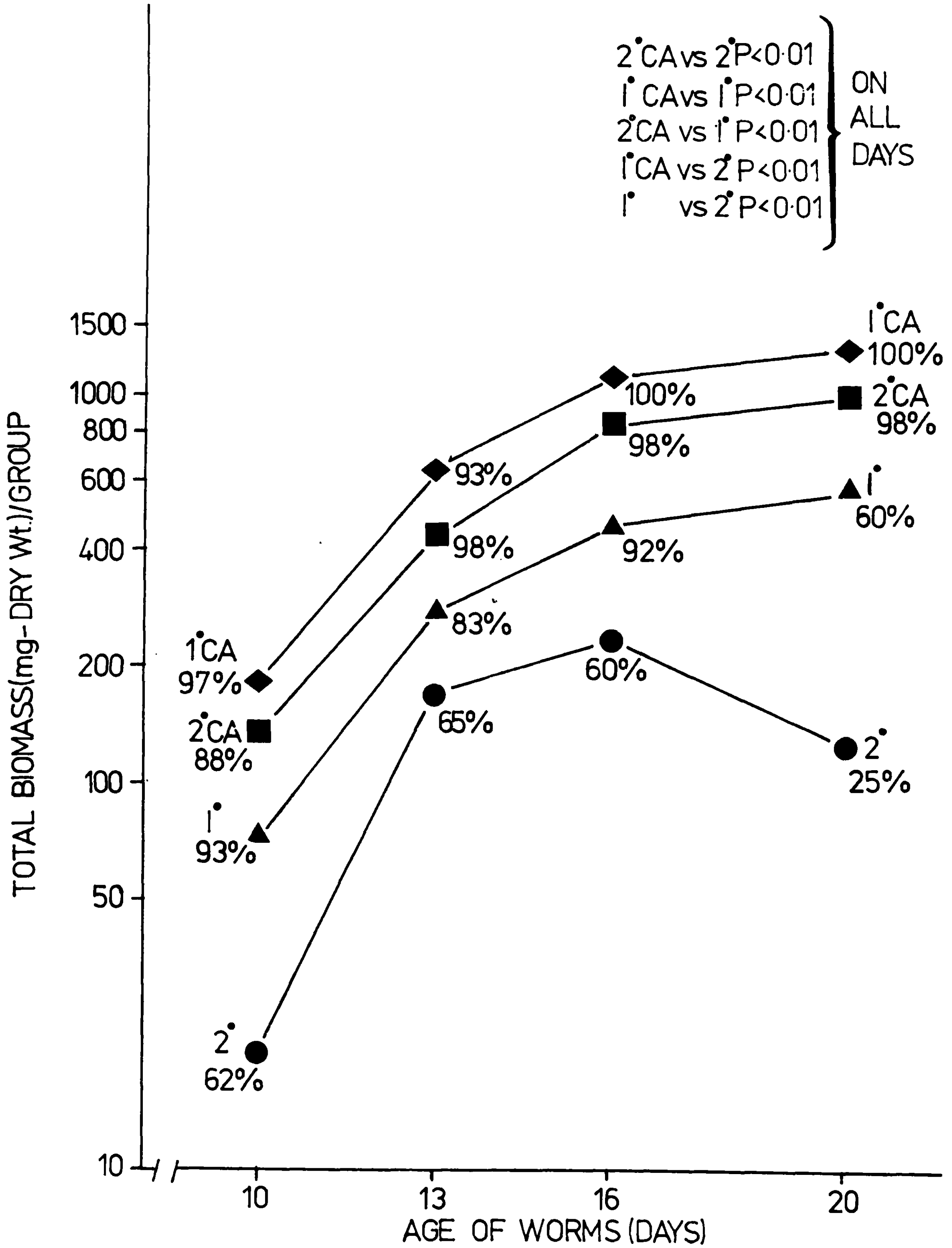
▲ - Primary infection (1°) untreated with CA

○ - Secondary infection (2°) untreated with CA

Each point represents the total weight of worms >0.2 mg per group.

Index indicates the percentage recovery of >0.2 mg worms per group.

n = 10 mice per group.



2° CA vs 2° P<0·01
1° CA vs 1° P<0·01
2° CA vs 1° P<0·01
1° CA vs 2° P<0·01
1° vs 2° P<0·01
ON ALL DAYS
1500
1000
800
600
400
200
100
50
10
TOTAL BIOMASS (mg-DRY Wt.)/GROUP
10
13
16
20
AGE OF WORMS (DAYS)
1° CA 97%
2° CA 88%
1° 93%
2° 62%
93%
98%
83%
65%
100%
98%
92%
60%
1° CA 100%
2° CA 98%
1° 60%
2° 25%

indicate that treatment of sensitised mice with cortisone acetate depresses the immune response to challenge - stunting/destrobilation was alleviated. There was virtually no worm loss in the cortisone treated groups.

DISCUSSION

The results demonstrate that treatment of mice with cortisone acetate prolonged the survival and enhanced the growth of a primary 6 cysticercoid H. citelli infection.

Hopkins and Stallard (1974) using cortisone, demonstrated that growth of H. citelli worms was enhanced in treated mice in comparison with control, untreated mice, and additionally postulated that the rejection of a primary infection was immunologically-mediated. Their results have been confirmed in this

study; and moreoever, delaying the administration of cortisone to mice harbouring a 6 cysticercoid primary infection until day 11 post infection, was equally effective in prolonging the survival of worms. This was interesting, because by day 11, the afferent arm of the response had presumably been stimulated as shown in Fig. 3-1, and yet the growth of worms in the treated mice was regained thereafter. Worms from cortisone treated mice were consistently heavier than worms from untreated control mice, except on day 15 in the category of mice in which treatment was not commenced until day 11 post infection.

The increase in growth of the worms from the cortisone-treated mice maybe attributable to: (a) non-specific suppression of the immune response (see review by Claman, 1975) and/or (b) the stimulation of the exocrino-enteric circulation of the host - resulting in increased feeding by the mice thereby affecting (enhancing) growth of worms (cf. Hopkins and Stallard, 1976 and Moss, 1972). Worms from cortisone-treated mice continued to grow until day 24, i.e. for as long as studied.

Similar enhancement in growth of worms from cortisone-treated mice has been reported for H. microstoma and H. diminuta infections, by Moss (1972) and Howard (1976b), and Hopkins et al., 1972b respectively. Moss (loc. cit.) using a 2 hour Passive Cutaneous

Anaphylaxis (P.C.A.) test reported negative results from serum collected on day 17 from cortisone-treated, H. microstoma infected CFLP mice. He concluded that this indicated an absence of circulating IgA (presumably he meant IgG_1!) and attributed the observed increase in growth of H. microstoma worms from cortisone-treated mice partly to a suppression of antibody production.

The persistence of primary H. citelli worms in mice given cortisone treatment, is similar to the increased survival of nematodes in cortisone-treated hosts (Ogilvie, 1965 for Nippostrongylus brasiliensis; Wakelin, 1970 for Trichuris muris and Moqbel, 1976 for Strongyloides ratti). Wakelin and Selby (1974) demonstrated an immunological unresponsiveness to Trichuris muris in mice after administering cortisone, at the time when the immune system was responding to the worm antigens. Immune tolerance was induced by injecting mice with cortisone acetate on days 8, 10, 12 and 14 post infection or by giving a single large dose on day 11 post infection. Enhanced survival of Aspiculuris tetraptera in mice, was obtained by short-term hydrocortisone treatment by (Behnke, 1975). Behnke (loc. cit.) suggested that a state of tolerance, due to the suppression of the recognition of antigens by the host was responsible for the protracted longevity. The results reported here have clearly demonstrated that cortisone acetate can suppress the rejection of a 6 cysticercoid primary

H. citelli infection even when administered as late as day 11 post infection. This is consistent with, and supports the hypothesis that, the rejection mechanism may be immune-mediated (Hopkins and Stallard, 1974).

The unequivocal demonstration of acquired immunity to homologous *H. citelli* challenge in CFLP male mice (see section 2 above) which is manifested mainly as stunting/destrobilation of secondary worms, and the refractoriness of deer-mice to re-infection as reported by Wassom *et al*. (1973 and 1974) led to the suggestion that retardation in growth of secondary worms was probably a consequence of an expression of a specific immune response. When immune mice were challenged and treated with cortisone acetate from Day 2 of the challenge infection and thereafter every 48 hours throughout the challenge infection, there was an enhanced survival of secondary worms as compared with challenge control worms (Fig. 3-2). 88%-98% of secondary worms were recovered as worms >0.2 mg from the cortisone-treated mice, whereas a 75% loss of worms (>0.2 mg) occurred in the challenge control mice by day 20. Virtually, no worm loss occurred in the primary, cortisone-treated control mice. These results are indicative of a complete suppression of the rejection mechanism (s) of mice by cortisone treatment.

The significance of immunosuppression can be visualised in natural populations of *H. citelli*; when during pregnancy and lactation, the immune status of hosts

maybe impaired (McLean *et al*., 1974) thereby increasing the longevity and burdens of worms, and indeed enhancing the susceptibility of resistant hosts. Studies on the immunodepressive effects of pregnancy and lactation in mice to *H. diminuta* (in relation to growth and survival) have already been described by Christie (1979), and there is room for such studies in *H. citelli* infections.

Stunting of secondary worms was suppressed by cortisone treatment; the biomass of worms from immune cortisone-treated mice was significantly higher on all days studied than in challenge control mice (Fig. 3-2). The biomass from immune mice on cortisone treatment was always lighter than that from primary control, cortisone-treated mice. These observations are indicative of a partial ablation of acquired immunity by cortisone treatment of immune mice. Probable explanations could be that a residual population of committed (sensitised) lymphocytes (see Claman, 1975) was still present in the immune mice treated with cortisone (cortisone resistant?) which weakly retarded growth, and/or that the enhanced growth in this category, was attributable to a compensatory nutritional effect due to increased feeding of the mice. Further studies are necessary to either refute or confirm this conjecture. However, the biomass from cortisone-treated immune mice was on all days studied higher than the biomass from primary, untreated control mice.

Evidence of corticosteroid treatment suppressing acquired immunity following a primary infection and resulting in successful establishment of challenge infections has been demonstrated in *Nippostrongylus brasiliensis* infections in rats (Ogilvie, 1965) , *Trichuris muris* in mice (Wakelin, 1970), *Strongyloides ratti* infections in rats (Moqbel and Denham, 1978) and *Nematospiroides dubius* infections in mice (Behnke and Parish, 1979a). It will be of interest to investigate whether a primary infection of *H. citelli* under cortisone treatment may completely or partially suppress the sensitisation of mice to *H. citelli* antigens. Thus impairing the acquisition of a protective response to a secondary infection.

The mechanism of action of the cortisone drug is multifaceted, it abrogates the immune response either by lysis, inhibition, depletion, suppression redistribution, destruction or otherwise of lymphocytic cell populations (North, 1971 and 1972, Claman, 1975, Cohen, 1971, Berenbaum, 1974, Dracott and Smith, 1979a and b). The specific action of cortisone on the obliteration of resistance to homologous *H. citelli* infections in mice, is as yet undefined. Wassom *et al*. (1974) suggested that resistance to *H. citelli* maybe a function of thymus-dependent lymphocytes in *Peromyscus maniculatus*, and it has also been demonstrated that there is thymus-dependency in immunity to *H. diminuta* (closely related parasite to

H. citelli); it may thus be partly plausible that the action of the drug interferes with the T-dependent step of the response. The possibility of the action of the drug on humoral responses is nevertheless not excluded, as Goodall (1973) and Befus (1975b) have discussed probable antibody roles in tapeworm immunity. Cortisone has other physiological effects, such as on the vascular activity which causes the inhibition of diapedesis (Ashton and Cook, 1952). No specific studies on the analysis of either cellular or humoral changes in the gut-associated lymphoid system were made during the course of this study. It is, however, not implausible that cortisone acetate exerted its effect on both the T and B-cell components of the response to H. citelli infections in mice. The role of specific suppression of T-cells e.g. by thymectomy and whole body irradiation and the use of anti-thymocyte serum, might help to elucidate the mechanism(s) of expulsion and that of acquired immunity in the H. citelli-mouse model.

SUMMARY

1. A literature review of the *Hymenolepis citelli* system is presented. The use of this model in the study of immunity to adult tapeworms is suggested.

2. The establishment, growth and survival of 1, 6, 12, and 24, cysticercoid primary infections are presented and discussed. The inherent variability in the system is also highlighted.

3. The dynamics of secondary infections are described. A primary infection of 1 and 6 cysts for 21 days immunise CFLP male mice against homologous challenge infections. The manifestations of acquired immunity is mainly as stunting/destrobilation of secondary worms. The severity of stunting of secondary worms depends on the intensity of the primary infection.

4. Sensitisation of mice for 7 days by 6 or 24 cysticercoid infections did not confer a measurable protective response; whereas sensitisation by the same regime for 21 days induces a significant protective response against a 6 cysticercoid homologous challenge.

5. Acquired immunity to homologous challenge wanes with time in the absence of the primary worms.

6. The growth and survival of a 6 cysticercoid primary infection is enhanced by the administration of the immunosuppressant cortisone acetate. Worms from cortisone-treated mice are heavier than those from untreated control mice. Acquired immunity to homologous challenge is partially ablated in cortisone treated mice. It is suggested that rejection of primary infections and stunting/destrobilation of secondary worms maybe immunologically-mediated.

CHAPTER 2

SECTION A

H. diminuta/H. citelli interaction

Introduction:

In the last two decades, a number of studies on cross-resistance interactions between parasites have been reported (Kazacos and Thorson, 1975, Kazacos, 1976). Singular infections are generally not the "norm" in nature, and the plurality of infections is of importance in understanding the interactions of parasites with each other, as well as with the protective mechanisms of hosts.

Cross-resistance studies between phylogenetically unrelated and related organisms in a host may have application in the control of zoonoses. Experimental demonstrations of cross-resistance between unrelated organisms have been reported, e.g. antibodies against the 'O' and 'H' components of typhoid organisms have been shown to be present in the sera of rabbits infected with *Trichinella spiralis* (Weiner and Price, 1956), and the existence of a heterogenetic cross-immune reaction between *T. spiralis* and *Salmonella typhi* somatic antigens has also been shown in mice and rats (Weiner and Neely, 1964). Increased resistance of pigs to *Ascaris suum* infection following the recovery from experimentally-induced transmissible gastro-enteritis (TGE) has

been observed - fewer foci of hepatic fibrosis and fewer adult worms were seen in TGE-infected pigs than in control pigs (Gaafar et al., 1973). Mice that were initially infected with Nippostrongylus brasiliensis have been shown to be resistant to subsequent Ascaris suum infection (Crandall et al., 1967a). Protection against Trichinella spiralis has been observed in rats primarily infected with N. brasiliensis (Louch, 1962 and Sinski, 1972), the reciprocal phenomenon has also been demonstrated by Kazacos (1975).

Cross-resistance studies between phylogenetically related organisms have also been reported, e.g. studies on homologous and heterologous immunisations against Taenia saginata and Taenia taeniaeformis in cattle and mice respectively have been described, using antigens obtained from both species of cestodes (Lloyd, 1979). Three month-old calves developed a protective immunity against T. saginata when immunised intramuscularly with the excretory/secretory products of oncospheres of the homologous or the heterologous parasite, T. taeniaeformis. Mice were protected against infection with T. taeniaeformis when immunised intramuscularly or orally with either a somatic antigen extracted from the metacestodes or an excretory/secretory antigen from the oncospheres of T. saginata or T. crassiceps. These observations may be relevant to the control of cysticercosis in cattle by active immunisation. Nelson

et al. (1968) utilising heterologous antigens demonstrated that an initial single exposure to either *Schistosoma bovis*, *S. mattheei* or *S. rhodainii* conferred a high degree of immunity against a challenge *S. mansoni* infection in mice. Smith *et al.* (1976) have shown cross-immunity between *S. mansoni* and *S. haematobium* in hamsters and furthermore, showed that antibodies in immune sera of hamsters from both infections were bound to common antigens on the surface of young schistosomulae of either species.

The maintenance of an ideal equlibrium in cestode host/parasite relationships is of paramount importance, relative to the survival of both host and parasite. This delicate balance can be disrupted by unfavourable conditions such as hibernation (Ford, 1972), anthelmintic treatment (Hopkins *et al.*, 1973) or/and by the induction and expression of host immunological responses (Gray, 1973, Andreassen *et al.*, 1978a, Bland, 1976a, Befus, 1975b and Howard, 1976b). Infection of a single host with two related cestodes, offers intriguing opportunities for studies on host/parasite interrelationships - the two parasite populations may interact

directly in competition for food (Read and Phifer, 1959) and/or location specificity signals (Hopkins, 1970; Hopkins and Allen, 1979, Crompton, 1973). An established infection may inhibit a challenge be it homologous or heterologous by limiting the establishment, growth or survival of the challenge, probably via an immune-mediated mechanism exerted by the host (cf. Premunition-Chandler, 1939). Studies on the immunological cross-reactivity between heterologous tapeworm infections in mice have been described by Weinmann (1966) for *H. nana*, *H. diminuta* and *H. citelli*, and also by Hopkins *et al*. (1977) between *H. diminuta* and *H. microstoma*. To provide further information on the nature of the cross-protection that may exist between closely-related *Hymenolepis spp*.; it was decided to investigate the *in vivo* interactions between *H. citelli*, *H. diminuta* and *H. microstoma*: in particular, whether heterologous protection exists between the species in mice.

Materials and Methods

No further description in addition to that already given in the General materials and methods is necessary here. 4-star CFLP male mice were purchased from Anglia Laboratories, Huntingdon, and used for experimentation when 6 weeks ±2 days old at time of infection.

Results

The objectives of the experiments were to investigate:

a) the nature of the cross-protection (if any) between <u>H. diminuta</u> and <u>H. citelli</u>,

b) the reciprocal of the response

and

c) the effect of a concurrent primary infection of <u>H. diminuta</u> and <u>H. citelli</u> on the survival of the latter parasite.

Immunisation protocols:

a) <u>H. diminuta vs. H. citelli</u>

Objective:

Does a primary 6 cysticercoid <u>H. diminuta</u> infection affect the growth and survival of a 1 and 6 cysticercoid <u>H. citelli</u> heterologous challenge?

Group	Day 0	D8	D16	D26	D36	D41
i)	-	-	Z	1Hc	K	K
ii)	-	-	Z	6Hc	K	K
iii)	6Hd	-	Z	1Hc	K	K
iv)	6Hd	K	Z	6Hc	K	K

K = Autopsy of 10 mice

Hc = <u>H. citelli</u> cysticercoid

Hd = <u>H. diminuta</u> cysticercoid

Z = 'Zanil' (250 mg/kg/mouse)

The results of the dry weight of worms per mouse in a group and the recovery (%) of >0.2 mg worms per group are shown in Fig. 4-1.

$^{60}/60$ worms wer recovered on day 8, indicating 100% establishment of the immunizing infection.

The results show that the biomass of single H. citelli challenge worms from H. diminuta immunised mice were not significantly different from controls on day 10, although by day 15, significantly ($p<0.05$) lighter worms were obtained from the immunised mice. The data from the 6 cysts challenge group indicate that immunised mice harboured significantly lighter worms than controls ($p<0.01$) and that the degree of suppression was greater on day 15 than on day 10 p.i.

b) Reciprocal response

H. citelli vs. H. diminuta

Objective:

Does a primary 6 cysticercoid H. citelli infection affect the growth and survival of a 3 cysticercoid H. diminuta heterologous challenge?

A third group of mice previously infected with H. diminuta cysts was added to the experimental groups (see below), so as to assess the relative immunogenicity of the homologous response.

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Figure 4-1

Dry weight of H. citelli from single and six cysticercoid challenge infections of CFLP male mice given a six cysticercoid H. diminuta primary infection.

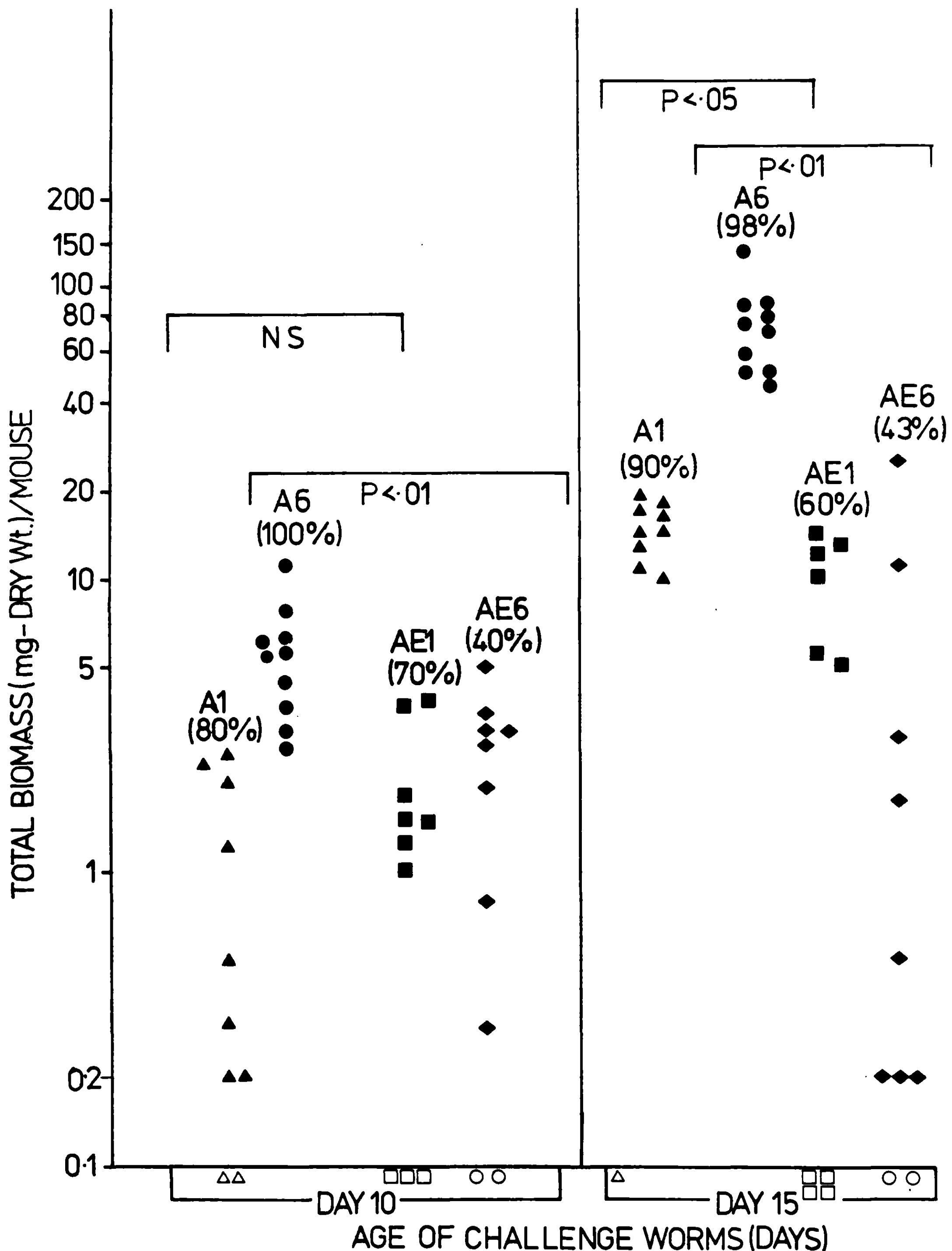
A1 (▲) = single cyst control

AE1 (□) = single cyst challenge

A6 (○) = six cysticercoid control

AE6 (◇) = six cysticercoid challenge

Index indicates percentage recovery of >0.2mg worms per group. Points below abscissa represent mice from which destrobilated/stunted worms totalling <0.2 mg were recovered.


TOTAL BIOMASS (mg- DRY Wt.)/MOUSE
200
150
100
80
60
40
20
10
5
1
0·2
0·1
NS
P<·01
A1 (80%)
A6 (100%)
AE1 (70%)
AE6 (40%)
P<·05
P<·01
A6 (98%)
A1 (90%)
AE1 (60%)
AE6 (43%)
DAY 10
DAY 15
AGE OF CHALLENGE WORMS (DAYS)

Protocol:

Group	Day 0	D8	D12	D21	D31	D39	D43
i)	-	-	-	Z	3Hd	K	K
ii)	6Hc	ND	K	Z	3Hd	K	K
iii)	3Hd	K	ND	Z	3Hd	K	K

K = Autopsy of 8 mice

Z = 'Zanil' (250 mg/kg/mouse)

ND = Not done

$^{23}/_{24}$ worms were recovered from the H. diminuta infection on day 8, and $^{46}/_{48}$ worms from the H. citelli infection on day 12, indicating 96% establishment of both immunizing infections.

The results of the total biomass and the recovery (%) of >0.2 mg worms per group for the challenge infections are shown on the histograms in in Fig. 4-2. The results demonstrate that mice previously immunised with H. citelli were protected against H. diminuta challenge, indicating the reciprocal response of the previous experiment. The protective response in the heterologous infection was 61% and 54%, and in the homologous infection (i.e. Hd vs. Hd) was 87% and 65% on days 8 and 12 respectively.

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Figure 4-2

Total biomass per group from three cysticercoid *H. diminuta* challenge infections of CFLP male mice given six cysticercoid *H. citelli* and three cysticercoid *H. diminuta* primary infections (1°).

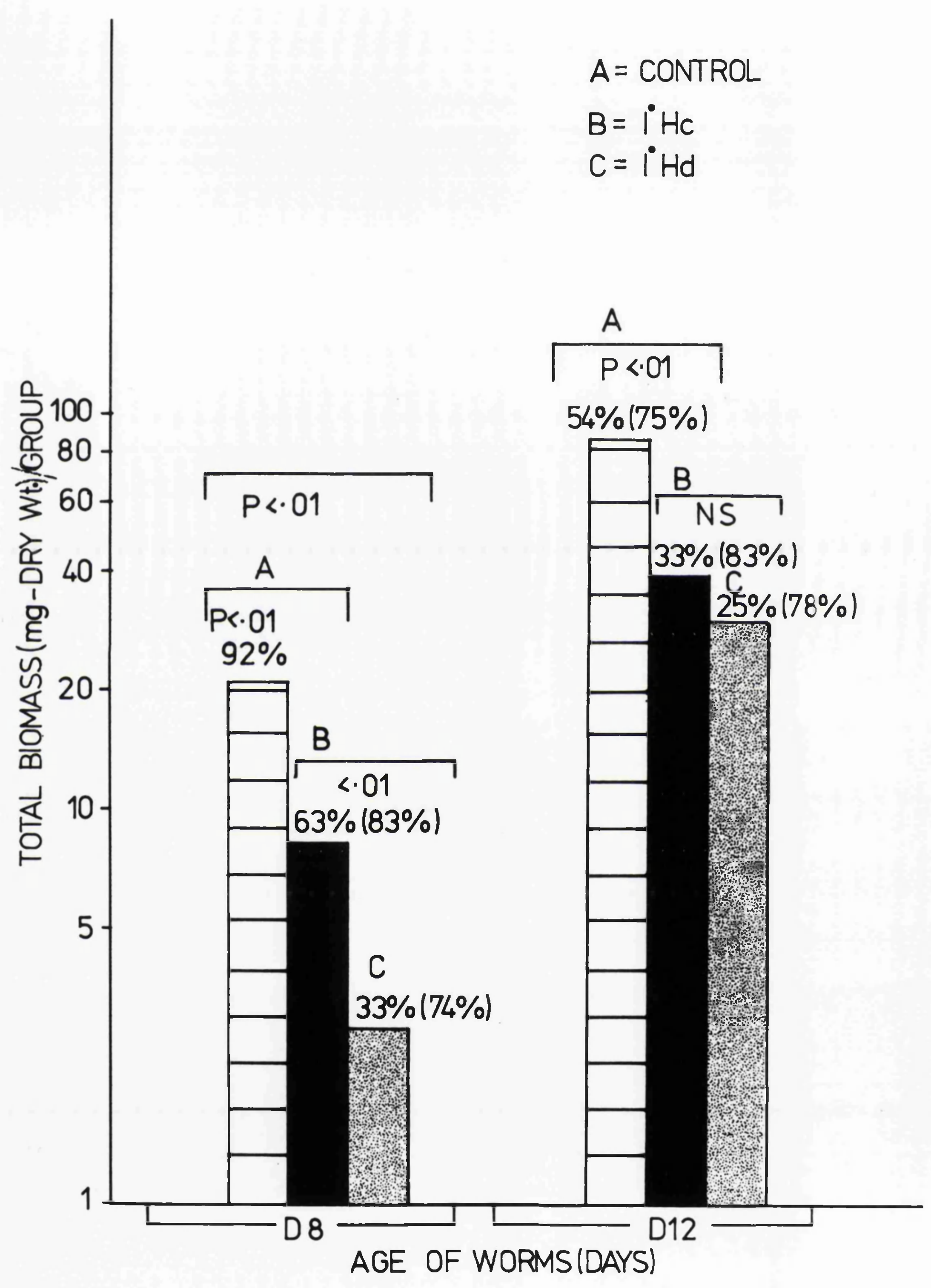
A = Control (naive)

B = 1° x 6 cysts *H. citelli*

C = 1° x 3 cysts *H. diminuta*

Index indicates percentage recovery of >0.2 mg worms per group. Numbers in parentheses indicate the total worm recovery (i.e. inclusive of worms <0.2 mg) per group.

n = 8 mice


A= CONTROL
B= I° Hc
C= I° Hd
TOTAL BIOMASS (mg-DRY Wt.)/GROUP
100
80
60
40
20
10
5
1
P<·01
A
P<·01
92%
B
<·01
63% (83%)
C
33% (74%)
A
P <·01
54% (75%)
B
NS
33% (83%)
C
25% (78%)
D8
D12
AGE OF WORMS (DAYS)

c) Concurrent infection (*Hd/Hc*)

The course of a primary *H. citelli* and *H. diminuta* infections has been studied in CFLP mice of similar age and sex. A primary 6 cysticercoid *H. citelli* infection is rejected more slowly (17-23 days - Hopkins and Stallard, 1974) than a primary 6 cysticercoid *H. diminuta* infection (13±2 days - Hopkins *et al.*, 1972a and Befus, 1975b). This may suggest that either *H. citelli* is a weaker immunogen, or that it stimulates an equally good response as that of *H. diminuta* but can partially evade the effector arm of the response (cf. *H. microstoma* - Howard, 1976b). In an attempt to investigate whether *H. citelli* can partially evade the effector arm of the response, a concurrent infection with *H. diminuta* was used to assess the susceptibility (i.e. survival) of the former parasite to the response initiated by the latter.

Before the actual concurrent experiment was conducted, a pilot experiment was done to firstly establish the course of a primary 3 cysts *H. diminuta* infection as this information was lacking for the time of year. Various workers at the Wellcome Laboratories, have observed (unpublished results), that for some, as yet unidentified reasons there is a slight seasonal variation in rejection times vis-a-vis *H. diminuta* infections.

The results obtained indicated that by day 15, there was a complete rejection of a primary 3 cysts *H. diminuta* infection. Based on this information, the following experimental protocol was undertaken. A fourth group of mice with a concurrent *Hd*/*Hc* infection was further treated with cortisone, so as to assess the probable involvement of an immune response.

Objective:

Does a primary 3 cysticercoid *H. diminuta* infection simultaneously throw out a concurrent primary 3 cysticercoid *H. citelli* infection during its rejection phase?

Protocol:

Group	Day 0	D4	D12	D15	D16
i)	3Hd	-	K	K	K
ii)	-	3Hc	ND	K	K
iii)	3Hd	3Hc	ND	K	K
iv)	3Hd	*3Hc	ND	ND	K

K = Autopsy of 10 mice

* Group (iv) mice treated with cortisone acetate (1.25 mg) every 48 hours commencing from Day 4 - D16.

ND = Not done

Hd = *H. diminuta*

Hc = *H. citelli*

The results of the survival of worms are shown below in Table 1.

Table 1

Group	D12	D15*	D16*
Hc (control)	-	67%	81%
Hd (control)	87%	13%	0%
Concurrent group (Hd/Hc)			
H. citelli	-	10%	3%
H. diminuta	-	20%	10%
Concurrent group treated with corticone			
H. citelli	-	-	95%
H. diminuta	-	-	85%

* Note age of H. citelli worms in the concurrent infection is D11 and D12 p.i.

The results show that during the rejection phase of H. diminuta a concurrent H. citelli infection is simultaneously thrown out. It is concluded that H. citelli worms are susceptible to, and unable to evade the effector arm of the response initiated by a generically closely-related parasite. The immunosuppressive effects of cortisone in enhancing the survival of both parasites suggests that an immunological interaction may have been involved.

DISCUSSION

The results reported above demonstrate that a cross-protective mechanism exists in mice previously infected with H. diminuta or H. citelli against heterologous challenge by either species. The phenomenon of cross-protection in tapeworm infections is best explained by assuming the development of immunological memory for specific antigen(s) common to the two parasites. Coleman et al. (1968) have demonstrated the sharing of at least 3 similar antigens between H. diminuta and H. nana, and Goodall (1973) has reported cross-reacting antibodies between H. citelli and H. diminuta using antisera from immunised rabbits.

The data from the experiment in which H. diminuta was used to immunise mice against H. citelli (Fig. 4-1), showed a considerable range in the ability of mice to respond to heterologous challenge. The variability is not unexpected in mice from an outbred colony (Wakelin, 1975b). In the 6 cysts H. citelli challenge on day 15, all the mice showed a degree of protection which led to 94% decrease in the biomass of secondary worms relative to controls; whereas on day 10, the protection (66%) although significant ($p < 0.01$) was less marked (5 of the 8 immunised mice harbouring worms > 0.2 mg, had a biomass comparable to that of controls). In the single cyst challenge, the response evoked on day 15 was just signifi-

cantly different ($p < 0.05$) from controls and comparatively weaker than that observed in the 6 cysts challenge. The difference in the degree of protection observed with the challenge level may be interpreted as showing that the response evoked by H. citelli in H. diminuta immunised mice, is related to the number of worms in the secondary infection; a challenge with 1 cyst being much less effective than with 6 cysts.

The degree of protection stimulated against H. diminuta when mice were immunised by a heterologous H. citelli infection, was much less than that induced by a homologous H. diminuta infection (Fig. 4-2). The data suggest that H. citelli may be a weaker immunogen than H. diminuta, as the level of protection recorded even from a 6 cysticercoid H. citelli immunisation was less than that from a 3 cysticercoid H. diminuta immunisation. This may be because the two worms do not have, either qualitatively or quantitatively, precisely the same "protective" antigens. Alternatively, the worms may share similar antigens but the position they occupy in the gut (in the case of H. citelli, from mid-jejunum to the ileum, and H. diminuta rather more anteriorly) could mean that the uptake or processing of antigenic material, or the response elicited is different in these regions. Whatever the explanation, it has been noted by various workers that immunizing with a heterologous species is less effective than with a homologous species. For example, Dineen et al. (1977)

have shown that when 6-8 months-old Merino ewes were vaccinated with irradiated *Trichostrongylus colubriformis* and then challenged, a higher level of protection (81%) was obtained against homologous infection than against challenge with the generically related *T. vitrinus* (34%).

In view of the fact that specific and/or non-specific factors may be involved in immunological protective mechanisms (Larsh and Race, 1975; Dineen *et al*., 1977; Wakelin and Wilson, 1979a and b) it is suggested that the cross-resistance between *H. citelli* and *H. diminuta* observed during this work, may have arisen from a specific immunological interaction involving the sharing of common antigens. In conclusion, the information reported above should prove useful in the design of vaccination experiments in order to distinguish between specific or non-specific factors that might be involved in the protective mechanism(s) of mice against tapeworm infections. The use of heterologous immune cell-transfer studies is strongly advocated so as to augment the concept of a true immunological interaction.

SECTION B

H. microstoma/H. citelli interaction

H. microstoma is closely related to H. diminuta and H. citelli, with its scolex attached in the bile duct in a mouse (Lumsden and Karin, 1970; Howard, 1976b). Whether this site of attachment is sequestered from the host's immune response is unknown. The parasite is long-lived (Litchford, 1963) and although not normally rejected in a primary infection (<5) in a mouse, is strongly immunogenic (Howard, 1976a). In homologous challenge infections in mice, worm growth is severely retarded during the first 4 days of infection but thereafter once the scolex is inside the bile duct, the growth rate of secondary worms progressively becomes identical to that of primary worms (Howard, 1976b). This Howard (1977) suggested as an "adaptation" of the secondary worms to the host's immune response.

The objectives of the experiments described below, were to investigate whether:

a) H. citelli worms can survive and grow in mice immune to H. microstoma

and

b) the extent to which the reciprocal response is manifested.

Materials and Methods

No further description in addition to that already given in the General materials and methods is necessary here. 4-star CFLP male mice were purchased from Anglia Laboratories, Huntingdon, and used for experimentation when 6 weeks ± 2 days old at time of infection.

Results

The question posed was: Does a primary 6 cysticercoid H. microstoma infection affect the growth and survival of a 6 cysticercoid heterologous H. citelli challenge?

Protocol

Group	Day 0	D15	D21/22	D32	D42	D45	D48	D50
i)	-	-	Z/Z	6Hc	K	K	K	K
ii)	6Hm	K	Z/Z	6Hc	K	K	K	K

Hm = H. microstoma cysticercoid

Hc = H. citelli cysticercoid

K = Autopsy of 10 mice

58/60 worms (97%) were recovered on day 15, as a check on the establishment of the immunizing infection.

The results of the total biomass per group recovered from control and immunised mice are represented on the histograms in Fig. 4-3. The recovery (%) of >0.2 mg worms per group is also indicated. The results show how strongly mice immunised with H. microstoma are protected against H. citelli challenge. The reduction in biomass of all worms recovered from immune mice (days 10-19) vis-a-vis control mice was 91% ($p<0.01$). The results of the recovery of H. citelli maybe indicative of a reduced establishment e.g. on day 10 (see Fig. 4-3) in the

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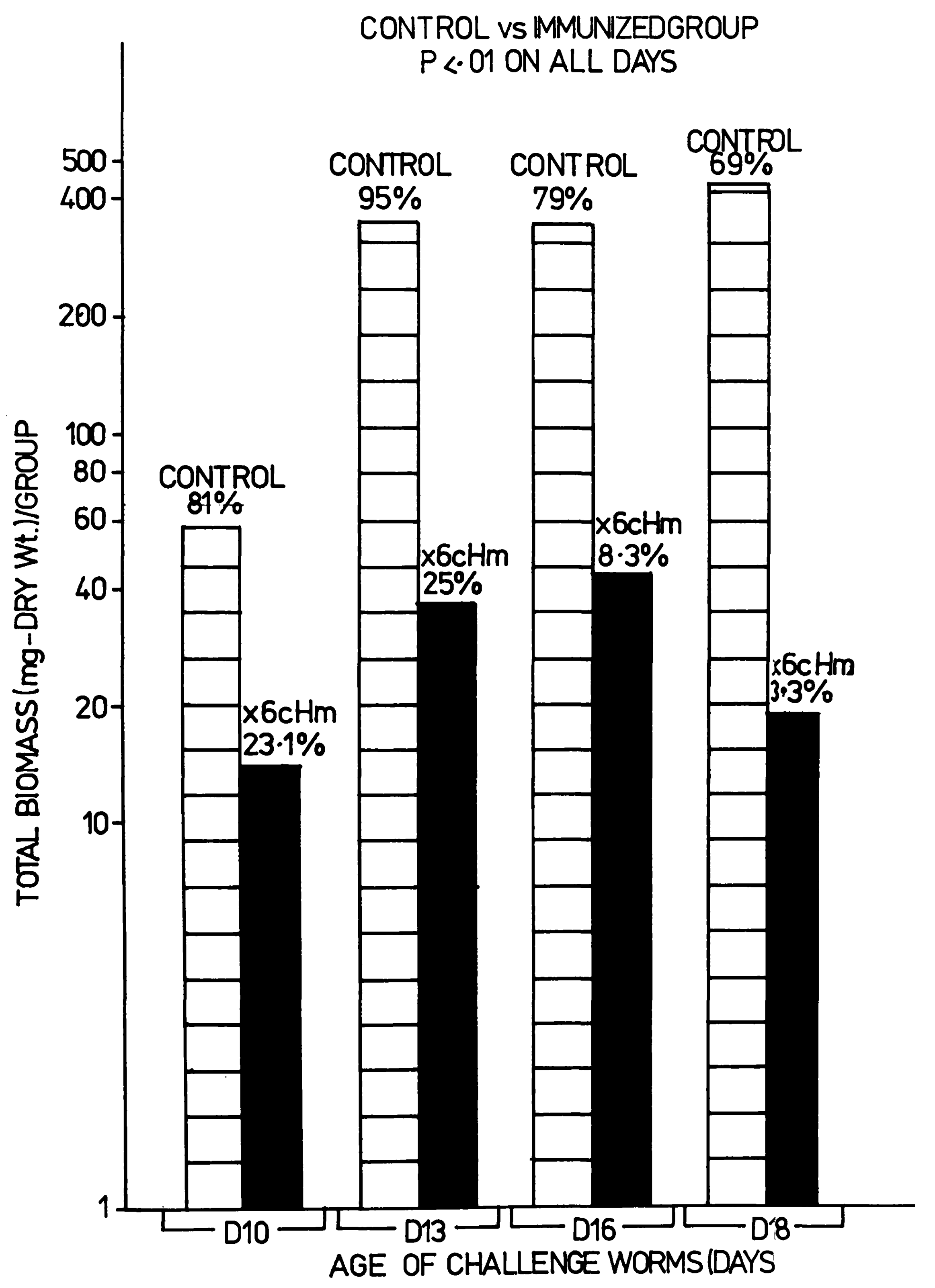
Figure 4-3

Total biomass per group from six cysticercoid *H. citelli* challenge infections of CFLP male mice given a six cysticercoid ( ) *H. microstoma* primary infection and controls ( ).

Index indicates percentage recovery of >0.2 mg worms per group.



CONTROL vs IMMUNIZEDGROUP
P <·01 ON ALL DAYS
500
400
200
100
80
60
40
20
10
1
TOTAL BIOMASS (mg-DRY Wt.)/GROUP
CONTROL
81%
x6cHm
23·1%
CONTROL
95%
x6cHm
25%
CONTROL
79%
x6cHm
8·3%
CONTROL
69%
x6c Hm
3·3%
D10
D13
D16
D18
AGE OF CHALLENGE WORMS (DAYS

immunised group only 23% of >0.2 mg worms were recovered in comparison with 81% from the control group.

To verify whether this low recovery was indeed reflective of a reduced establishment, a subsequent experiment was conducted using the same immunisation protocol as before. This time, however, each of the immunised mice was given cortisone acetate (1.25 mg/mouse - see General methods) commencing on day 0 of the challenge infection until day 10 post infection every 48 hours.

The results obtained from this experiment showed that when both groups of control and immunised mice were killed on day 10 p.i., 93% ($^{56}/60$) of the challenge worms were recovered, whereas from the control group (not given cortisone) 90% of the administered worms were recovered. As was expected, the worms from cortisone treated immunised mice were significantly heavier than those from control mice. Thus the data demonstrate that in mice previously immunised with H. microstoma, the establishment of H. citelli challenge worms was comparable to that of naive (uninfected) mice. It is concluded that the previous low recovery of >0.2 mg worms from H. microstoma immunised mice (Fig. 4-3) was due to severe stunting/destrobilation of H. citelli challenge worms.

Reciprocal response:

H. citelli vs H. microstoma

The objective was: Does a 6 cystcercoid primary *H. citelli* infection affect the growth and survival of a 6 cysticercoid *H. microstoma* challenge?

In view of the suggested "evasiveness" of *H. microstoma* worms to the immune response initiated by a homologous infection (Howard, 1976b), two other groups of mice were each infected (as indicated in the protocol below) with:

a) 12 *H. citelli* cysticercoids to assess whether *H. microstoma* may be more susceptible to a relatively stronger *H. citelli*-stimulated response

and

b) 6 *H. microstoma* cysticercoids, to evaluate the relative immunogenicity of the homologous response.

Protocol

Group	Day 0	D14	D21/22	D32	D42	D47
a)	-	-	Z/Z	6Hm	K	K
b)	6Hc	-	Z/Z	6Hm	K	K
c)	12Hc	-	Z/Z	6Hm	K	K
d)	6Hm	-	Z/Z	6Hm	K	K

Hc = *H. citelli* cysticercoids

Hm = *H. microstoma* cysticercoids

K = Autopsy of 10 mice

Z = 'Zanil' (250 mg/kg/mouse)

The results of dry weight of >0.2 mg worms recovered per mouse and the recovery (%) of >0.2 mg worms per group are shown in Fig. 4-4. The results indicate that mice previously immunised with H. citelli were protected against H. microstoma challenge infections. Although the protective response in the 6 cysts H. citelli immunised category was not statistically significant on day 10, the percentage reduction in the total biomass recovered from the immunised mice relative to that from control mice (i.e. protective response) was 31%. The protective response in the 12 cysts H. citelli immunised category was 35%, and from the H. microstoma immunised category (i.e. homologous challenge) it was 50%. By day 15, the protective response had diminished to 18%, 16% and 33% in the 6Hc, 12Hc and 6Hm immunised categories respectively, vis-a-vis the control group. This indicates the probable "adaptation" of H. microstoma worms to the protective response initiated either by a heterologous (H. citelli) or a homologous (H. microstoma) infection.

The worm recoveries (>0.2 mg) in all the categories, throughout the experiment were similar.

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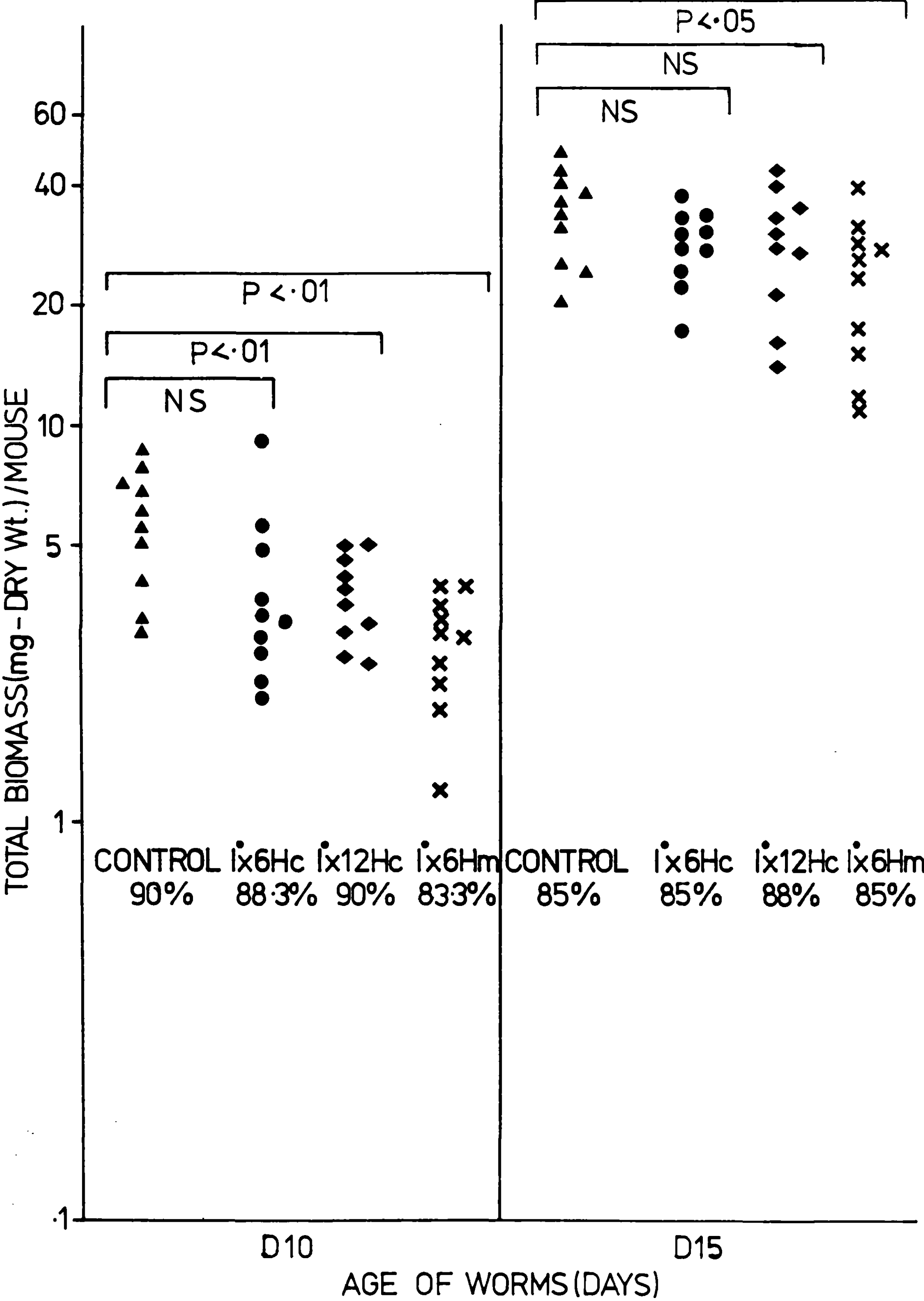
Figure 4-4

Dry weight of H. microstoma from six cysticercoid challenge infections of CFLP male mice given a six (6Hc) and twelve (12Hc) H. citelli and a six (6Hm) H. microstoma primary infections (1°) and controls.

Percentage recovery of worms >0.2 mg per group is indicated on graph.



TOTAL BIOMASS(mg-DRY Wt.)/MOUSE
60
40
20
10
5
1
·1
P <·01
P<·01
NS
P<·05
NS
NS
CONTROL
90%
Ix6Hc
88·3%
Ix12Hc
90%
Ix6Hm
83·3%
CONTROL
85%
Ix6Hc
85%
Ix12Hc
88%
Ix6Hm
85%
D10
D15
AGE OF WORMS (DAYS)

DISCUSSION

The results demonstrate that a cross-protective response exists between H. microstoma and H. citelli in mice. The strong protection of mice previously infected with H. microstoma and challenged with H. citelli indicates the relative susceptibility of H. citelli to the protective response initiated by another Hymenolepis sp.. The present observation is comparable to the response initiated by a 6 cysticercoid H. microstoma infection against a 1 cyst H. diminuta challenge (Hopkins et al., 1977): in which the total biomass recovered from H. microstoma immunised mice weighed less than 5% that of control mice.

Interestingly, the response induced by H. citelli against H. microstoma was comparatively weaker than that initiated by H. microstoma against H. citelli. Also of interest was the decline in the protective response with time, in the experiment (Fig. 4-4) where H. citelli and H. microstoma were used to immunise mice against H. microstoma; lending support to the results of Howard (1976b) in which he proposed that H. microstoma can partially evade the effector arm of the secondary homologous response. The intriguing mechanism(s) by which H. microstoma evades the immune response stimulated by both a heterologous (this work) and homologous infections is yet unidentified, although

Hopkins et al. (1977) suggested possible explanations. These included a) the probable "sequestration" of the scolex in the bile duct (although it does not explain why the rest of the strobila hanging in the small intestinal lumen is unaffected by an immune response), and b) that H. microstoma may be able to repair "immune damage" and camouflage its surface. Befus (1975b) has reported the occurrence of surface immunoglobulins on H. microstoma worms, but whether these molecules are antiparasitic is equivocal. Further work is indeed necessary to elucidate the precise mechanism(s) by which this strongly immunogenic tapeworm probably "disguises" itself against attack by effector molecules or cells.

The above results reflect the significance of the cross-protective responses that exist between H. citelli and H. microstoma. It is suggested that, in the furtherance of work on immunity to tapeworms, the use of a mixed inoculum of "Hymenolepis antigens" in the protocol of vaccination studies against tapeworms could be worthwhile - in that it may have a broad protective spectrum.

SECTION C

Nematospiroides dubius/H. citelli interaction

INTRODUCTION

Nematospiroides dubius causes a chronic primary infection in the small intestine of the mouse, in which host it survives up to 8 months without any reduction in fecundity (Ehrenford, 1954; Bartlett and Ball, 1972; Hagan, personal communications). In the last decade much information has been documented about the sequence of events which initiate and effect the immune expulsion of some parasitic nematodes (Ogilvie and Love, 1974; Wakelin, 1975a) although detailed analyses of the evasive mechanisms employed by parasites to circumvent host immune responses are still forthcoming (Ogilvie and Wilson, 1976). It has been demonstrated that mice with N. dubius infections, have depressed responses to concurrently administered unrelated antigens, in comparison with uninfected control mice. Both the primary and anamnestic antibody responses to orally or parenterally administered sheep erythrocytes have been shown to be markedly depressed during infection with N. dubius (Shimp, Crandall and Crandall, 1975); equally the immune response to influenza virus in mice has been shown to be depressed (Chowaniec, Wescott and Congdon, 1972). It could be that the non-specific immunosuppressive effects may be a side effect of a mechanism for increasing the survival of the parasite in a host.

The demonstration of acquired immunity to homologous challenge in N. dubius infections (Prowse et al., 1978a and b; Behnke and Parish, 1979a and b; Hagan, 1980) shows that the afferent arm of the response is stimulated and that it is the effector mechanism of the response that is blocked or interfered with in a primary infection, causing a prolonged survival of the parasite (Ogilvie and Love, 1974; Behnke and Parish, 1979b). In outbred mice, immunity is acquired after repeated infections (Cypess and Zidian, 1975). Behnke and Wakelin (1977), Prowse et al. (1979) and Hagan (1980) have shown that inbred mice acquire resistance to this parasite, and their findings are indicative of the immunogenicity of larvae. Jenkins (1977) has reported the immunologically-mediated rejection of adult N. dubius from the intestine of jirds, and importantly demonstrated that truncated adult infections stimulate acquired immunity in this host.

It was decided to study the phenomenon of N. dubius immunosuppression relative to its effect on a cestode, in particular using the nematode infection as a tool (i.e. biological suppressor) to analyse the response to H. citelli. Both the nematode and the cestode parasites live in the small intestine, favouring an interaction between the two parasites. The aim of this study was to determine, a) whether in a concurrent infection in mice, N. dubius could interfere with the immune expulsion of H. citelli, in particular whether

the efferent arm of the response initiated by the cestode could be impaired; (in order to prevent non-specific interference with the establishment of the cestode in this experiment, infection with N. dubius was given 7 days post H. citelli, by which time the tapeworm would have established normally).

b) whether the induction of a primary response against the cestode may be partially or completely inhibited

and

c) whether the expression of acquired immunity to H. citelli may be abrogated by a concurrent secondary H. citelli/N. dubius infection in NIH male mice.

In the latter two experiments, infection with N. dubius was on the same day as H. citelli).

Materials and Methods

No further description of infection and autopsy procedures for the cestode infection is necessary here (see General methods). 3-star, male NIH mice were ordered from Hacking and Churchill, Huntingdon, when 5 weeks ± 2 days old and infected when 6 weeks ± 2 days old.

Nematospiroides dubius: The strain of *N. dubius* used was obtained in 1975 from the Wellcome Research Laboratories, Beckenham. The parasite was maintained as stock infection in outbred CFLP male mice at the Wellcome Laboratories (Glasgow). Mice were orally infected with the required number of third stage larvae in 0.2 ml suspension. Infectivity of the cultures used in these experiments was assessed by worm counts, 10-12 days after infection. Mice were killed as described in the General methods and worms recovered by a Baermann technique (Wakelin and Lloyd, 1976). After the incubation period (4-6 hours), 1 ml of formalin was added to each flask and these were then stored at 4°C until examined. Worms were transferred to a Petri dish and counts made under a binocular dissecting microscope.

Anthelmintic: The drug used for the removal of adult *N. dubius* from infected mice was Pyrantel embonate (Strongid-P paste, Pfizer Ltd.) administered orally as an aqueous suspension at a dose of 100 mg/kg body weight (Hagan, 1980).

Results

The objective of the experiment was: Does a concurrent *N. dubius* larval infection affect the growth and survival of a 6 cysticercoid primary *H. citelli* infection in NlH mice?

Protocol

Group	Day 0	D7	D11	D14	D20	D25	D31
a)	6Hc	-	K	K	K	K	K
b)	6Hc	340 N.d	K	K	K	K	K
c)	-	340 N.d	K	K	K	K	K

Hc - cysticercoid infection (*H. citelli*)

N.d - Third stage larval infection (*N. dubius*)

K = Autopsy of 8 mice

In all of the experiments reported here, between 90-98% of the administered *N. dubius* dose established. *H citelli* had no apparent effect on the establishment or recovery of the nematode in either the concurrent primary or secondary infections. The results of counts from the *N. dubius* infections have therefore been omitted from the results.

The results of worm recovery vis-a-vis the cestode infection are shown in Fig. 4-5. The data show a delayed rejection of a six cysticercoid primary *H. citelli* infection in mice concurrently infected with

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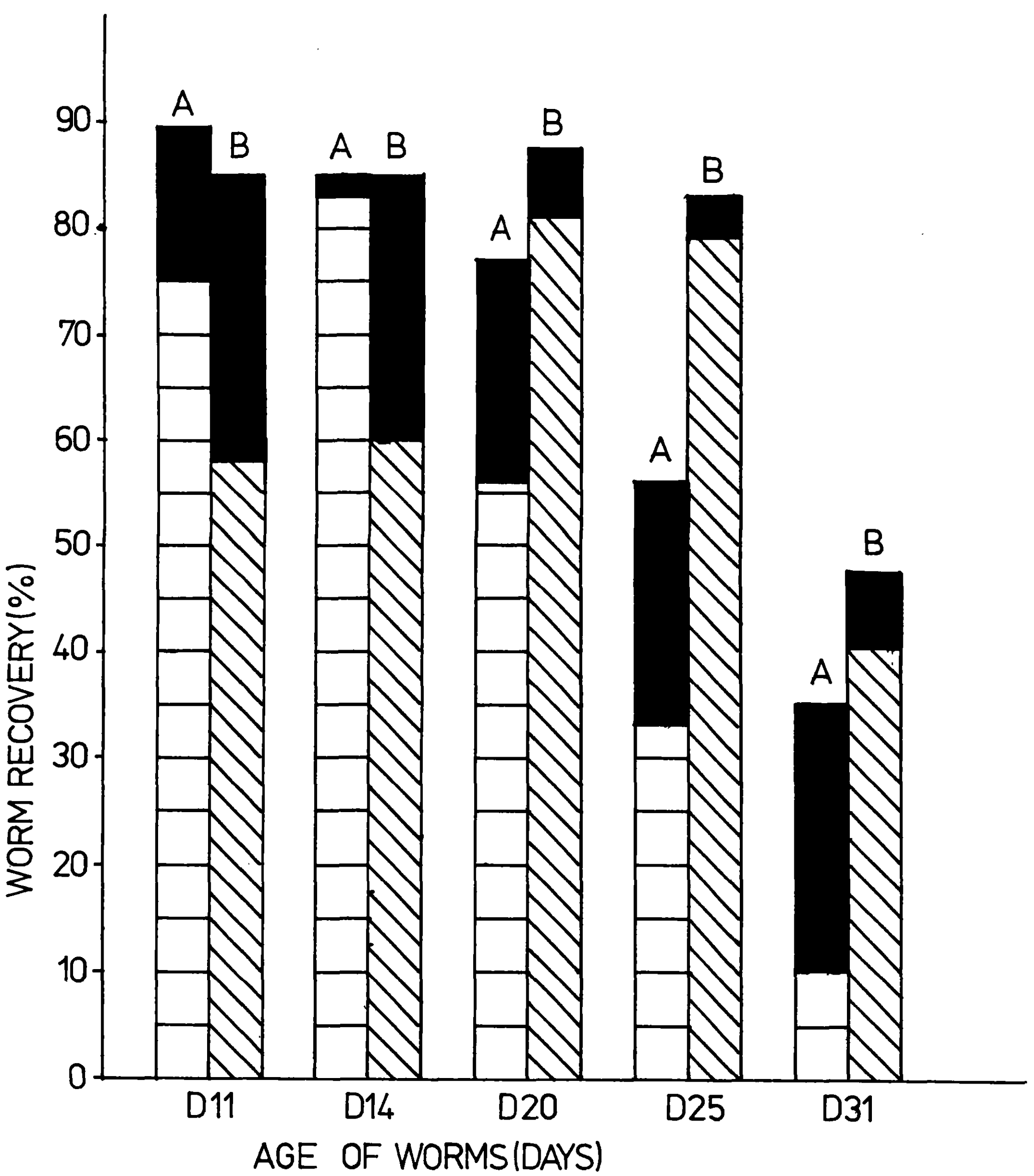
Figure 4-5

Percentage recovery of *H. citelli* worms >0.2 mg from a six cysticercoid primary infection of NIH male mice concurrently infected with 340 *Nematospiroides dubius* larvae.

A = Control (i.e. *H. citelli* only)

B = Concurrent group (i.e. *Hc*+*Nd*)

Shaded area represents recovery of worms <0.2 mg per group.



A = CONTROL GROUP
B = CONCURRENT GROUP
WORM RECOVERY (%)
0
10
20
30
40
50
60
70
80
90
A
B
D11
D14
D20
D25
D31
AGE OF WORMS (DAYS)

N. dubius. By day 31, control mice harboured only 35% of the initial inoculum with less than one-third of the worms greater than 0.2 mg. Concurrently infected mice had more worms in total, and more worms greater than 0.2 mg than controls.

Growth:

The mean biomass per mouse (± standard error) is shown in Fig. 4-6. The results show that growth of the cestode was poorer in concurrently infected mice (though not significantly, $p > 0.05$) and this effect was rapid, being evident within 4 days of the N. dubius infection. Maximum biomass in the controls was reached on day 20, whereas in the concurrent group it was reached on day 25.

The results obtained with the concurrent infection were interesting as will be discussed later. From the data, the following questions were posed: a) can a concurrent H. citelli/N. dubius infection affect the afferent arm of the primary response against the cestode, if so, to what extent is the effect manifested against a homologous challenge? b) can N. dubius affect the growth and survival of secondary worms by suppressing the expression of acquired immunity to homologous H. citelli challenge?

In an attempt to answer these questions, the following experiments were designed and carried out.

a) Objective: To prove whether a primary six cysticercoid H. citelli infection under N. dubius suppression can affect the growth and survival of a homologous challenge.

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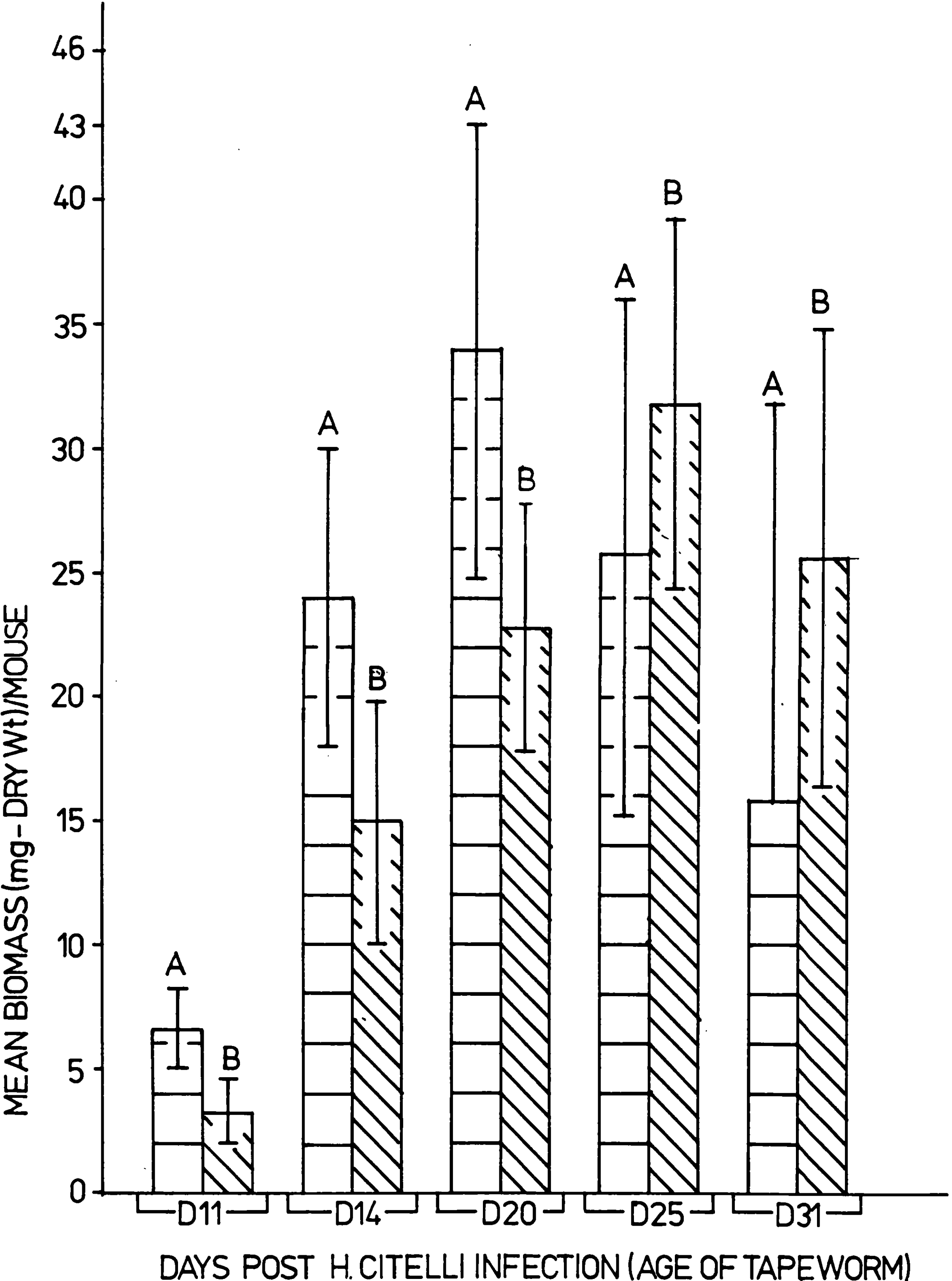
Figure 4-6

Mean biomass per mouse (± standard error) from six cysticercoid primary *H. citelli* infection of NIH male mice concurrently infected with 340 *Nematospiroides dubius* larvae.

A = Control (*H. citelli* only)

B = Concurrently infected group


MEAN BIOMASS (mg - DRY Wt)/MOUSE
46
43
40
35
30
25
20
15
10
5
0
A
B
D11
D14
D20
D25
D31
DAYS POST H. CITELLI INFECTION (AGE OF TAPEWORM)

<u>Protocol</u>

Group	Day 0	D12	D21/23	D33	D45
a)	-	-	Z + Py	6Hc	K
b)	6Hc + 340 N.d	-	Z + Py	6Hc	K
c)	6Hc	-	Z + Py	6Hc	K

Z = Zanil (250 mg/kg) on days 21 and 23

Py = Pyrantel embonate (100 mg/kg) on days 21 and 23

K = Autopsy of 16 mice

b) <u>Objective</u>: To prove whether <u>N. dubius</u> can affect the growth and survival of a homologous <u>H. citelli</u> challenge infection in concurrently infected mice.

<u>Protocol</u>

Group	Day 0	D12	D21	D31	D43
a)	-	-	Z	6Hc	K
b)	6Hc	-	Z	6Hc + 340 N.d	K
c)	6Hc	-	Z	6Hc	K

K = Autopsy of 16 mice

The dry weights of worms per mouse recovered and the recovery(%) of >0.2 mg worms per group , for both experiments (a) and (b) are shown in Fig. 4-7a and 4-7b.

The results of the concurrent primary infection on the sensitisation of mice to <u>H. citelli</u> (Fig. 4-7a) show that mice were not protected against

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Figure 4-7a

Dry weight of *H. citelli* from six cysticercoid challenge infections of NIH male mice given a concurrent primary infection (1°) of six *H. citelli* cysticercoids and 340 *Nematospiroides dubius* larvae (B').

Figure 4-7b

Dry weight of *H. citelli* from concurrent challenge infections (2°) of six cysticercoid and 340 *N. dubius* larvae (B'') of NIH male mice given six cysticercoid primary *H. citelli* infections.

A = Control (▲)

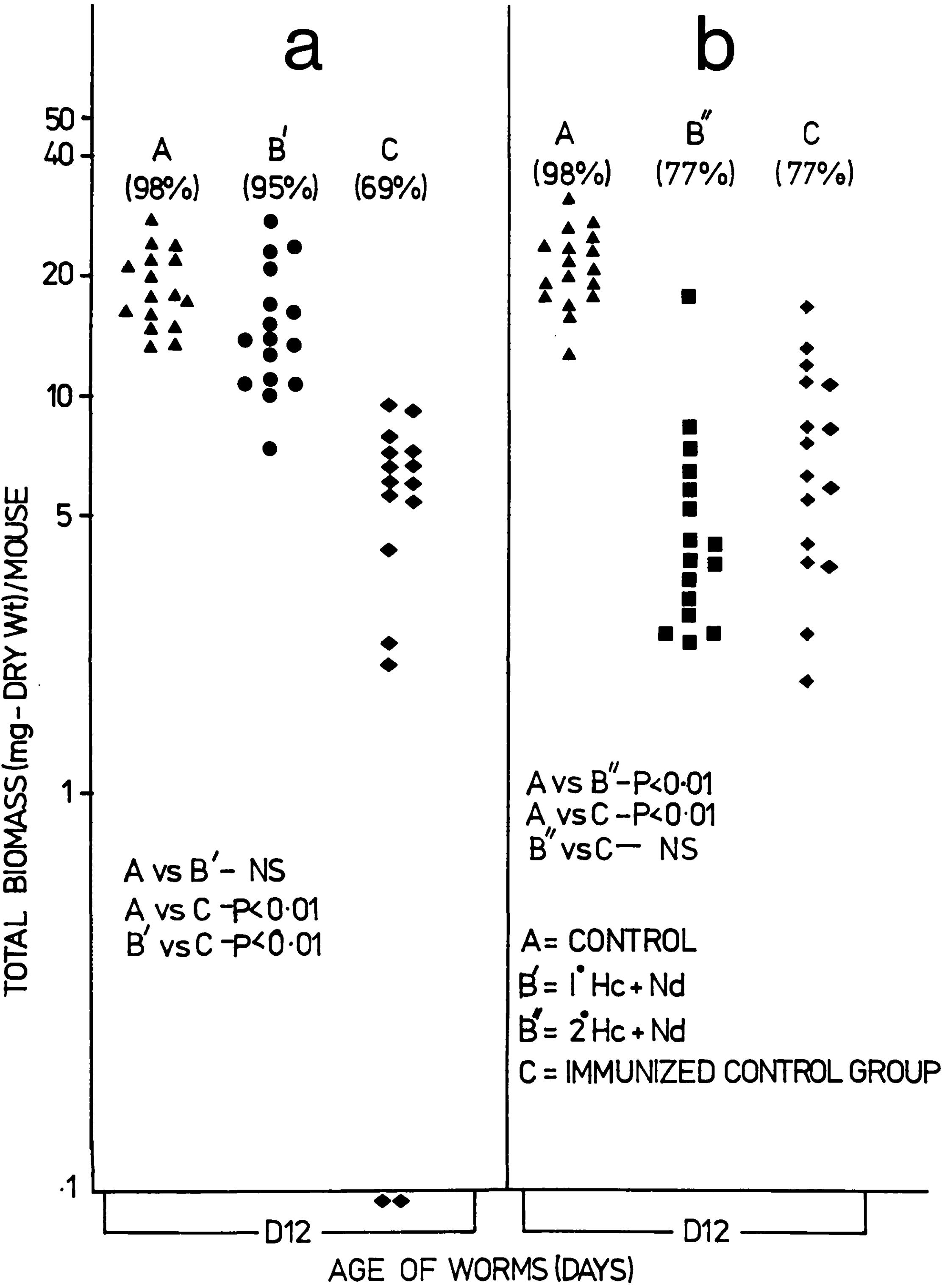
B' = 1°*Hc* + *Nd* (○)

B'' = 2°*Hc* + *Nd* (□)

C = Immune control group (◇)

Points below abscissa represent mice from which no worms were recovered.

Index indicates percentage recovery of $>$0.2 mg worms per group.


a
b
50
40
20
10
5
1
·1
A
(98%)
B′
(95%)
C
(69%)
A
(98%)
B″
(77%)
C
(77%)
A vs B′– NS
A vs C –P<0·01
B′ vs C –P<0·01
A vs B″–P<0·01
A vs C –P<0·01
B″ vs C— NS
A= CONTROL
B′= 1° Hc + Nd
B″= 2° Hc + Nd
C = IMMUNIZED CONTROL GROUP
TOTAL BIOMASS (mg - DRY Wt)/MOUSE
D12
D12
AGE OF WORMS (DAYS)

homologous challenge. Growth of worms in the concurrent group (B') was not significantly different from control mice (A). Growth of worms in the immune control category (C) was significantly ($p<0.01$) retarded in comparison with controls (A) and the concurrent category (B'). Worm recovery per group, was essentially similar in the control and concurrent groups. It is difficult to assess the significance of the 29% difference in worm recovery between the control and immune categories because of the difficulty in finding very stunted/destrobilated worms.

The data from the secondary concurrent experiment (Fig. 4-7b) show that mice in the concurrent category (B'') were significantly protected ($p<0.01$) against homologous challenge; demonstrating that the protective response was not suppressed by an N. dubius concurrent infection. Mice from the immune control group (C) were significantly protected ($p<0.01$) in comparison with control mice. There was no significant difference between the concurrent secondary (B'') and immune control (C) groups. The validity of the difference in worm recoveries between the immune categories and control is again difficult to assess.

DISCUSSION

The results indicate that the rejection of *H. citelli* was delayed in a concurrent infection with *N. dubius* in NIH mice (Fig. 4-5). A similar impairment of rejection of *T. spiralis* (i.e. enhanced survival of the parasite for at least 4 weeks), and in the delayed expulsion of *T. muris* in mice concurrently infected with *N. dubius* has been reported by Behnke *et al*. (1978) and Jenkins and Behnke (1977) respectively. Interestingly, the *N. dubius* infection was able to delay the rejection of the cestode even when given 7 days post-*H. citelli* infection, by which time the mice may have been sensitised to the tapeworm antigens. This suggests that the impairment may have been on the efferent arm of the immune response to *H. citelli*. It is also possible that the delayed rejection may occur because the worms grow more slowly in concurrently infected mice than controls and therefore initially present a weaker antigenic stimulus. However, this interpretation presupposes that the rate of rejection is related to the mass of strobilar present (see discussion on page 57).

The timing of the two infections meant that both larval and adult *N. dubius* would have been present during the course of the *H. citelli* infection. It is possible that both stages of *N. dubius* exerted a depressive effect on the response initiated by the tapeworm, as it is known that both larval and adult *N. dubius* can depress the immune response to *T. spiralis* (Behnke *et al*., 1978); whereas, as suggested by Jenkins and Behnke (1977), it is the larval stage which is most effective in relation to *T. muris* infections in mice.

N. dubius and *H. citelli* share the same habitat (small intestine) and it is conceivable that the inflammatory response produced by *N. dubius* (Hagan, 1980) might alter the local environment of *H. citelli* in such a way as to promote its survival. However, this seems unlikely. Castro *et al*. (1973) have shown that the pathophysiological alterations accompanying the inflammatory changes induced by *T. spiralis* (e.g. altered fluid-flux, reduction of pH) are deleterious to the worm and may contribute to its expulsion. Expulsion of *T. spiralis* is known to coincide with marked inflammatory changes (Larsh and Race, 1975, Wakelin and Wilson, 1979b).

Further evidence against the interpretation that the enhanced survival of *H. citelli* may be due to an environmental effect, comes from the work of Behnke, Bland and Wakelin (1977) on the effect of the expulsion phase of *T. spiralis* on *H. diminuta* (a closely related parasite to *H. citelli*). They demonstrated that the rejection phase of *T. spiralis* had a marked effect upon the growth and survival of the cestode. The cestode either failed to establish or grow. However, if the worms were already strobilate when inflammation developed then destrobilation occured. Similarly, Howard *et al*. (1978) suggested that the deleterious effects on the growth and survival of *H. microstoma* in a concurrent infection with *T. spiralis*, were due to the non-specific inflammatory components of the host's response to the nematode.

If local environmental interaction can be excluded as the cause of the protracted survival of H. citelli in N. dubius infected mice, then it can be considered more probable that the cause lies in the generalised immunosuppressive effect the nematode is known to have, both against particulate, non-living antigens such as sheep erythrocytes (Shimp et al., 1975) and against infective organisms (Chowaniec et al., 1972 and Jenkins and Behnke, 1977). As the effect was induced by infection with N. dubius given 7 days after H. citelli, it can be proposed that the mechanism involves interference with the effector arm of the host's response.

Courtney and Forrester (1973) have reported interspecific interactions between H. microstoma and N. dubius in CD-1, female mice. They showed that the biomass from mice concurrently infected with the nematode was higher than that from control, cestode only, infected mice; regardless of whether the H. microstoma infections were initiated 3 weeks before or 2 weeks after the mice were infected with N. dubius. The results from the present study, showed that the biomass from the concurrently infected mice was lower, though not significantly so, up to day 20 (33-49% lighter between days 11-20), and thereafter was heavier (19-38% on days 25 and 31 respectively) than the control mice; because of worm loss in the (H. citelli only) control group. The poor growth of H. citelli in the concurrent category could be attributable

to the inflammatory response initiated by the nematode (cf. - Behnke et al., 1977). Indeed, Baker (1955) has shown that N. dubius caused a marked anaemia and splenomegaly in mice (splenomegaly, enlarged lymph nodes and gross inflammation were observed during this study). Symons and Jones (1970, 1971) reported that mice infected with N. dubius ate little in comparison with uninfected control mice and that they additionally lost weight. Whether the latter factors may also have contributed to the poor growth of H. citelli in the concurrent-nematode infection and perhaps also exacerbated the depressed protective response of mice to H. citelli is conjectural.

The results from the effect of a primary H. citelli infection under N. dubius suppression (Fig. 4-7a) on the response of mice to homologous challenge, are indicative of an inhibition of sensitisation to the cestode infection. This observation lends credence to the previous suggestion that the enhanced survival of H. citelli in the concurrently infected mice may have been a consequence of a depressed immune response.

The data from the secondary H. citelli/N. dubius concurrently infected mice, show that acquired immunity was not ablated by the nematode infection (Fig. 4-7b). This could be taken to indicate that H. citelli sensitised lymphocytes are insusceptible to the effects of N. dubius suppression. However, further studies are

required to ascertain this premise: in particular, the role of immune lymphoid cells (replicating thymus-derived lymphocytes) should be evaluated to distinguish between a state of temporary hightened reactivity to H. citelli, and one which may persist long after the active response has subsided i.e. "immunological memory".

The mechanism of immunosuppression by N. dubius remains, as yet unidentified. It is, however, possible that cytotoxic factors acting against lymphoid cells (either alone or in combination with other host immune components) may be involved, as has been shown by Faubert (1976) for T. spiralis (resultant suppression in terms of response to sheep erythrocytes). The involvement of lymphoid cell activity in immunity to H. citelli (Wassom et al., 1974) suggests that this aspect of the response may be susceptible to the effects of N. dubius suppression. Increased IgG_1 has been shown by Cypess et al. (1977), Prowse et al. (1979) and Hagan (1980) to be a feature of N. dubius infection. Molinari, Ebersole and Cypess (1978) have reported that this hypergammaglobulinaemia increased and peaked by day 7 when nearly 45% was specific for this nematode. 30-35% of the IgA detected by 24-72 hours were specific,

although by day 14 only 20% were antiparasitic. Brown *et al.* (1976) proposed that increased IgG catabolism could be a possible factor involved in the immunosuppression produced in mice infected with *N. dubius*; whether the above phenomena are involved in the impairment of the response to *H. citelli* is speculative. Another possibility in explaining the delayed rejection of the cestode as a consequence of the concurrent *N. dubius* infection, could be the elicitation of suppressor cells by the nematode as has been suggested for *Ascaris suum* by Khoury *et al.* (1977), i.e. resulting in a depressed immune response to both the cestode and nematode infections.

In conclusion, the data presented and discussed above have some ecological significance: in that a concurrent *N. dubius*/*H. citelli* infection in wild populations, may enhance the survival of the cestode and probably enhance patency.

SUMMARY

1. *H. diminuta* immunised mice are protected against *H. citelli* challenge. The reciprocity of the response is demonstrated, although the protection recorded for *H. diminuta* when mice are immunised with *H. citelli* is weaker. *H. citelli* is expelled simultaneously during the rejection phase of *H. diminuta*, indicating the susceptibility of *H. citelli* to the response initiated by a generically related parasite.

2. *H. microstoma* immunised mice are strongly protected against a heterologous *H. citelli* challenge. The reciprocity of the response is much weaker: a statistically significant protection was obtained only after a 12 cysticercoid *H. citelli* primary infection, although a 6 cysticercoid *H. citelli* infection did weakly stunt the growth of *H. microstoma* challenge worms. The results indicate that *H. microstoma* may partially evade the heterologous and homologous sensitisation of CFLP male mice.

3. It is suggested that the *in vivo* interactions between *H. diminuta*, *H. microstoma* and *H. citelli* emanates from a specific immunological cross-protection and that this is due to the sharing of similar antigenic determinants.

4. The immune rejection of 6 cysticercoid primary *H. citelli* infection in NIH mice is impaired by an *N. dubius* infection (concurrent infection), resulting in a delayed rejection of the cestode. The induction of acquired immunity to homologous *H. citelli* infection is suppressed, although the expression of a secondary response is not abrogated by a concurrent *H. citelli*/*N. dubius* infection. Growth of *H. citelli* worms in the concurrent infections is poorer. Probable reasons for these observations are discussed.

CHAPTER 3

THE RAT

SECTION A

Primary Infections

Preface

The use of the rat as a model for studying immunity to *Hymenolepis sp*. was initiated by Chandler (1939) with *H. diminuta* infections. Since then, the growth and survival of *H. diminuta* in the rat has been investigated by various workers (Roberts, 1961, Roberts and Mong, 1968, Andreassen *et al*., 1974 and Chappell and Pike, 1976a). The cumulative information obtained from these studies have contributed to the understanding of the growth of a tapeworm in a natural host/parasite system and have led to the onset of studies into whether or not there is evidence of acquired immunity to this tapeworm in a rat (Hopkins, 1980).

H. diminuta in the rat is regarded as a good laboratory model, probably because of its long life span in this host in low level infections (at least 14 years when sequentially transplanted into naive rats - Read, 1967) and the absence of an effector (rejection) mechanism in primary infections of 5 or less cysts

(Andreassen and Hopkins, 1980). Thus it provides a wide scope for immunological studies, in elucidating the mechanisms that might be involved in regulating the expulsion of high level infections and the expression of acquired immunity in a natural host/parasite model.

With the exception of work by Goodall (1973) in which the growth and survival of primary H. citelli infections in rats during the first week of infection was observed, no other work, up to the time of writing this thesis has been reported on the growth and survival of this parasite in the rat. In the absence of such work, it was thought necessary to partially fill this vacuum in our knowledge by describing the characteristics of primary infections of varying intensities in the rat in order to:

a) establish the suitability of this host as a good laboratory model for studies on immunity to H. citelli

and

b) compare the growth of worms in this host with that in mice.

It is hoped that once the above aspects of the H. citelli/rat model have been delineated,

the information derived from such studies will augment the concept that the loss of Hymenolepis spp. in high level infections in the rat maybe immunologically-mediated.

Materials and Methods

No further description in addition to that described in the General methods will be given here. CFHB (Wistar-derived) male rats were bred at the Wellcome Laboratories (Glasgow) and used for experimentation when 8-9 weeks old at time of infection.

Results

The objective of the work was to obtain information that would reflect the relationship between the population density of H. citelli in the rat and its effect upon worm growth and survival in a primary infection. Since it was the effect of population density that was under study and not the precise timing of any effect, day 15 was selected as a starting point in the protocol of autopsies as preliminary experiments had indicated that growth of worms was continuing at day 13. Groups of rats were each infected with either 6, 20 or 50 cysticercoids, and rats were autopsied between days 15 and 76 post infection as indicated in the accompanying graphs.

Worm recoveries:

6 cysts infections

The results of the recovery of worms >0.2 mg are shown in Fig. 5-a (A), and (B) for a repeat experiment. Over 75% of the cysts administered became established and were recovered as weighable worms on day 15 post infection. Loss of worms was essentially similar in both experiments A and B. The reason for the 23% difference between the recoveries from experi-

ments A and B on day 76 is unknown, but may reflect variation in responses of outbred rats. The data show that worm loss was essentially exponential between days 25 and 61.

<u>20 cysts infections</u>

Over 85% of the cysts administered were recovered as worms >0.2 mg on day 15 (Fig. 5a-C). Worm loss was rapid between days 15 and 25 and by day 61 only 10% of the worms were recovered.

<u>50 cysts infections</u>

The recovery of >0.2 mg worms is shown in Fig. 5a-(D). The data show that over 85% of the cysts administered became established indicating that <u>H. citelli</u> in the rat was as infective when administered in doses of 50 cysticercoids as when administered as 6 or 20 cysticercoids per rat. The rate of worm loss was very precipitous between days 15 and 20. The occurrence of destrobilated/stunted worms, which are worms weighing less than 0.2 mg with the characteristic "darkened terminal protrusion" was more abundant in the 50 cysts infection than in the 20 or 6 cysts infection. Although no precise quantitative estimation of the numbers of these worms was undertaken, their abundance progressively reduced with the course of the infection, presumably reflecting their loss from the host gut with time.

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Figure 5-a

Percentage recovery of *Hymenolepis citelli* worms ($\geq$0.2 mg) from CFHB male rats given a six, twenty and fifty cysticercoid primary infections.

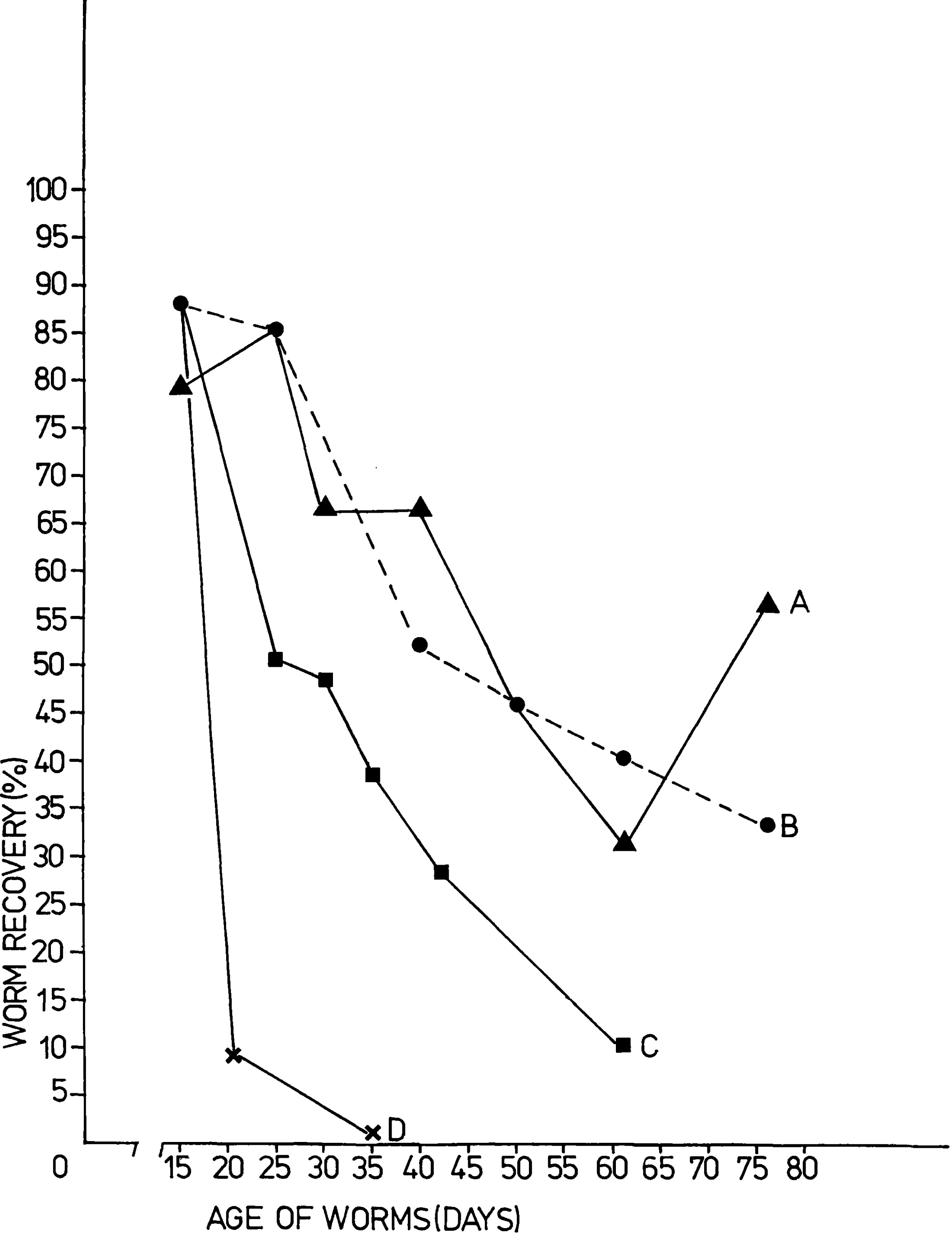
A = six cysticercoids

B = six cysticercoids (repeat)

C = twenty cysticercoids

D = fifty cysticercoids

n = 8 rats/group



WORM RECOVERY (%)
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
15 20 25 30 35 40 45 50 55 60 65 70 75 80
AGE OF WORMS (DAYS)
A
B
C
D

Facing page 130

Figure 5-b

Total biomass per group of H. citelli worms ≥ 0.2 mg from CFHB male rats given a six, twenty and fifty cysticercoid primary infections.

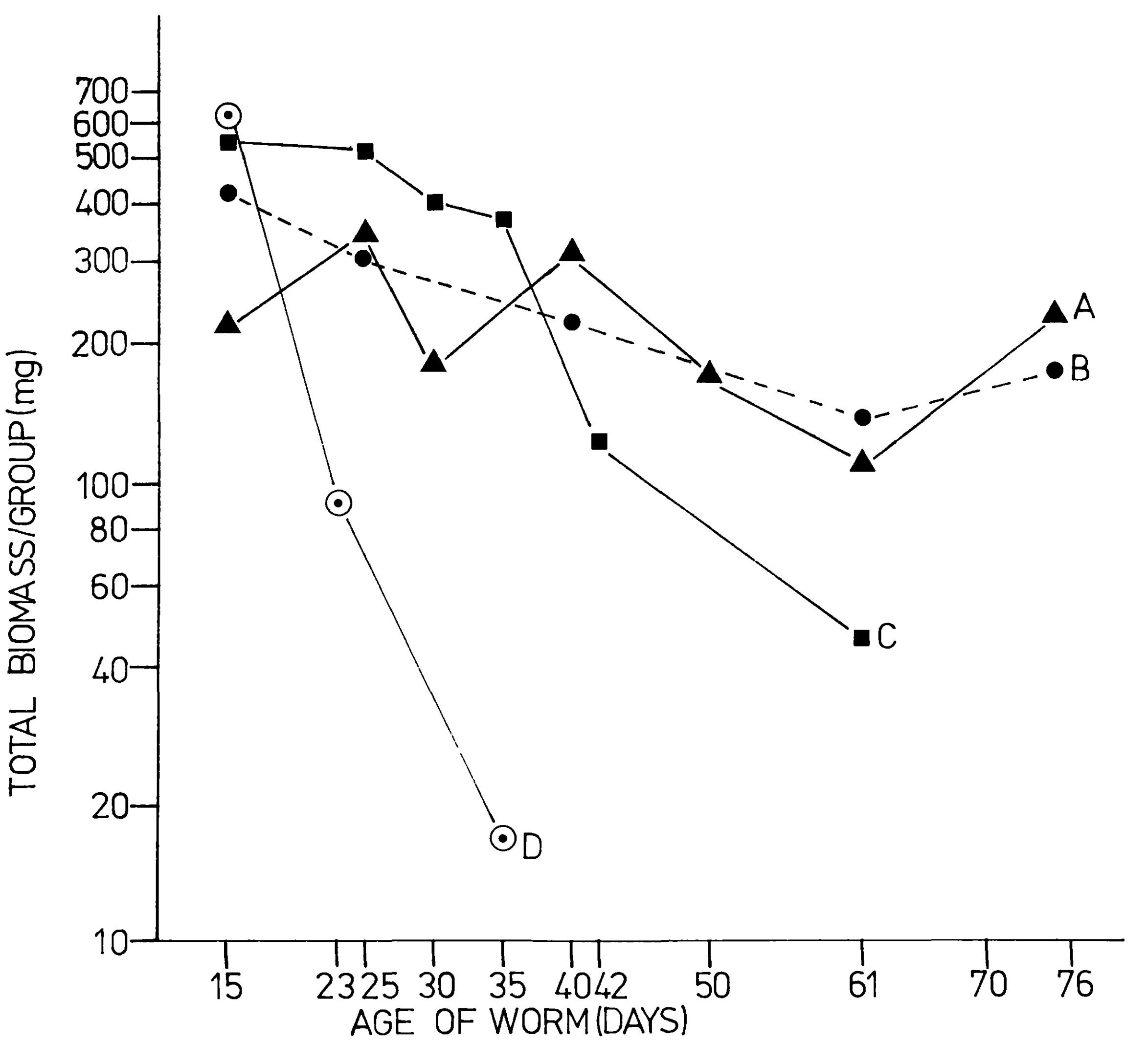
A = six cysts

B = six cysts (repeat)

C = twenty cysts

D = fifty cysts

n = 8 rats per group


TOTAL BIOMASS/GROUP (mg)
700
600
500
400
300
200
100
80
60
40
20
10
15
23
25
30
35
40
42
50
61
70
76
AGE OF WORM (DAYS)
A
B
C
D

Growth:

6 cysts infections

The total biomass per group is shown in Fig. 5b-(A and B) for the two experiments. The biomass in Experiment A fell from 216.8 mg on day 15 to 108.9 mg on day 61, albeit variably and thereafter increased to 239.2 mg on day 76. The increase is partly a reflection of the higher recovery of worms (see Fig. 5a-A). In Experiment B the biomass gradually fell from 423.3 mg on day 15 to 175.8 mg on day 76. The mean worm weights (Fig. 5-c) were variable but were essentially the same and showed no significant decrease between days 15 and 76.

20 cysts infections

The total biomass obtained per group in comparison with those from the 6 and 50 cysticercoid infections is shown in Fig. 5b-(C). The data show that the biomass gradually fell to 45.1 mg on day 61. The fall is associated with worm loss. Mean worm weight (Fig. 5c) peaked on day 25 (6.62 mg) and thereafter was variable, dropping to 2.8 mg on day 42 and then stabilises. The mean worm weight in the 20 worm infection was consistently less than in the 6 worm infections.

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Figure 5-c

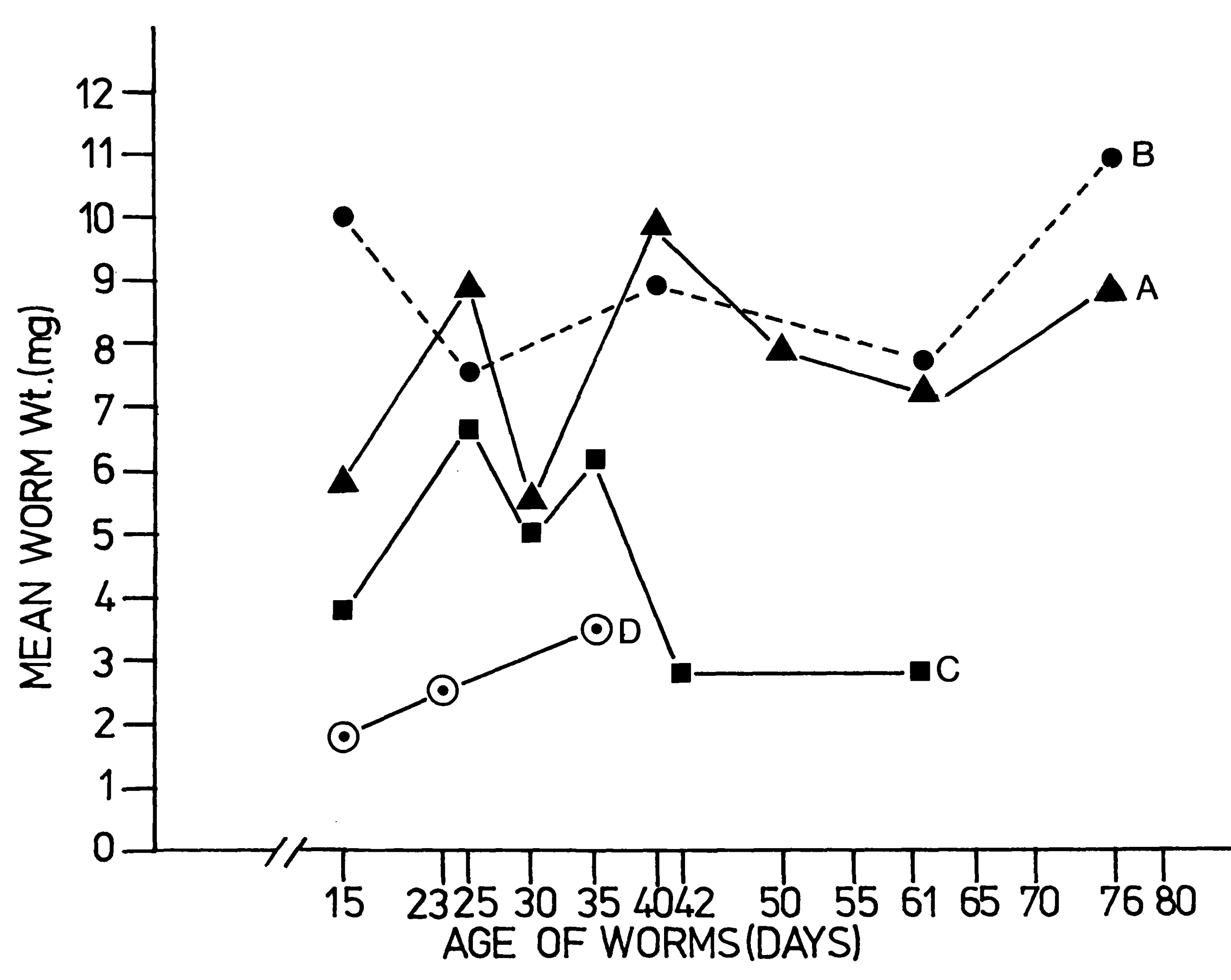
Mean worm dry weights of H. citelli from six, twenty and fifty cysticercoid primary infections from CFHB male rats.

A = six cysts

B = six cysts (repeat)

C = twenty cysts

D = fifty cysts



MEAN WORM Wt.(mg)
0
1
2
3
4
5
6
7
8
9
10
11
12
15
23
25
30
35
40
42
50
55
61
65
70
76
80
AGE OF WORMS (DAYS)
A
B
C
D

50 cysts infections

The total biomass per group is shown in Fig. 5b-(D). The biomass fell sharply from day 15 to 17.2 mg on day 35. This decrease is associated with the rapid worm loss. Mean worm weight (Fig. 5c) increased from 1.8 mg on day 15 to 3.4 mg on day 35, indicating that the surviving worms were still growing. Growth of worms throughout this work was poorer in the 50 worm infections than in the 20 or 6 worm infections.

DISCUSSION

The results show that subsequent to the establishment and growth of H. citelli cysticercoid infections in CFHB rats, worms were lost from the rat at a rate which is proportional to the intensity of the infection. Multiple infections in the rat are characterised by progressive worm loss. The rate of worm loss was faster in the 50 worm infections than either in the 20 or 6 worm infections (Fig. 5a). The decrease in the mean worm weights of H. citelli as the density of the parasite population increases in the rat (Fig. 5c) may be attributable to an intraspecific competition for utilisable carbohydrate (Roberts, 1961; Read, 1959 and Chappell and Pike, 1976a) and/or an immunological interaction between the worm population and the host. The data therefore, as presented here, suggests that the relationship between parasite density and the rat host may be precariously balanced by an immunological as well as a physiological interaction. Both worm loss and the decrease in the mean worm weights as the density of the parasite increases in a rat could be a consequence of these interactions.

Comparable worm loss from the rat in H. diminuta infections has been reported by Chandler (1939), Roberts (1961) and Roberts and Mong (1968).

Hesselberg and Andreassen (1975) showed that expulsion of heavy (50 and 100 cysts) H. diminuta infections in Wistar rats occurred before day 56 post infection. They recorded a decrease in the lengths and weights of worms with an increase in the number of cysts administered (10, 12 or 20 cysts) up to day 56 post infection in Wistar male rats. They also observed that the mean worm positions in 10, 12 and 20 worm infections were significantly posterior to that in 1, 2 and 5 worm infections and that egg production per rat decreased with increasing worm populations (using 1-5 worms per rat). A mean worm recovery of 65%-100% in 1-20 cysts infections compared with 2%-13% in 40-200 cysts infections was obtained in their studies, and Hesselberg and Andreassen (loc. cit.) suggested that a deleterious factor was operative during the 8 weeks after infection which affected both worm loss and growth. Previous to their work above, Andreassen et al. (1974) using cortisone acetate were able to suppress worm loss and postulated that loss of H. diminuta from the rat was immune-mediated. Chappell and Pike (1976b) have also reported a gradual loss of H. diminuta in 15 and 30 worm infections from Sprague-Dawley rats, and observed a decrease in worm weights from days 19-50 in 5, 15 and 30 worm infections. They reported that Hooded-Lister rats showed little worm loss in 50 and 100 worm infections over the same period and discussed

their results in terms of strain differences and suggested that their data could fit either a competitive or immunological model.

The above works lend support to the observations described in this study with H. citelli, in that it augments the evidence that Hymenolepis spp. are lost in the rat in heavy primary infections. Why are worms lost in primary H. citelli and H. diminuta infections at a rate which is associated with the intensity of the parasite population in the rat? In the light of the reports discussed earlier, the reason seems to be equivocal in being attributable to either an immunological or physiological competition. Indeed Harris and Turton (1973) argued that worm loss was not related to immunity but to a competitive interaction. Alternate factors such as "disturbances" in location specificity signals which might affect the migratory behaviour of worms rendering them more susceptible to peristalsis might also play a part in this concept. The "disturbances" may or may not have been correlated with an increase in worm burdens. Although no observations on the migratory behaviour of worms were done in the present study, Goodall (1973) found that H. citelli worms migrated from the anterior to the posterior half of the small intestine in rats and mice between days 4-6 post infection. Walder (1978) reported no significant forward shift in the position of H. citelli worms

in hamsters when compared with the position of *H. diminuta*. He hypothesised that the circadian migratory behaviour of *H. diminuta* in hamsters was correlated with intestinal glucose levels. Read and Kilejian (1969) have suggested that the migratory behaviour of tapeworms in multiple infections in the rat maybe related to a competitive interaction for location specificity signals, although Hopkins and Allen (1979) have reported circadian migratory behaviour in single *H. diminuta* infections (in the absence of any intraspecific worm competition) and cautioned against inferences drawn from multiple infections. It may thus be plausible that a combination of immunological and physiological factors and/or "disturbances" in location specificity signals which affected an anteriad or posteriad movement in heavy *H. diminuta* and *H. citelli* infections respectively, could have precipitated worm loss.

The observations in the present study effectively establishes the rat as a suitable laboratory model for studies on immunity to *H. citelli* infections. Comparing the growth of *H. citelli* worms in the mouse system (see Chapter 1, above) with that in the rat model, it is apparent that growth of worms in the latter host is poorer. This confirms the results of Goodall (1973) in which he reported the growth of *H. citelli* worms in rats and mice, only during the first week of infection, and concluded

that growth was better in the mouse host. Probable reasons for such differences could be genetic (Wakelin, 1978b), immunological (Hopkins, 1980) or physiological. The use of immunosuppressants as a tool to elucidate whether the increase in the rate of worm loss and the decrease in mean worm weights as the population density increases in a rat were immunologically-mediated could be worthwhile.

It is however, of greater interest to the author to demonstrate whether or not there is evidence of acquired immunity to homologous and heterologous challenge infections in the rat, thus rendering the involvement of an immune response more tenable. Studies in this regard are presented in the next section.

SECTION B

Secondary Infections

Preface

Studies on secondary *Hymenolepis sp.* infections in the rat were initiated by Chandler (1939) when he described the effects of numbers and age of *H. diminuta* worms on the development of primary and secondary infections. He used the term "premunition" (i.e. "protection against re-infection as the result of an existing one") and suggested that it was due to "crowding" rather than to immunity in the ordinary sense. The concept that *H. diminuta* is non-immunogenic in the rat has been perpetuated in the literature (Heyneman, 1962). Roberts and Mong (1968) studied the development of *H. diminuta* as affected by a pre-existing homologous infection. They reported that superimposed infections with 10 cysts in rats with mature primary infections of various intensities, resulted in the weights of individual secondary worms being inversely proportional to the numbers of primary worms present. Recovery of secondary worms was slightly lower than controls, although the establishment was good in all cases. Roberts and Mong (loc. cit.) discussed their results in terms of host resistance and cautioned against the use of the term "premunition".

In the last decade, evidence that refutes the alleged non-immunogenicity of *H. diminuta* in the rat has been presented. Harris and Turton (1973) showed evidence for the presence of circulating antibodies in rat serum in 5 and 25 worm *H. diminuta* infections. The level of circulating antibody titres in their work did not however correlate with the intensity of the infection. Andreassen, Hindsbo and Hesselberg (1974) reported the probable involvement of an immune response in 100-worm infections using cortisone treatment. Andreassen, Jespersen and Roepstorff (1978b) have shown that inbred Wistar rats infected with 5 *H. diminuta* cysts for 28 days, expelled a 100-worm challenge a week later (concomitant immunity). Only about 9.4% of destrobilated/stunted worms in comparison with about 97% of primary control worms were recovered. In another experiment using a 5-worm infection for 3 weeks, then treating the rats with anthelmintic and challenging a week later, Andreassen *et al.* (1978b) demonstrated acquired immunity to a 100-worm challenge on day 7 post-infection. More recently Andreassen and Hopkins (1980) have reported acquired immunity in the absence of the primary worms, their results show that the protective response initiated by 50 *H. diminuta* worms wanes with time in the rat host.

No work has been published on studies concerned with acquired immunity in *H. citelli* infections in the

rat. In view of the successful use of the rat as a model for studying immunity to *H. citelli* infections (Section A above), it was decided to observe the growth and survival of secondary worms in rats that have experienced primary infections at various levels of intensity.

The questions posed were:

a) Is there evidence of acquired immunity in a heavy (50 cysts) infection in the absence of the primary worms?

b) Is the response evoked in a challenge infection related to the intensity of both the primary and secondary infections?

c) Does acquired immunity to homologous challenge infections wane with time i.e. how long after the anthelmintic removal of the primary infection can a protective response be measured?

d) Is there evidence of heterologous protective responses between *H. citelli* and *H. diminuta* in the rat?

Results

a) The objective of the experiment was to observe the effect of a primary 50 H. citelli cysticercoid infection on the growth and survival of a 50 cysticercoid homologous challenge.

Protocol

Group	Day 0	D21/22	D32	D42
a)	-	Z/Z	50Hc	K
b)	50Hc	Z/Z	50Hc	K

K = Autopsy of 8 rats

Hc = H. citelli cysticercoids

Z = 'Zanil' (170 mg/kg/rat) on days 21 and 22

The dry weight of worms per rat and the total biomass per group are shown in Fig. 6-a. The recovery of >0.2 mg worms from the control group was 92% and from the immunised group was 33%. This might indicate either a reduced establishment or an accelerated expulsion of secondary worms. However, the total recovery from the immunised group was 74% (i.e. it included 41% stunted/destrobilated worms). These small worms are easily missed, so the true number of worms present was probably not significantly different from that in the controls (92% in total).

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Figure 6-a

Dry weight of H. citelli from fifty cysticercoid challenge infections of CFHB male rats given a fifty cysticercoid primary infection.

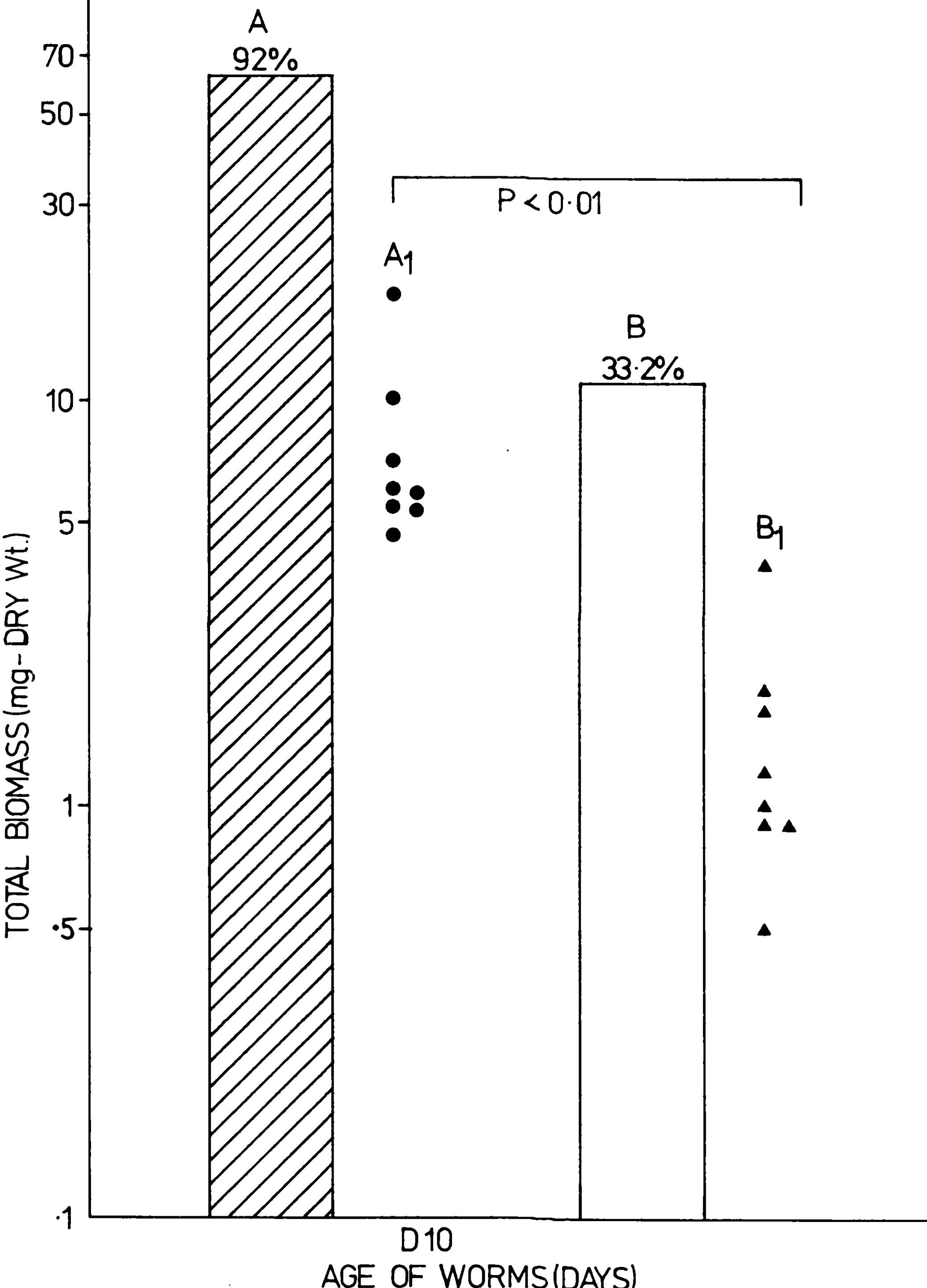
A = total biomass/group - control

A' = dry weight/rat - control

B = total biomass/group - immune group

B' = dry weight/rat - immune group

Index indicates percentage recovery of ≥ 0.2 mg worms per group.



A
92%
P < 0·01
A1
B
33·2%
B1
70
50
30
10
5
1
·5
·1
TOTAL BIOMASS (mg - DRY Wt.)
D 10
AGE OF WORMS (DAYS)

The results demonstrate that rats previously exposed to primary H. citelli infections were significantly protected ($p < 0.01$) against homologous challenge infections: in terms of percentage, the reduction in the biomass of secondary worms relative to primary worms was 83%.

From the above observations, it was subsequently decided to evaluate the quantitative aspects of the secondary response in the rat by varying the intensities of the primary and secondary infections, in order to observe below which level of primary and secondary infections a measurable protective response cannot be obtained. Indeed this would be of technical significance as a lower cysticercoid requirement would make experimental designs in measuring protective responses much easier. The data described below thus indicate the quantitative aspects of the response against challenge infections in rats that have previously experienced a primary H. citelli infection.

b) Objective

Effect of a primary 6, 12, 20 and 50 H. citelli cysticercoid infection on the growth and survival of a 6 cysticercoid homologous challenge.

Protocol

Note: The protocol used below was the same for

subsequent experiments described henceforth, except that the level of the challenge infections was increased to 12 and 20 cysticercoids. No further description will thus be given, however objectives will be stated in each experiment.

Group	Day 0	D21/22	D32	D44
a)	-	Z/Z	6Hc	K
b)	6Hc	Z/Z	6Hc	K
c)	12Hc	Z/Z	6Hc	K
d)	20Hc	Z/Z	6Hc	K
e)	50Hc	Z/Z	6Hc	K

Hc = H. citelli cysticercoids(c)

Z = 'Zanil' (170 mg/kg/rat)

K = Autopsy of 8 rats

The dry weight of worms >0.2 mg per rat and the recovery(%) of >0.2 mg worms per group are shown in Fig. 6-b. The mean worm weight from the control group was 2.24 mg, and from the 6, 12, 20 and 50 cysticercoid immunised groups was 0.99 mg, 0.48 mg, 0.40 mg and 0.27 mg respectively. In terms of percentage, the reduction in the biomass of secondary worms vis-a-vis the control, primary worms (i.e. protective response) was 57%, 80%, 83% and 88% ($p < 0.01$ in all cases) in the 6c, 12c, 20c and 50c immunised groups respectively.

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Figure 6-b

Dry weight of H. citelli from six cysticercoid challenge infections of CFHB male rats given six, twelve, twenty and fifty cysticercoid primary infections (1°).

A = control (naive)

B = 1° x 6 cysts

C = 1° x 12 cysts

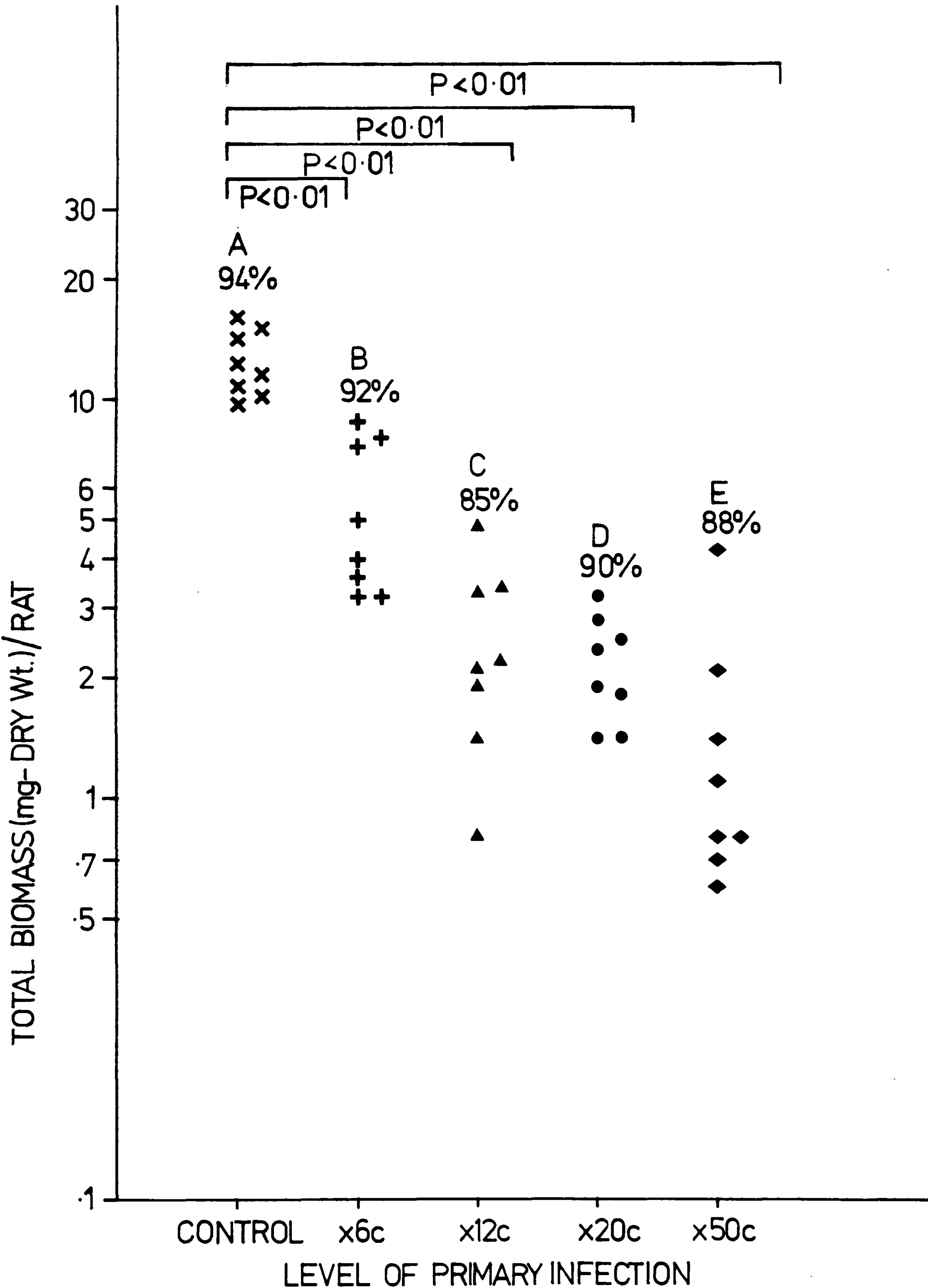
D = 1° x 20 cysts

E = 1° x 50 cysts

Index indicates percentage recovery of $\succ$0.2mg worms per group.

*Probability (P) Values

B vs C	$P < 0.05$	C vs D	N.S.
B vs D	$P < 0.01$	C vs E	N.S.
B vs E	$P < 0.01$	D vs E	$P < 0.05$


P<0·01
P<0·01
P<0·01
P<0·01
A
94%
B
92%
C
85%
D
90%
E
88%
30
20
10
6
5
4
3
2
1
·7
·5
·1
TOTAL BIOMASS (mg- DRY Wt.)/RAT
CONTROL
x6c
x12c
x20c
x50c
LEVEL OF PRIMARY INFECTION

The results demonstrate that rats were significantly protected and that the protective response increases with an increase in the intensity of the primary infection. The total worm recovery (i.e. all worms) from the control, 6c, 12c, 20c and 50c immunised groups was 98%, 96%, 92%, 96% and 96% respectively, indicating no appreciable worm loss in comparison with the recovery of weighable worms (Fig. 6-b).

c) <u>Objective</u>

Effect of a primary 6, 12, 20 and 50 cysticercoid infection on the growth and survival of a 12 cysticercoid challenge.

The dry weight of worms per rat and the recovery of >0.2 mg worms per group are shown in Fig. 6-c. The mean worm weights from control, 6c, 12c, 20c and 50 cysts immunised groups were 1.85 mg, 1.26 mg, 0.79 mg, 0.90 mg and 0.61 mg respectively. Total worm recoveries from the same categories were 98%, 90%, 98%, 96% and 98% respectively again indicating good establishment of secondary worms in a 12 cysticercoid challenge. The protective response (i.e. percentage reduction in biomass of secondary worms relative to control worms) in the 6c, 12c, 20c and 50 cysts immunised categories was 48%, 64%, 62% and 74% respectively. The response is reflective of the intensity of the primary immunizing infection.

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Figure 6-c

Dry weight of <u>H. citelli</u> from twelve cysticercoid challenge infections of CFHB male rats given six, twelve, twenty and fifty cysticercoid primary infections(1°).

A = control

B = 1° x 6 cysts

C = 1° x 12 cysts

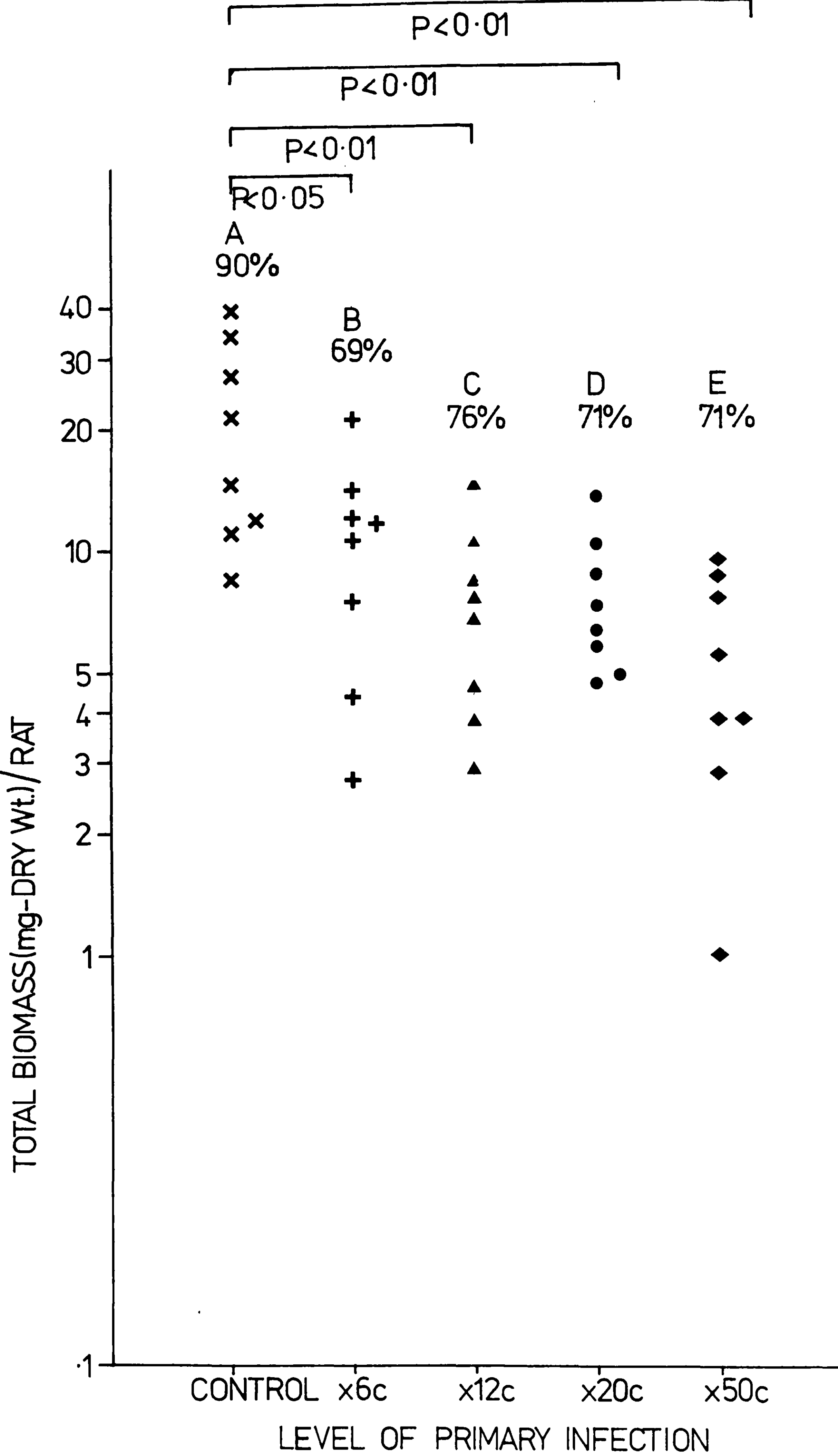
D = 1° x 20 cysts

E = 1° x 50 cysts

Index indicates percentage recovery of $>$0.2mg worms per group.

*<u>Probability (P) Values</u>

B vs C	N.S.	C vs D	N.S.
B vs D	N.S.	C vs E	N.S.
B vs E	N.S.	D vs E	N.S.


P<0·01
P<0·01
P<0·01
P<0·05
A
90%
B
69%
C
76%
D
71%
E
71%
40
30
20
10
5
4
3
2
1
·1
TOTAL BIOMASS(mg-DRY Wt.)/RAT
CONTROL
x6c
x12c
x20c
x50c
LEVEL OF PRIMARY INFECTION

d) <u>Objective</u>

Effect of a primary 6, 12, 20 and 50 cysticercoid infection on the growth and survival of a 20 cysticercoid challenge.

The dry weight of worms per rat and the recovery of >0.2 mg worms per group are shown in Fig. 6-d. The mean worm weights of these worms of >0.2mg. from the control, 6c, 12c, 20c and 50 cysts immunised categories were 1.0 mg, 1.0 mg, 0.89 mg, 0.50 mg and 0.35 mg respectively. The total worm recoveries from the respective categories were 91%, 73%, 73%, 68% and 73% indicating that in comparison with the other levels of challenge (i.e. 6c and 12c) the abundance of very stunted/destrobilated worms was greater at this level. The protective response vis-a-vis control rats was 38%, 44%, 71% and 85% in the 6c, 12c, 20c and 50c immunised groups respectively.

The above results unequivocally demonstrate the quantitative relationship between the intensity of the primary infection and the degree of manifestation of acquired immunity. The protective response observed in this study is summarised in Fig. 6-e. The data indicate the progressive increase in the protective response with an increase in the intensity of the sensitising infection. The 6 cysts challenge gave a consistently higher reduction in biomass (in percentage

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Figure 6-d

Dry weight of H. citelli from twenty cysticercoid challenge infections of CFHB male rats given six, twelve, twenth and fifty cysticercoid primary infections (1°).

A = control

B = 1° x 6 cysts

C = 1° x 12 cysts

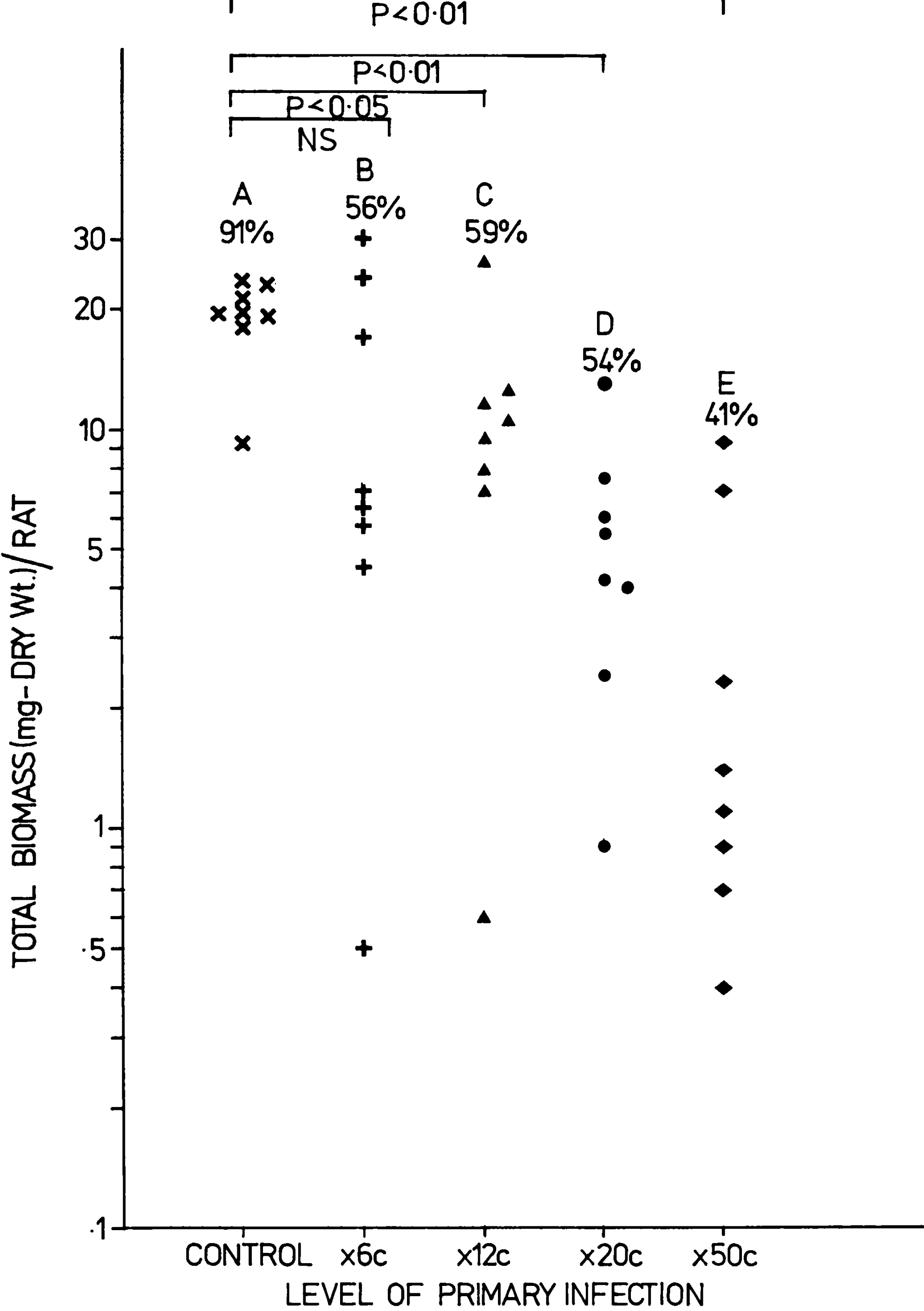
D = 1° x 20 cysts

E = 1° x 50 cysts

Index indicates percentage recovery of ≥ 0.2 mg worms.

*Probability (P) Values

B vs C	N.S.	C vs D	N.S.
B vs D	N.S.	C vs E	$P < 0.05$
B vs E	N.S.	D vs E	N.S.


P<0·01
P<0·01
P<0·05
NS
A
91%
B
56%
C
59%
D
54%
E
41%
30
20
10
5
1
·5
·1
TOTAL BIOMASS (mg-DRY Wt.)/RAT
CONTROL
x6c
x12c
x20c
x50c
LEVEL OF PRIMARY INFECTION

terms) in comparison with the 12c and 20c challenge. It is concluded that in measuring the protective response in immunised rats, a 6 cysticercoid challenge may be more "sensitive" than a 12 or 20 cysticercoid challenge in reflecting the expression of an "anamnestic response". A probable reason could be because growth is better at this comparatively lower level of challenge.

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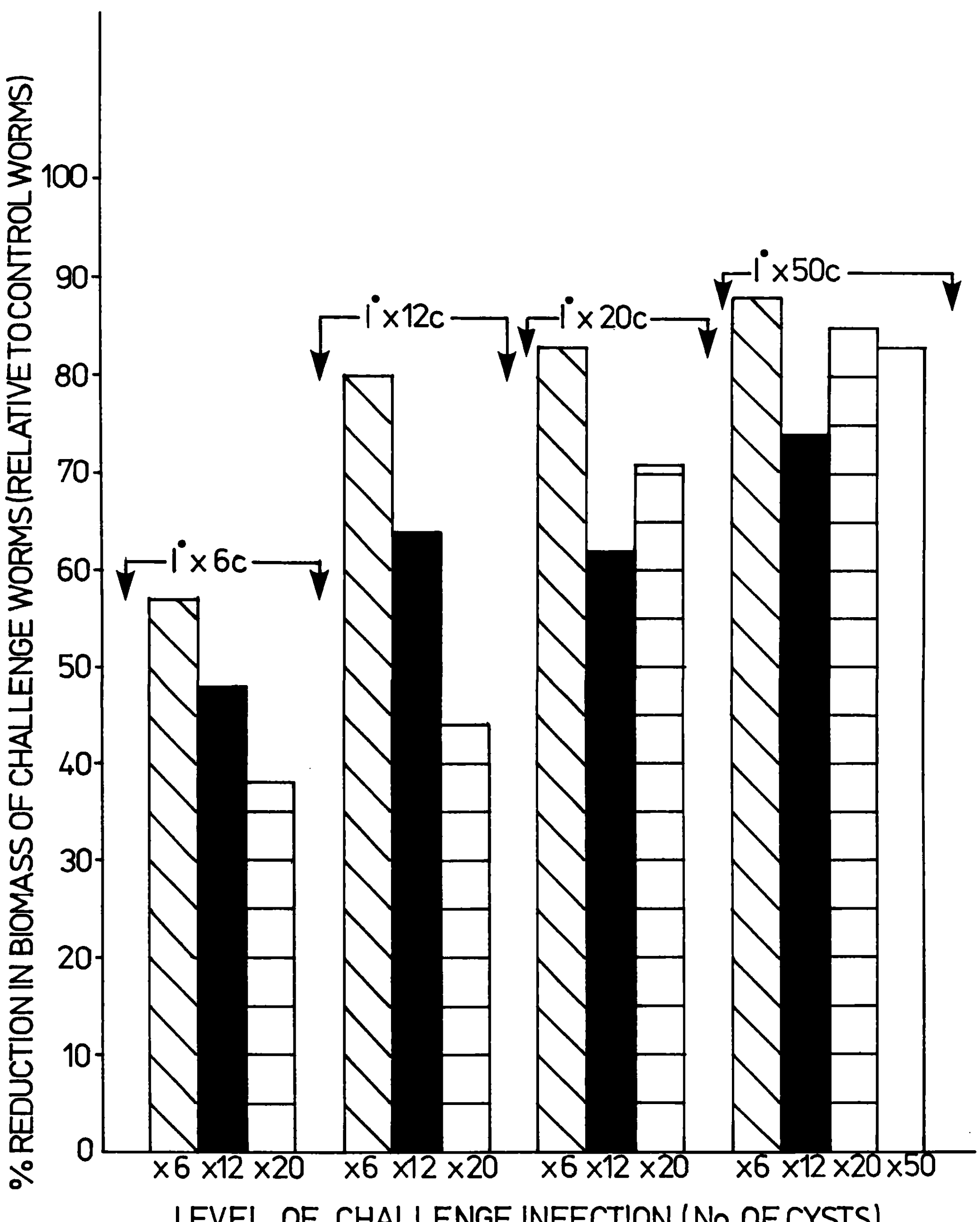
Figure 6-e

Percentage reduction in the biomass of six, twelve, twenty and fifty cysticercoid challenge infections of CFHB rats given six, twelve, twenty and fifty cysticercoid primary infections vis-a-vis control, primary worms.


% REDUCTION IN BIOMASS OF CHALLENGE WORMS (RELATIVE TO CONTROL WORMS)
100
90
80
70
60
50
40
30
20
10
0
1° x 6c
1° x 12c
1° x 20c
1° x 50c
x6 x12 x20
x6 x12 x20
x6 x12 x20
x6 x12 x20 x50
LEVEL OF CHALLENGE INFECTION (No. OF CYSTS)

e) Effect of delaying the challenge infection

Rats were usually challenged 10 days after clearing the primary infections with an anthelmintic. It is probable that the primary infection had altered the intestinal environment rendering it "hyper-responsive" to any subsequent infection given shortly afterwards. In order to investigate how long after the removal of the immunizing infection an "anamnestic response" could be measured, the time interval between anthelmintic treatment and challenge was delayed to 42 days. A 6 cysticercoid challenge was employed, as this level had previously reflected the highest protective response (Fig. 6-e).

Protocol

Group	Day 0	D21/22	D64	D76
a)	-	Z/Z	6Hc	K
b)	6Hc	Z/Z	6Hc	K
c)	12Hc	Z/Z	6Hc	K
d)	20Hc	Z/Z	6Hc	K
e)	50Hc	Z/Z	6Hc	K

*Key as page 145

The mean biomass per rat ($\pm$ standard error) recovered is shown in Fig. 6-f. Mean worm weights from the control, 6c, 12c, 20c and 50 cysticercoid immunised groups were 2.87 mg, 2.07 mg, 2.04 mg, 1.54 mg and 1.18 mg respectively. The reduction in biomass of secondary

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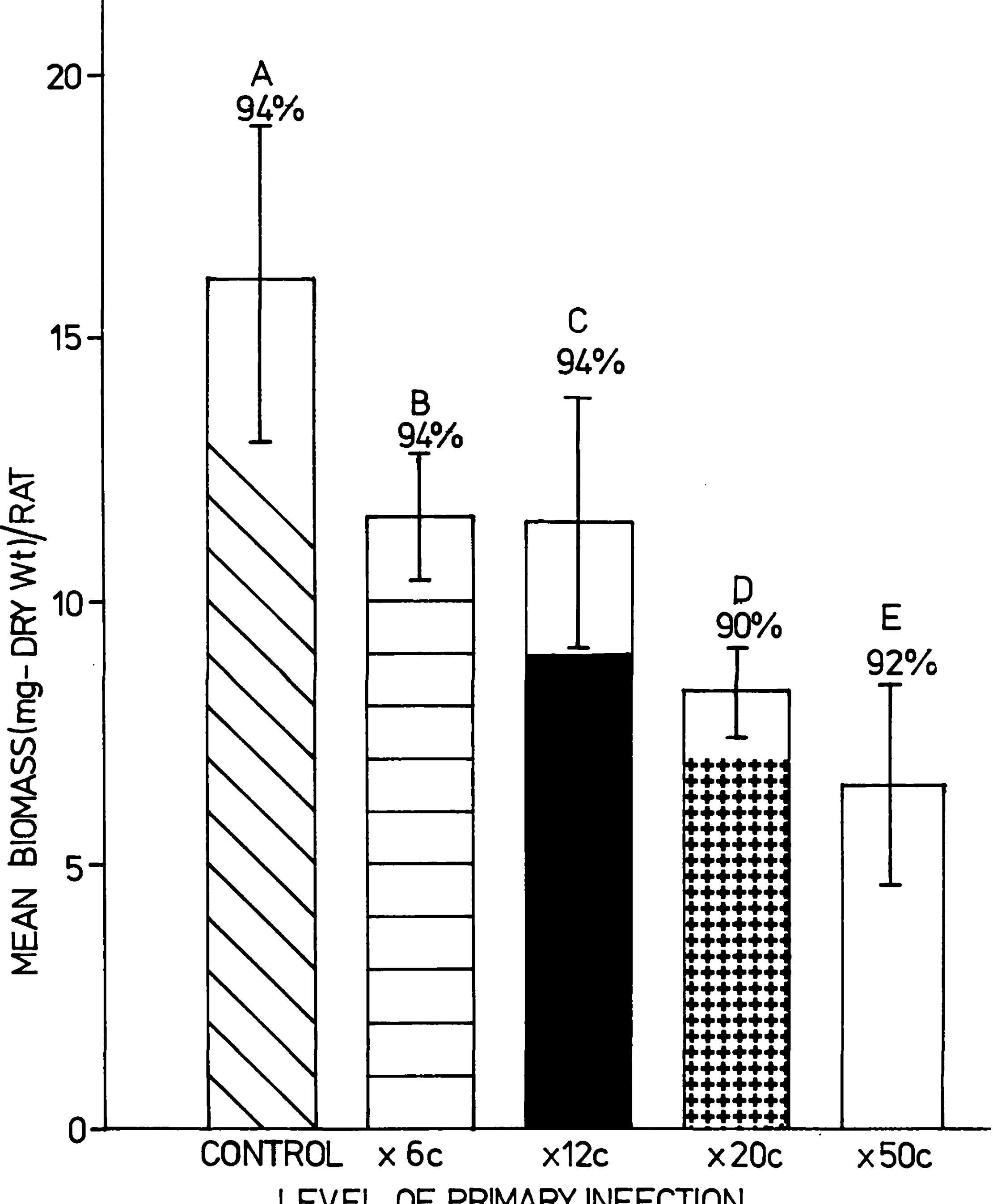
Figure 6-f

Mean biomass ($\pm$ standard error) per rat of H. citelli worms from six cysticercoid challenge of CFHB rats given six (B), twelve (C), twenty (D), fifty (E) cysticercoid primary infections and control (A); when challenged 42 days after 'Zanil' treatment.
Index indicates percentage recovery of $>$0.2 mg worms per group.

n = 8 rats



MEAN BIOMASS(mg-DRY Wt)/RAT
20
15
10
5
0
A
94%
B
94%
C
94%
D
90%
E
92%
CONTROL
x 6c
x12c
x20c
x50c
LEVEL OF PRIMARY INFECTION

worms relative to control worms was 28% (N.S), 29% (N.S), 49% ($p<0.01$) and 60% ($p<0.01$) in the 6c, 12c, 20c and 50c immunised groups respectively. When compared with the protective response obtained in the previous experiment in which rats were challenged 10 days after anthelmintic treatment (Fig. 6-b), the data is indicative of a diminution in the protection. In percentage terms the difference between the "42 days challenge response" and the "10 days challenge response" in the 6c, 12c, 20c and 50 cysts immunised categories was 51%, 64%, 41% and 32% respectively.

Heterologous Infections

The objective of the experiments described below was to investigate whether there is evidence of cross-protection between H. diminuta and H. citelli in the rat, and if so, how does it compare with the interactions observed in the mouse (Chapter 2).

Results

Wistar male rats were purchased when 7-8 weeks old from the University of Nottingham, Joint Animal Breeding Unit, Loughborough and used for experimentation when $8\frac{1}{2}$-9 weeks old at time of infection.

H. diminuta vs. H. citelli

Protocol

a) Objective: Does a 5 and 50 cysticercoid primary H. diminuta infection affect the growth and survival of a 6 cysticercoid heterologous H. citelli challenge?

Group	Day 0	D21/22	D32	D47	D62
1)	-	Z/Z	6Hc	K	K
ii)	5Hd	Z/Z	6Hc	K	K
iii)	50Hd	Z/Z	6Hc	K	K

Hd = H. diminuta cysts

Hc = H. citelli cysts

K = Autopsy of 8 rats

Z = 'Zanil' (170 mg/kg/rat)

The results of the dry weight of worms >0.2 mg recovered per rat and the recovery (%) of >0.2 mg worms per group are shown in Fig. 6-g. There was no appreciable worm loss on day 15 between immunised and the control groups. The significance of the 22% and 33% difference in worm recovery between control and immunised groups on day 30 is difficult to assess, but may be a reflection of the difficulty in finding stunted/destrobilated worms. The results show that rats immunised by H. diminuta were significantly ($p < 0.01$) protected

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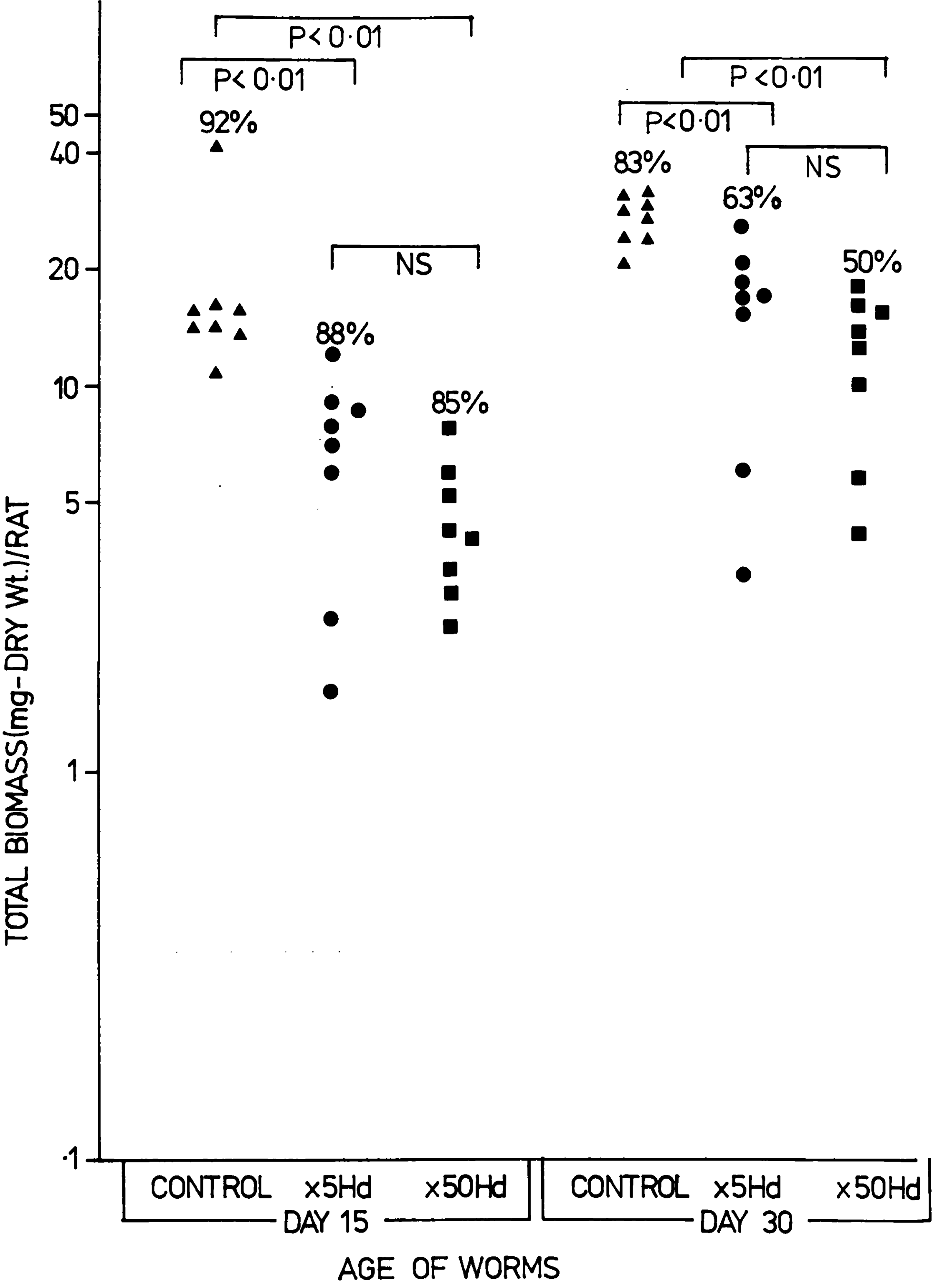
Figure 6-g

Dry weight of H. citelli from six cysticercoid challenge of Wistar male rats given five (5Hd) and fifty (50Hd) H. diminuta cysticercoid primary infections and controls.

Index indicates percentage recovery of >0.2 mg worms.


TOTAL BIOMASS (mg-DRY Wt.)/RAT
50
40
20
10
5
1
·1
P< 0·01
P< 0·01
92%
NS
88%
85%
P <0·01
P<0·01
NS
83%
63%
50%
CONTROL
x5Hd
x50Hd
DAY 15
CONTROL
x5Hd
x50Hd
DAY 30
AGE OF WORMS

against a heterologous *H. citelli* challenge. The protective response was greater in the 50 cysts (73% and 57%) than in the 5 cysts (58% and 43%) immunised groups on days 15 and 30 respectively.

Reciprocal response:

H. citelli vs. *H. diminuta*

Objective: Does a 50 cysticercoid primary *H. citelli* infection affect the growth and survival of a 6 cysticercoid heterologous *H. diminuta* challenge?

A group of rats immunised with *H. diminuta* cysticercoids was also included (as indicated in the protocol below) so as to assess the relative immunogenicity of the two tapeworms.

Protocol

Group	Day 0	D21	D32	D40	D46
i)	-	Z/Z	6Hd	K	K
ii)	50Hc	Z/Z	6Hd	K	K
iii)	50Hd	Z/Z	6Hd	K	K

K = Autopsy of 8 rats

Z = 'Zanil' (170 mg/kg/rat)

Hc = *H. citelli* cysticercoids

Hd = *H. diminuta* cysticercoids

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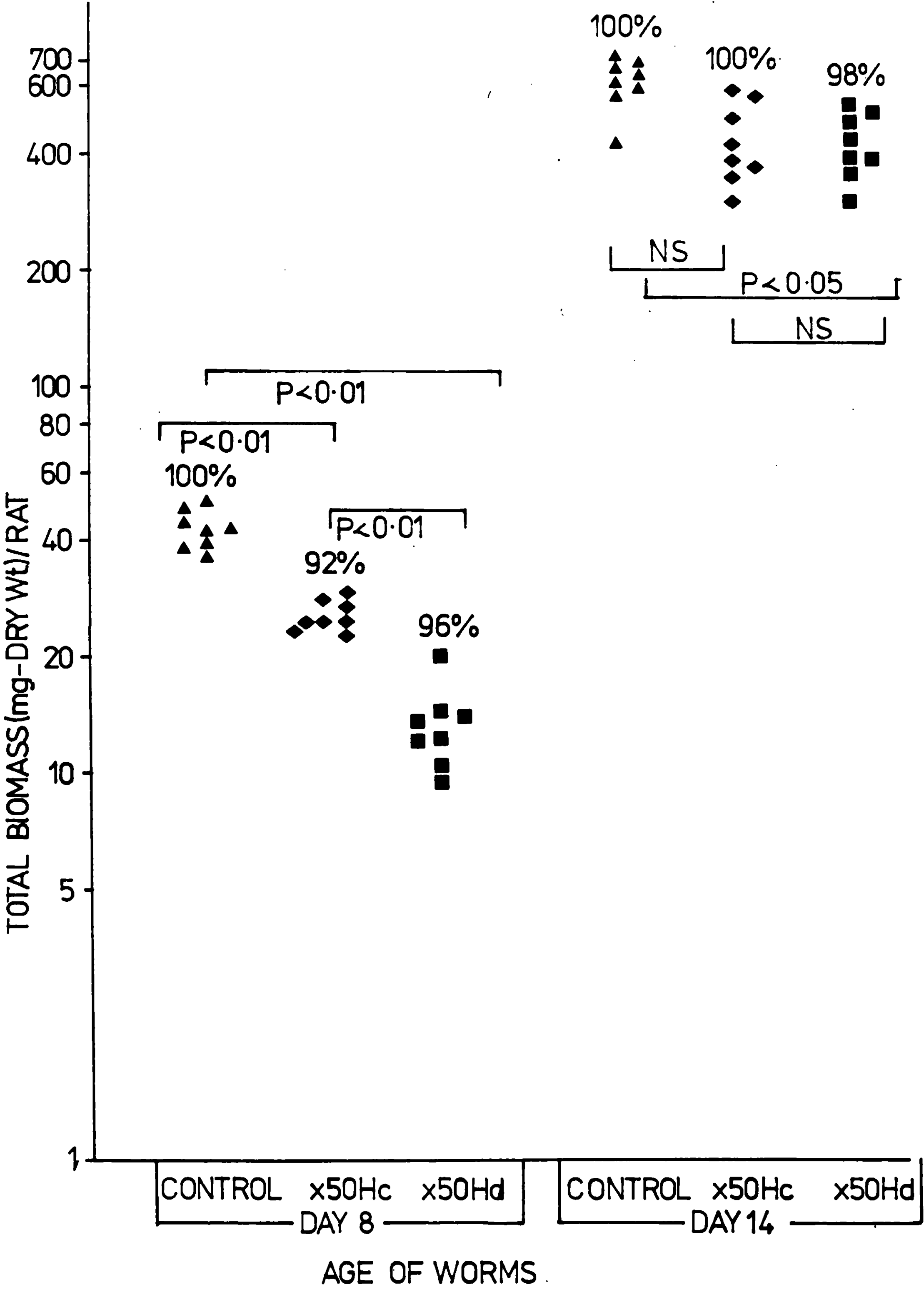
Figure 6-h

Dry weight of H. diminuta from six cysticercoid challenge of Wistar male rats given fifty (50 Hc) H. citelli and fifty (50 Hd) H. diminuta cysticercoid primary infections and controls.

Index indicates percentage recovery of >0.2 mg worms.



TOTAL BIOMASS (mg-DRY Wt)/RAT
700
600
400
200
100
80
60
40
20
10
5
1
100%
100%
98%
NS
P<0·05
NS
P<0·01
P<0·01
100%
P<0·01
92%
96%
CONTROL
x50Hc
x50Hd
DAY 8
CONTROL
x50Hc
x50Hd
DAY 14
AGE OF WORMS

The results of the dry weight of worms >0.2 mg recovered per rat and the recovery (%) of >0.2 mg worms per group are shown in Fig. 6-h. There was no significant worm loss among the groups in either the immunised or control categories throughout the experiment. Comparing the degree of protection recorded in the homologous challenge group (i.e. Hd vs Hd) with that from the heterologous challenge (i.e. Hc vs Hd) category, it is clear that the protection was better in the homologous challenge on day 8 ($p<0.01$). By day 14, the difference between the immunised and control groups had decreased, though remaining just significant ($p<0.05$) in the homologous challenge; whereas in the heterologous challenge the difference was no longer significant.

DISCUSSION

Following a primary infection, CFHB rats exhibited a protective response to homologous challenge with H. citelli infections. The response was manifested mainly as severe stunting of secondary worms with destrobilation an accompanying feature. The results demonstrate the quantitative relationship between the intensity of the primary infection and the severity of the protective response (Fig. 6-e). At least down to a 6 worm primary and secondary infection, there was no indication of a threshold response; the level below which no protective response can be detected. Increasing the intensity of the primary infection increases the percentage reduction in the biomass of secondary worms relative to controls (Fig. 6-b, 6-c, 6-d); probably indicating the importance of antigen dose on the degree of expression of the protective response. The present observations are similar to the results obtained in the mouse system (see Chapter 1) in which it was also demonstrated that the degree of the protective response against homologous challenge infections was related to the intensity of the immunizing infection. Similar evidence for the probable involvement of an immunologically-mediated response to secondary H. diminuta infections in the rat has been given by Andreassen and Hopkins (1980). Their results show that a 50 worm

primary infection (after clearing with an anthelmintic) retarded the growth of secondary worms, and that even a primary infection of 5 worms (which is itself not normally expelled in the rat - Hopkins, 1980) depressed the growth of an 8 day-old secondary infection by over 80%. Interestingly in this study, the protective response induced by primary infections appreciably diminished with time (cf. Figs. 6-b and 6-f). A similar waning of the response with time has been observed in the mouse system (see Chapter 1) and also reported in <u>H. diminuta</u> secondary infections in the rat by Andreassen and Hopkins (1980).

It is suggested that stunting in growth of secondary worms in the rat may be in part immunologically-mediated. The use of immuno-suppressants in an attempt to ablate acquired immunity to secondary infections as has been reported for <u>H. citelli</u> infections in the mouse (Chapter 1, Section 3 herein) using cortisone acetate, helps to clarify the involvement of an "anamnestic response". The results of Harris and Turton (1973) on the occurrence of antibodies in the serum of rats infected with 5 and 25 <u>H. diminuta</u> primary worms, probably lends support to the involvement of an immune

response to Hymenolepis sp. infections in the rat, although they argued that because the titre levels were similar at both levels of infection, a competitive interaction rather than an immunological event was operative. It would be interesting to investigate whether the level of antibodies to H. citelli in the rat (unlike H. diminuta, where the rat is the natural host) correlates with the intensity of the infection.

The results from the interaction between H. diminuta and H. citelli show that the protective response induced by H. diminuta had a considerable effect against H. citelli resulting in retardation in the growth of the latter (Fig. 6-g). The reciprocal response was also demonstrated (Fig. 6-h). It follows that there is evidence of cross-immunisation between the two tapeworms which is similar to that observed in the H. citelli/mouse system.

In conclusion, evidence describing the parameters in measuring acquired immunity to H. citelli in rats has been presented which emphasises the role of the host's resistance in tapeworm infections. It yet remains to investigate further the cellular and/or humoral mechanisms (and the possibility

of suppression of these mechanisms) that might be involved in the expression of acquired immunity to challenge infections.

SUMMARY

1. The use of *Hymenolepis citelli* infections in the rat as a model for studying immunity to adult cestodes is suggested.

2. The characteristics of establishment, growth and survival of worms in primary infections of varying intensities are described.

3. Acquired immunity to homologous *H. citelli* challenge infections was demonstrated. The effectiveness of the protective response in suppressing the growth of challenge worms is related to the intensity of the primary infection.

4. Evidence demonstrating that the protective response diminishes with time is presented.

5. It is demonstrated that cross-protective responses exist between *H. citelli* and *H. diminuta* infections in the rat. The nature of the interaction is described.

6. Further investigation of humoral or cellular components of immunity that might be instrumental in the expression of an anamnestic response to homologous infections is urged.

CHAPTER 4

Local immune response to H. citelli infections

Preface:

The primary function of the gastro-intestinal tract (G.I. tract) is concerned with the digestion and absorption of nutrients. The intestinal mucosa is continually exposed to antigens in/as food and from the normal intestinal microflora. The investigations of Walker and Isselbacher (1974) and Hemmings and Williams (1978) have clearly shown that enterally applied proteins can be absorbed from the gut, not only in enzymatically degraded form, but also as intact macromolecules which are potentially immunogenic (Thomas and Parrot, 1974). It is thus not surprising that an efficient immunological mechanism has evolved to regulate the absorption of such macromolecules.

About 25% of the gut mucosa is lymphoid, and of cells covering the intestinal surface, 1 in 6 is a lymphocyte (Ferguson, 1972). Immunisation by the oral route usually leads to stimulation of the immunological system and to the appearance of specific antibodies in intestinal secretions and serum (Ogra, 1971; Bienenstock, 1974; Guy-Grand et al., 1974; Pierce and Gowans, 1975, Husband, 1978 and Hall, 1979). These reviews re-appraised the role of the mucosal surface in providing the host with an effective barrier against

antigenic bombardment from the normal intestinal microflora and ingested antigens in food. An "immunological homeostasis" is thus maintained between host responses and antigenic stimulation from the intestine. The concept of a local mucosal immune system, as distinct from that of the general and peripheral system is valuable, in that the defense mechanisms interact with antigenic material under enzymatic and physiological conditions that are different from anywhere else in the body (Tomasi and Bienenstock, 1968).

There is ample information on humoral responses to infections localised at mucosal surfaces (see Tomasi and Grey, 1972; Brandtzaeg, 1973; Bienenstock, 1974; Befus and Podesta, 1976; Parrot, 1976; Bazin, 1977 and Hall, 1979). The existence of a local cellular immunity on secretory surfaces has been recognised only recently, probably because of the difficulty in obtaining lymphoid cells in sufficient quantities from tissues (Ferguson, 1972). However, as this study will be concerned mostly with humoral immunity at the intestinal surface, the reader is referred to Ganguly and Waldman (1979) for a comprehensive review on the concept of local cell-mediated immunity.

At this juncture a brief description of the local intestinal immune system is necessary, as this is relevant to the understanding of the immunoglobulin-

associated immune responses on mucous surfaces. The lymph nodes, spleen and Peyer's patches together form the peripheral lymphoid organs (Roitt, 1978). Below the intestinal epithelium are numerous immunocompetent cells, including plasma cells in the lamina propria shown to be derived from precursors in Peyer's patches (Craig and Cebra, 1975 and Befus, O'Neill and Bienenstock, 1978), macrophages, and eosinophils and mast cells. Surrounding the crypts of Lieberkühn and in the lamina propria of each villus is a preponderance of plasma cells. The Peyer's patches have been shown to be populated by T and B lymphocytes (Ferguson and Parrot, 1972) and evidence to justify their classification as lymphoid organs has been demonstrated after sensitisation by antigens (Levin et al., 1976). Craig and Cebra (1971) have shown that on sensitisation, cells in the Peyer's patch proliferate, migrate via the lymphatics and the blood stream to repopulate the lamina propria of the gut predominantly with IgA-producing cells.

The presence of immunoglobulin—containing cells in gut tissues has been demonstrated by immuno-fluorescent studies, using fluorescein-labelled antisera specific for different immunoglobulin classes. These immunocytes mostly have the appearance of mature plasma cells (Craig and Cebra, 1971 and Cebra et al., 1977). The immunoglobulins secreted by these cells may pass across the basement membrane and epithelium and enter the

gut lumen or pass into the collecting terminal of the intestinal lymphatics (Parrot, 1976). Immunoglobulins may also reach the gut lumen by transudation from serum (Bienenstock, 1974).

The free and cellular immunoglobulins found in the gastro-intestinal tract are IgA, IgM, IgG and small traces of IgE (Tomasi and Grey, 1972). $IgD^{+}ve$ cells have also been found in low numbers in the gut lamina propria (Shearman et al., 1972). The predominant immunoglobulin found both in plasmacytoid cells in the lamina propria, as well as in secretions is IgA (see Table 1, Brandtzaeg, 1973 and Hall, 1979). Herein, only a brief description of secretory IgA (sIgA) will be given. The reader is referred to immunological texts (e.g. Roitt, 1978 or Hobart and McConnell, 1978) for the sake of brevity for the structure and function of the other secretory and serum immunoglobulins.

It has been estimated that the human GI tract may contain 50 g of immunoglobulin containing lymphoid tissue; equivalent to the total immunoglobulin-secreting cell content of the spleen (Brandtzaeg, 1974). This produces about 3 g of IgA per day, 50% of which is secreted. The majority of $IgA^{+}ve$ cells are located towards the bases of the villi and have an average half-life of 4.7 days in the mouse (Mattioli and Tomasi, 1973). The IgA secreted in most animals and man is dimeric, with the α-chains linked by a J-chain. Its molecular weight

is 385,000 which is higher than that of serum IgA (Tomasi and Grey, 1972). The J-chain is added to the molecule before it leaves the plasma cell and has an affinity for a glycoprotein called the "secretory piece". The secretory component is synthesised by serous glandular as well as columnar epithelial cells of the gastrointestinal tract (Brandtzaeg, 1974). The presence of the secretory piece appears to alter the susceptibility of the IgA molecule to peptic and tryptic enzymatic degradation (Brown et al., 1970 and Steward, 1971), and may thus be important in preserving the functional integrity of the IgA molecule in the gut. A detailed assessment of the union of the J-chain moiety with the secretory piece has been given by Brandtzaeg and Baklien (1977). Whether the dimeric structure of sIgA in man and animals has a special transport advantage (stabilising the structure?) is unclear. One of the ascribed features of the secretory component in the pre-ruminant calf is its affinity for mucin (Porter and Allen, 1972), probably ensuring that secreted IgA is bound in high local concentration to the surface of the villous epithelium, thus erecting an effective local barrier to infection. It is interesting to note that the intestinal secretions of the pre-ruminant calf contain higher levels of IgM than IgA, mainly because the IgM is less effectively bound to the mucin and therefore is more readily released into the lumen (Porter and Allen, 1972).

The predominant form of serum IgA in man is monomeric, although about 80% of mouse serum IgA is dimeric (Tomasi and Grey, 1972). The immunoglobulin producing cells of the gut probably contribute to serum immunoglobulin levels as dimeric IgA has been detected in the serum of normal humans and in higher concentrations in disease states characterised by mucosal abnormalities e.g. ulcerative colitis and coeliac disease (Ferguson, 1976). Presumably the dimeric IgA found in serum may reflect the escape of exocrine IgA into the circulation via the lymphatics. Recently, immunological evidence for the transport of dimeric IgA by hepatocytes from the blood to the bile has been reported (Orlans et al., 1978), and quantitatively the most important pathway by which IgA gains access to the gut, is via the biliary tract (Lemaitre-Coehlo et al., 1977 and Hall et al., 1979). The functions of both secretory and serum IgA are multifaceted and Lamm (1976) has given a comprehensive review on the subject. The major role of IgA antibodies in the gut is to prevent access of foreign antigenic material to the internal millieu, by specific inhibition of intestinal absorption of antigens (Walker et al., 1973 and 1974; Stokes et al., 1975, Andre et al., 1974, 1975 and 1978a and Hemmings, 1978). The above works suggest that antigens are complexed to secretory IgA antibodies thereby regulating the absorption of "free antigens" in the gut wall.

The above description attests that local intestinal mucosal surfaces, being normally exposed to antigenic stimulation remain in a state of constant activation and that an immunoglobulin-associated response efficiently functions in protective immunity against viral, bacterial, parasitic and food antigens. It is thus conceivable that local antibodies acting either as sole mediators (Lamm, 1976 and Husband _et al_., 1977) or offering a mechanism that relies on the tolerogenic effect of antigen-antibody complexes (Andre _et al_., 1978a Bazin, 1977 and Thomas and Parrot, 1974) are effective in immune responses. A co-operative response thus exists between humoral, cell-mediated and non-specific immunity, i.e. involvement of macrophages (Ganguly and Waldman, 1979) which plays a significant role in host protection.

The objective of the work to be described here was to provide information that might establish whether there was a local immune response to _H. citelli_ infections in the mouse. In particular to establish whether the 6 cysts immunisation regime used in most of the experimental work described in this thesis, resulted in a quantitative increase of antigen-specific immunoglobulin-containing immunocytes in the gut lamina propria of mice, as there is no published work on this aspect. The technique chosen was that of immunofluorescence, using fluorescein-labelled antisera. To provide further

information on the host's secretory immune response, it was necessary also to determine whether or not immunoglobulins could be detected on the surfaces of *H. citelli* worms *in vivo*. For comparative purposes, infections of *H. diminuta* and *H. microstoma* in mice were studied simultaneously.

Materials and Methods

Mice:

4-star CFLP male mice were purchased from Hacking and Churchill, Huntingdon, when 5 weeks ±2 days old. Experimental mice were each infected when 6 weeks ±2 days old, with 6 cysticercoids of the appropriate parasite (i.e. H. citelli, H. diminuta and H. microstoma).

Preparation of tissues:

Prior to treatment with the immunofluorescent reagents, the tissues were prepared for either
a) Cryostat sectioning (Johnson and Holborow, 1973), or
b) paraffin wax embedding (Sainte-Marie, 1962).

a) Cryostat sectioning:

Prior to sectioning, mice were killed (see General methods) and 1-2 cms long segments of the gut (10-20 cms from the pylorus) were cut and put dry into bijou bottles and stored frozen at -30°C until required. If the mouse to be studied was infected with H. citelli, additional segments from the proximal jejunum, mid-jejunum and the distal ileum were also obtained. However, if the mouse to be studied was infected with H. microstoma, the hypertrophied bile duct (Howard, 1976b) as well as duodenal sections were obtained. The segments from all the infected mice were selected so that worm

tissues were present in the lumen. For comparative control purposes, appropriate intestinal and bile duct segments were obtained from uninfected mice and treated similarly as above.

When required, the sections were removed from the freezer and immediately (making sure that they did not thaw), put on to a metal chuck (Johnson and Holborow, 1973) on which a drop of OCT embedding medium (Ames Co., U.S.A.) had been previously placed. The metal chuck together with the suitably oriented embedded tissue was then immediately snap-frozen in liquid nitrogen for at least one minute. The metal chuck was then put inside the freezing cabinet (-27°C) of the cryostat (Slee, London) for 10-15 minutes prior to sectioning. Serial sections were cut transversely at 4 μm thick, attached to clean glass slides (76 x 26 mm, thickness 1.0/1.2 mm), and air dried for 30-40 minutes. The slide was then gently washed twice in phosphate buffered saline (PBS, ph 7.2) for 2-3 minutes in each wash, and then fixed in cold ether:alcohol (50:50) for 10 minutes. Thereafter, the tissue was again fixed in cold 100% methanol (Analar) for a further 20 minutes, removed and washed three times in PBS for 1-2 minutes in each wash, prior to incubation with the antisera.

b) Paraffin embedding: (Sainte-Marie, 1962)

For fixing and blocking the following procedure was carried out, after the appropriately selected intestinal regions have been cut into 1-2 cms. sections as previously described. The wax used was pastillated 'Difco' polywax with a melting point of 57°C.

1st Day: Samples put in bijou bottles containing 95% ethanol at 4°C. Left overnight at 4°C in a refrigerator.

2nd Day: Samples changed into absolute ethanol at 4°C with 4 changes for a total period of 6-7 hours. Samples were then put in xylene and left at 4°C overnight.

3rd Day: Samples were put in wax I at 58°C for 3 hours. Thereafter the samples were removed and put in wax II at 58°C for a further $1\frac{1}{2}$-2 hours and then blocked out as usual.

4th Day: Blocked tissues were cut on a microtome (Leitz 1501, Rotary) at 5 μm, floated off sections put on slides and then dried for 30-40 minutes at 37°C. Thereafter, the tissue was placed in xylene (5 minutes) and subsequently passed through a descending series of ethanol. The sample was washed three times in PBS (7.2) prior to staining with the immunological reagents.

Immunofluorescent technique:

1. Antisera

a) Unconjugated: Class specific (IgA, IgM and IgG_1) unconjugated, goat anti-mouse sera were purchased from Gibco, Europe Ltd., Paisley, Scotland and used for the indirect immunofluorescent technique.

b) Conjugated: Rabbit anti-goat fluorescein-conjugated (FITC) antiserum was purchased from Nordic, Sera Service Ltd., Maidenhead. The protein concentration was 10.0 mg/ml, with a FITC/protein ratio of 3.4. The working dilution was 1:10.

For direct immunofluorescence, goat anti-mouse IgA-conjugated antiserum obtained from Gibco Ltd., Paisley, was used. The working dilution was 1:10, the protein concentration was 9.8 mg/ml, with an FITC/protein ratio of 3.4.

2. General procedures

a) For the indirect immunofluorescent technique, sections of gut tissue on slides were covered with a drop of an appropriate anti-sera (IgA, IgM or IgG_1) and incubated in a moist chamber at room temperature for 45 minutes. Following this, the excess antiserum was washed off the tissue by rinsing three times (5-8 minutes per wash) in PBS (7.2) in a Coplin jar. Conjugated rabbit anti-goat sera was then added to the tissue and the slide incubated for 30-45 minutes in a humid chamber. Thereafter, the excess antiserum was washed off three times in PBS before glycerol:PBS (70:30) was added as a mounting medium. A coverslip was then added to the slide which was now ready for viewing.

The specificity of the reaction was controlled by

a) use of unconjugated rabbit anti-goat (RAG) prior to incubation with the labelled antisera, to block the subsequent combination of labelled immunoglobulin.

b) use of section without the addition of the conjugated antisera

c) use of unconjugated rabbit anti-dog serum (RAD) prior to incubation with the conjugated antiserum.

d) use of conjugated antiserum of differing specificity from that of rabbit anti-goat, namely sheep anti-dog-SAD, and goat anti-dog-GAD.

The antisera used to control the specificity of the reactions, i.e. unconjugated RAG, RAD and conjugated SAD and GAD were gifts most kindly donated by Professor N.G. Wright (Anatomy/Histology Department, Veterinary School, Glasgow).

The "blocking control" (a) above did not completely obliterate the specific staining, as has been reported by Johnson and Holborow (1973). After the specificity of the reaction had been firmly established, only control (b) above was invariably used in the examination of tissues from both infected and uninfected mice, as the use of all the above antisera controls was not only very expensive, but time consuming.

b) For direct immunofluorescence examination using conjugated goat anti-mouse IgA, sections on slides were incubated with a drop of the conjugate (GAM-IgA), for 45 minutes. Excess antisera was washed off 3 times in PBS (7.2), 5 minutes in each wash, and then prepared for examination as previously described.

c) Surface immunoglobulins on worms

Mice infected with the appropriate tapeworms were killed and worms extracted as described in the General methods. The worms were then washed once in HBSS (Hanks' balanced salt solution - see General materials) to remove adherent debris from the intestine, and again washed twice in PBS (7.2). Worms were then cut into 3 portions (i) scolex plus neck region, (ii) mid-strobilar section and (iii) distal strobilar section. These sections were then put into wells of a microtiter tray and incubated with unconjugated goat anti-mouse IgA, IgM or IgG_1 for 45-60 minutes at 37°C. Excess antisera was removed by using a Pasteur pipette and the worms were washed twice in PBS. Excess PBS was again sucked off and the worms incubated with conjugated rabbit anti-goat (RAG-FITC) serum for 45 minutes, thereafter they were removed and washed twice in PBS and mounted in glycerol:PBS (50:50) ready for viewing.

3. Microscopy and Photography

The preparations were examined on a Leitz Orthoplan microscope fitted with a Ploem incident-light fluorescent system (Koch, 1972) with a 75W ultra high pressure lamp in a Leitz 100Z lamp housing. BG38 red suppression filter, GG475 edge filter, FITC KP490 selective excitation filter and a K530 suppression filter built into the system together with a heat filter in the lamp housing, were routinely used.

A Wild Photoautomat camera was used for photography with a Kodak Ektachrome ASA 400 colour film.

Results

1. Intestine:

The investigation was aimed at determining the participation of intestinal plasma cells in the response of the mouse to primary and secondary *H. citelli*, *H. diminuta* and *H. microstoma* infections. The direct and indirect immunofluorescent techniques were employed to (a) assign antibody-producing cells to three different immunoglobulin classes (IgA, IgM and IgG_1) and (b) determine the number of immunocytes with specific cytoplasmic fluorescence in the lamina propria between 2 crypts, including that in the villus above i.e. a villus crypt unit.

a) Primary infections

Preliminary observations were made using the direct immunofluorescent technique on cryostat-sectioned tissues. In order to be more confident about the

sensitivity/specificity of the fluorescence obtained, the indirect technique was also subsequently employed.

The results described below are those obtained with the indirect method using paraffin-embedded tissue sections which show the distribution and abundance of brilliantly fluorescing cells more clearly than do tissues after cryostat-sectioning. Positive staining cells were classified morphologically as plasma cells on the basis of their oval, mononuclear appearance. As preliminary studies had shown no marked variations in the numbers and location of reactive cells in the lamina propria of the villus between different regions of the intestine, the results presented below are those of sections taken from the duodenum.

IgA: The mean number of IgA-positive cells per villus crypt unit counted between days 7 and 21 post infection for control uninfected, H. citelli, H. diminuta and H. microstoma infected mice are shown in Fig. 7-1. The results show the considerable variation in the numbers of cells.

Control uninfected mice:

The mean number of IgA-positive immunocytes between days 7 and 21 remained virtually unchanged. Most of the cells showing clearly defined cytoplasmic staining (see Plate 1, p 187) were in the lamina propria

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Plate 1

Section of a villus showing specific cytoplasmic fluorescence in IgA-containing immunocytes of the lamina propria of H. citelli infected mouse; paraffin-wax embedded tissue (approx. X580). [Under the microscope, the fluorescence appeared apple-green. After photographic reproduction the fluorescence shows as yellow against the green non-staining background of the tissues].

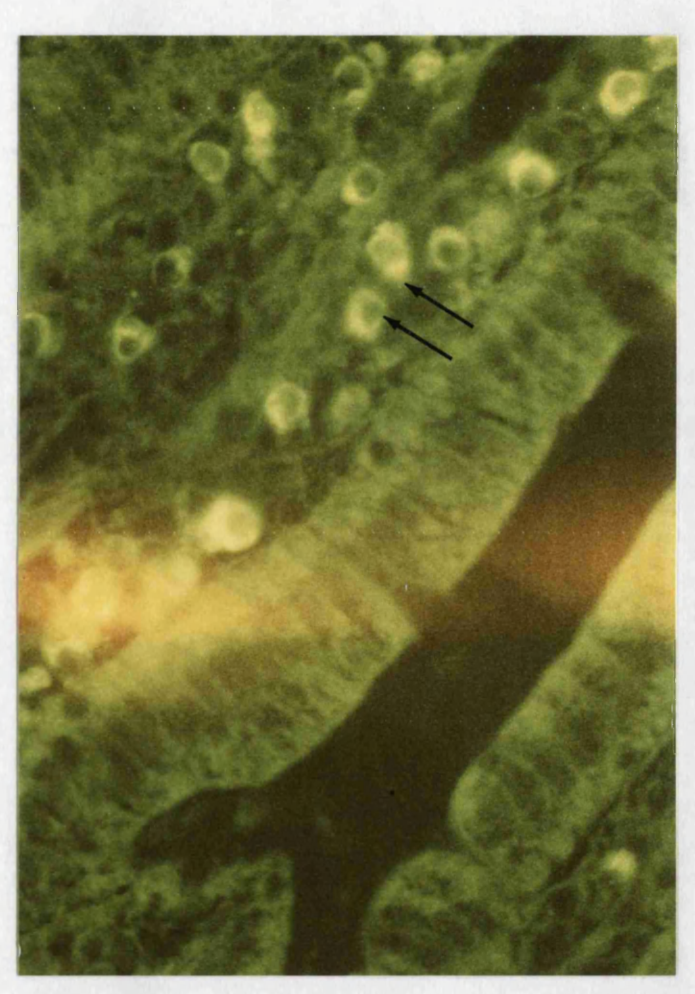

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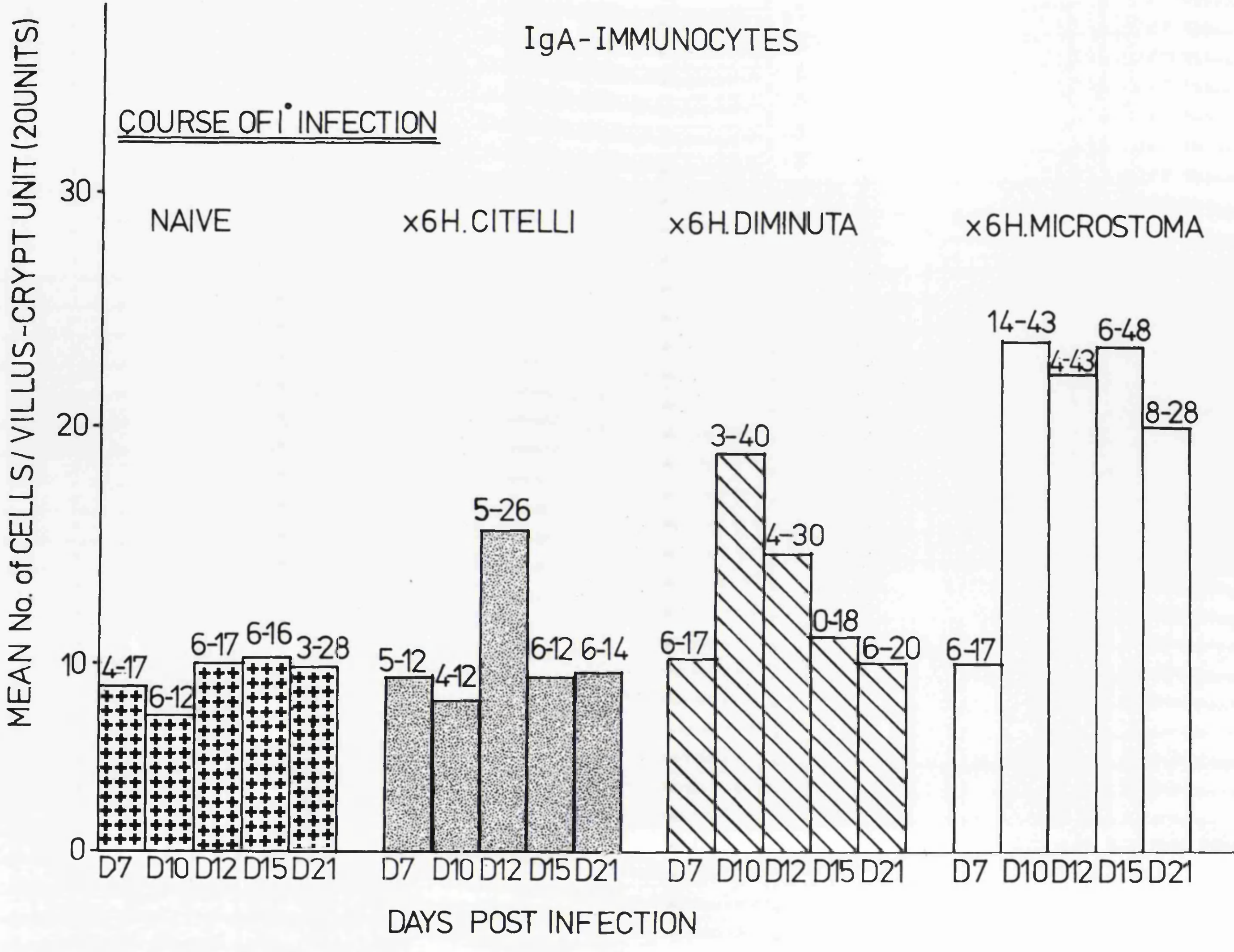
Figure 7-1

Mean number of IgA positive immunocytes per villus-crypt unit in the lamina propria of uninfected, and *H. citelli*, *H. diminuta* and *H. microstoma* infected mice.

Index above histograms indicates the range of immunocytes counted in 20 villus-crypt units.

* Mice were each infected with 6 cysticercoids of the appropriate species.


IgA-IMMUNOCYTES
COURSE OF I° INFECTION
NAIVE
x6H. CITELLI
x6H. DIMINUTA
x6H. MICROSTOMA
MEAN No. of CELLS/ VILLUS-CRYPT UNIT (20UNITS)
30
20
10
0
4-17
6-12
6-17
6-16
3-28
5-12
4-12
5-26
6-12
6-14
6-17
3-40
4-30
0-18
6-20
6-17
14-43
4-43
6-48
8-28
D7 D10 D12 D15 D21
D7 D10 D12 D15 D21
D7 D10 D12 D15 D21
D7 D10 D12 D15 D21
DAYS POST INFECTION

of the villi. No fluorescence was seen within or between epithelial cells of the villi or crypts, however, very occasionally strands of fluorescence were seen in the centre of the villus and on the surface of both longitudinal and circular muscle layers. Particles in the intestinal lumen did show both specific and non-specific (yellowish colour) fluorescence, these particles were mostly of plant origin from food. In a few instances between days 12 and 21, non-specifically fluorescing clones of 4-6 cells were seen at the bases of villi, whether these were eosinophils is unknown.

H. citelli infected mice: The results show no difference in the mean number of immunocytes when compared with uninfected mice. The increase on day 12 is due to the wide variation in counts. The distribution of cells showing positive fluorescence was essentially similar to that in uninfected mice.

H. diminuta infected mice: The results show an increase in the mean number of immunocytes on day 10 and thereafter a decline till day 21. Whether the decrease is associated with the rejection mechanisms is conjectural, as by day 12 a 6 cysts H. diminuta infection is already being expelled in a primary infection. The results again show the considerable variation in the numbers of positive cells. There was no consistent difference

in the distribution or abundance of fluorescence between sections from uninfected, H. citelli infected and H. diminuta infected mice.

H. microstoma infected mice: The results show an increase in the mean numbers of cells in comparison with control uninfected mice during days 10 to 21. The majority of the positive immunocytes were present in the lamina propria and their distribution was comparable to that from uninfected and H. citelli infected mice. As H. microstoma migrates from the small intestine to the bile duct by day 4-5 p.i., the distribution and abundance of fluorescing cells in the bile duct was studied. Intra-cellular and intercellular fluorescence was extensively seen throughout the duct and counts of immunocytes were impossible because of masking. There was marked evidence of inflamation and hypertrophy of the villi. However, the fluorescence was diffused showing a bright-green colour. Streaks of positive fluorescence were seen in the centre of the villus probably indicating IgA present in the arterioles and lacteals.

Throughout the present study, no IgA specific fluorescence was observed on the surfaces of sectioned worms in the intestinal lumen of H. citelli, H. diminuta

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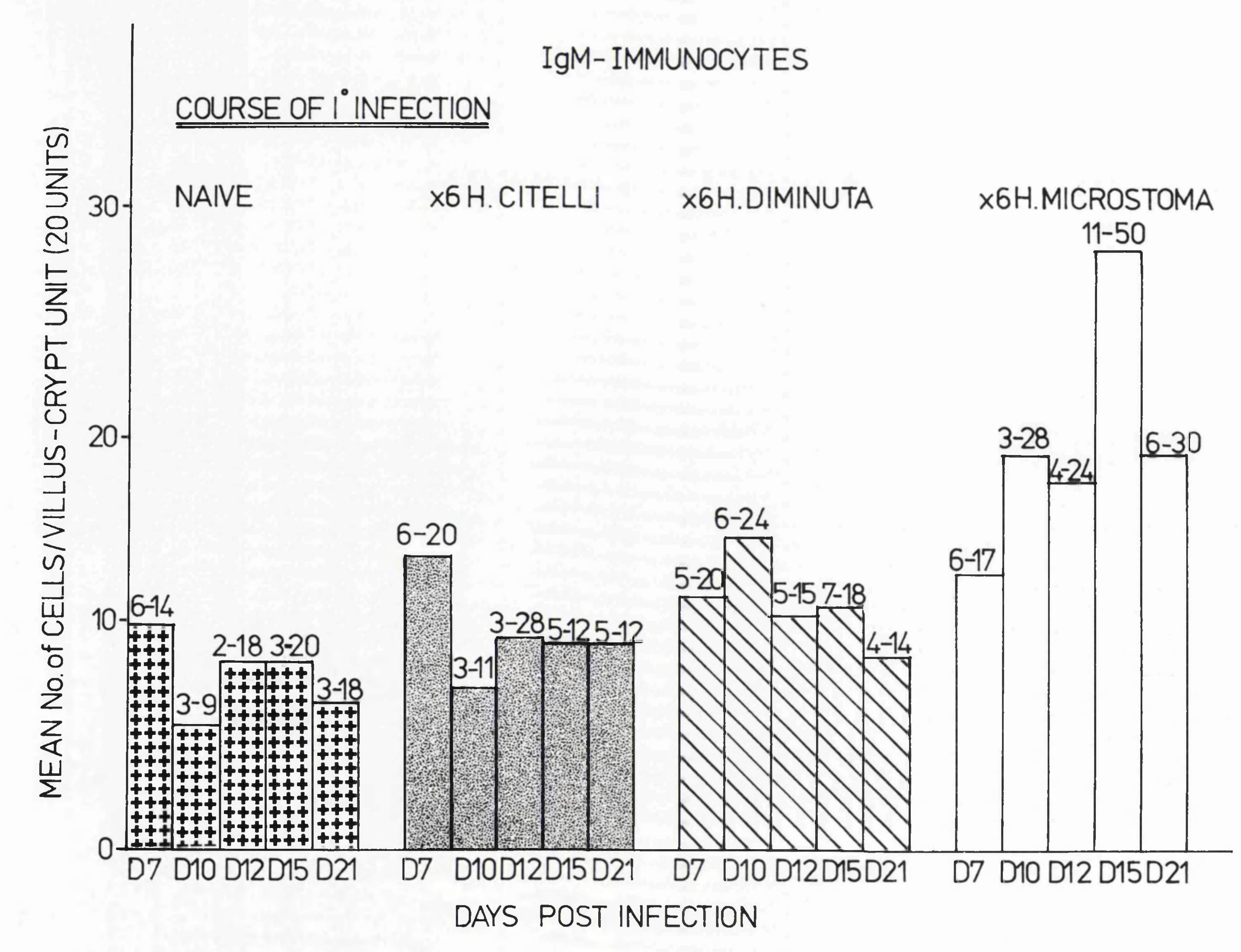
Figure 7-2

Mean number of IgM positive immunocytes per villus-crypt unit in the lamina propria of uninfected, and H. citelli, H. diminuta and H. microstoma infected mice.
Index above histograms indicates the range of immunocytes counted in 20 villus-crypt units.
* Mice were each infected with 6 cysticercoids of the appropriate species.


IgM- IMMUNOCYTES
COURSE OF I° INFECTION
NAIVE
x6 H. CITELLI
x6 H. DIMINUTA
x6 H. MICROSTOMA
MEAN No. of CELLS/VILLUS-CRYPT UNIT (20 UNITS)
30
20
10
0
6-14
3-9
2-18
3-20
3-18
6-20
3-11
3-28
5-12
5-12
5-20
6-24
5-15
7-18
4-14
6-17
3-28
4-24
11-50
6-30
D7 D10 D12 D15 D21
D7 D10 D12 D15 D21
D7 D10 D12 D15 D21
D7 D10 D12 D15 D21
DAYS POST INFECTION

intensity of fluorescence in the lamina propria was less than in IgA stained tissues. Again as in IgA stained tissues, non-specific fluorescence probably of eosinophils was evident. IgM specific streaks of fluorescence were also seen in the central arterioles of the villi.

H. citelli infected mice: The results show no difference between the numbers of IgM positive cells in H. citelli infected mice in comparison with control mice. Groups of immunocytes were slightly more localised at the bases of the villi in comparison with uninfected mice. The intensity of the fluorescence was similar in both infected and uninfected mice, although in H. citelli infected mice non-specific fluorescence of plant material was slightly more evident than in uninfected mice. There was no fluorescence on the worm surfaces in the sections.

H. diminuta infected mice: In comparison with uninfected and H. citelli infected mice, the data show no appreciable difference in the quantitative appearance of IgM positive cells between days 7 and 21. The distribution and intensity of fluorescence was comparable in all three groups, although the fluorescence of the luminal contents was less than in H. citelli infected mice. Clusters of non-specifically fluorescing cells were evident near the apices of the villi, albeit variably throughout the infection.

H. microstoma infected mice: The results show an increase in the mean numbers of IgM positive cells in comparison with uninfected, H. citelli and H. diminuta infected mice. The increase on day 15 was striking, although there was a wide variation in counts on that day, it is possible that the increase might be reflecting events in the synthesis of IgM occurring before day 15. However, the lower counts on day 21 might indicate that whatever these events were, they were short-lived. Essentially, the distribution and intensity of fluorescence in the tissues were comparable to the other tissues. The fluorescence from the bile duct was again diffused as was with IgA stained duct tissues, thus making cell counts impossible.

IgG_1: The results for uninfected, H. citelli, H. diminuta and H. microstoma infected mice are shown in Fig. 7-3. The data collectively indicates the rarity of occurrence of positively reacting IgG_1 immunocytes in comparison with the results from IgA and IgM positive

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Figure 7-3

Mean number of IgG_1 positive immunocytes per villus-crypt unit in the lamina propria of uninfected and *H. citelli*, *H. diminuta* and *H. microstoma* infected mice.

Index above histograms indicates the range of immunocytes counted in 20 villus-crypt units.

* Mice were each infected with 6 cysticercoids of the appropriate species.

IgG$_1$ IMMUNOCYTES

COURSE OF 1° INFECTION

MEAN No. of CELLS / VILLUS CRYPT UNIT (20UNITS)

20

10

0

NAIVE

3-9 1-5 0-14 0-8 0-8

D7 D10 D12 D15 D21

x6 H. CITELLI

2-7 0-5 0-6 0-8 0-8

D7 D10 D12 D15 D21

x6 H. DIMINUTA

2-8 0-4 0-8 0-7 0-7

D7 D10 D12 D15 D21

x6 H MICROSTOMA

2-7 0-5 0-10 0-6 0-6

D7 D10 D12 D15 D21

DAYS POST INFECTION

cells (Figs. 7-1 and 7-2). The fluorescence of tissues was not as bright as with IgA or IgM staining, and there was no difference in either the distribution or abundance of the fluorescence observed relative to the duration of the respective infections.

b) Secondary infections

The objective of the work was to establish whether re-stimulation of the gut 10 days after removal of the primary infection, elicited an "anamnestic" IgA and IgM response in mice infected with *H. citelli*, *H. diminuta* or *H. microstoma*. The indirect technique was employed for the IgM studies, and for the IgA study the direct method was used.

Immunisation Protocol

	Day 0	D21	D31	D38
a)	-	Z	-	K
b)	6Hc	Z	*6Hc	K
c)	6Hd	Z	*6Hd	K
d)	6Hm	Z	*6Hm	K

Hc = *H. citelli* cysticercoids

Hd = *H. diminuta* cysticercoids

Hm = *H. microstoma* cysticercoids

Z = 'Zanil' (250 mg/kg/mouse)

*Note: Primary controls were carried through for each infected category. A group of mice was additionally treated with cortisone to observe the effect of the immunosuppressant on the quantitative appearance of IgA positive cells in uninfected and *H. citelli* infected mice.

Results

IgA: The data presented in Fig. 8-1 collectively show that there was no difference in the numbers of IgA positive cells in secondary H. citelli, H. diminuta and H. microstoma infections in comparison with their respective primary controls.

The results from the cortisone treated infected mice showed no difference either from untreated primary and secondary H. citelli infected mice or from cortisone treated, uninfected mice. The intensity of the fluorescence in cortisone treated mice tissues was however, less brilliant when compared with tissues from untreated mice.

IgM The results are shown in Fig. 8-2. It is concluded that there was no increase in the numbers of IgM positive cells from secondary H. citelli, H. diminuta and H. microstoma infections in comparison with their respective primary infections. The results from H. microstoma showed a slight increase over that of uninfected mice.

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Figure 8-1

Mean number of IgA positive immunocytes per villus-crypt unit in the lamina propria of mice that have experienced six cysticercoid secondary _H. citelli_, _H. diminuta_ and _H. microstoma_ infections for 7 days, and uninfected and primary infected, control mice.

Also showing mean number of IgA immunocytes per villus-crypt unit in cortisone treated mice with primary and secondary _H. citelli_ infections and control, cortisone treated mice.

Index above histograms indicates the range of immunocytes counted in 20 villus-crypt units.

* See text for immunisation protocol.

IgA ≡ IMMUNOCYTES D7 POST INFECTION

2° INFECTION

1° – PRIMARY INFECTION

2° – SECONDARY INFECTION

CA – CORTISONE TREATMENT

MEAN No. of PLASMA CELLS/VILLUS CRYPT UNIT (20VCU)

26 24 22 20 18 16 14 12 10 8 6 4 2 0

NAIVE

5–20

NAIVE CA

H. CITELLI

4–25

6-24

H. DIMINUTA

6–16

6–16

H.MICROSTOMA

5-30

6–34

H.CITELLI

5–15

3–21

1° 2° 1° 2° 1° 2° 1°CA 2°CA

TYPE OF INFECTION

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Figure 8-2

Mean number of IgM positive immunocytes per villus-crypt unit in the lamina propria of mice that have experienced six cysticercoid secondary *H. citelli*, *H. diminuta* and *H. microstoma* infections for 7 days, and uninfected and primary infected, control mice. Index above histograms indicates the range of immunocytes counted in 20 villus-crypt units.

* See text for immunisation protocol.

IgM IMMUNOCYTES D7 POST INFECTION
2° INFECTION

1° PRIMARY INFECTION
2° SECONDARY INFECTION

MEAN No of PLASMA CELLS/ VILLUS CRYPT UNIT (20VCU)

24
22
20
18
16
14
12
10
8
6
4
2
0

NAIVE
2-19

H. CITELLI
0-14 2-12
1° 2°

H. DIMINUTA
3-12 2-14
1° 2°

H. MICROSTOMA
8-19 5-24
1° 2°

TYPE OF INFECTION

2. Surface immunoglobulins on worms

The objective was to determine whether immunoglobulins (IgA and IgM) could be detected on worm surfaces *in vivo*, by the use of the indirect immunofluorescent technique (see Methods) in primary and secondary *H. citelli*, *H. diminuta* and *H. microstoma* infections.

Results

The results are shown in Table 2. The intensity of the fluorescence observed is represented on a scale as indicated below:

a) +++; Bright-green (specific)
b) ++; Fairly bright
c) +; Weak
d) +-; Yellow (non-specific)
e) -ve; Negative

TABLE 2

DAYS POST INFECTION

	7		8		9		10		12		15		21	
	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM
1°														
H. citelli	-	-					-	-	+-	-	+-	+	+	+
H. diminuta			+	+			+	+	+	+				
H. microstoma	+	-					++	+-	++	+			++	+
2°														
H. citelli	-	-							+	-				
H. diminuta	+	-+			+	+-								
H. microstoma	+	-			++	-			++	+				

The results (Table 2) show the relative abundance of IgA and IgM on the tegument of the tapeworms. The intensity of fluorescence when observed in H. citelli and H. diminuta infections was usually very weak.

The intensity of fluorescence was always greatest on H. microstoma worms and tended to increase with the duration of the infection. The results for H. microstoma show that IgA was more abundant than IgM between days 10 and 21 in the primary infection and first appeared (IgA) on day 7 post infection. The IgM detected on day 10 showed non-specific fluorescence. On all days studied, the distribution of fluorescence showed no pattern on the tegument of H. microstoma worms and no fluorescence was observed on the scolex of the worms.

In primary H. citelli infection, fluorescence was first detected on day 12 although the reaction was non-specific, while IgM was first detected on day 15. There was no increase in the abundance of immunoglobulins in secondary H. citelli infections. When fluorescence was detected on the tegument the distribution was invariably patchy, usually occurring on mature terminal proglottids. Fluorescence was never detected on the scolex or around the neck region.

In *H. diminuta* infections, IgA and IgM were first detected on day 8 in primary infections and neither the distribution nor the abundance of immunoglobulins increased with the duration of the infection. In secondary infections IgA was first detected on day 7, the IgM detected on the same day on the worms showed non-specific fluorescence. The distribution of the immunoglobulins was as patches on both mature and immature proglottids, and there was no evidence of fluorescence either specific or non-specific on the scoleces of worms throughout the infection.

DISCUSSION

The results in the present work describe immunofluorescent studies which were designed to obtain evidence that following the exposure of mice gut to primary and secondary H. citelli, H. diminuta and H. microstoma infections, there is antigen-induced local proliferation of antibody-containing cells in the intestinal lamina propria. The data from H. citelli and H. diminuta infections did not reflect any significant difference in the numbers of immunocytes producing IgA, IgM and IgG_1 in the gut of infected mice when compared to that of uninfected mice, probably because of the large variation in cell numbers per villus-crypt unit encountered. These observations probably suggest that there was no major involvement of IgA, IgM or IgG_1 plasma cells in the development of functional intestinal immune responses to the two tapeworms; even though it has been unequivocally demonstrated that their primary and secondary infections in mice are characteristically immunogenic (Hopkins et al., 1972a and b; Befus, 1975b and this thesis). The results from H. microstoma showed some evidence for the involvement of IgA and IgM specific antibody-producing cells (Figs. 7-1 and 7-2) in the primary response of infected mice when compared with control, uninfected mice. Whether there was an appreciable increase in plasma cell numbers in the hypertrophied

bile duct (in which the scolex of the worm is attached causing an inflammatory response - Howard, 1976b and Lumsden and Karin, 1970) in H. microstoma infected mice could not be determined, because of the intense intercellular fluorescence observed in the intestinal lamina propria and the sub-epithelium of the bile duct. It is likely that the increase in the abundance of fluorescence in the bile duct might have been (in addition to local production of immunoglobulins) due to the presence of immunoglobulins in the bile and leakage of IgA, IgM and IgG molecules from the systemic circulation as a result of villous atrophy. Increased production of these molecules at sites of chronic inflammation such as in the bile duct of H. microstoma infected mice, is a phenomenon known as "pathotopic potentiation" (Brandtzaeg, 1973). In this process immunoglobulins are supplied to the local site by exudation from serum and by local synthesis in immunocytes whose precursors probably are derived at random from the recirculating lymphocyte pool and perhaps from local lymphoid follicles (Craig and Cebra, 1975 and Befus, O'Neill and Bienenstock, 1978).

The localisation of immunoglobulins in sections of stained intestinal tissue showed no immunoglobulins in villous epithelial cells at any of the intestinal regions examined in this study. The distribution of positive cells in the lamina propria of

infected mice was essentially similar in IgA, IgM or IgG_1 - containing immunocytes. In contrast to the findings for IgA and IgM, the numbers of IgG_1 positive cells in both uninfected and infected mice were very small, an observation that is not at variance with those obtained by Befus (1975b). This finding does not exclude the participation of homocytotropic IgG_1 in local immunological responses as it is likely that systemic IgG_1 can gain access to the mucosal wall either by transudation or leakage (Brandtzaeg, 1973). Crandall et al. (1967b and 1974) have reported an increase in IgG_1 -containing cells relative to cells containing immunoglobulins of other classes in the immunocyte populations of mice infected with T. spiralis and N. dubius respectively.

The IgA and IgM plasma cell responses (Fig. 8-1 and 8-2) showed no marked differences between the primary and secondary responses in all three tapeworm infections, suggesting that secondary H. citelli, H. diminuta and H. microstoma infections in mice do not elicit an anamnestic local antibody response. This observation does not necessarily mean that there is an absence of local "memory-type" response in the mouse gut to infections as Andre et al. (1978b) have demonstrated an anamnestic IgA-producing cell response in the mouse gut after repeated intragastric immunisation with sheep erythrocytes, and Pierce and Gowans (1975) have also

demonstrated immunological memory in the local intestinal mucosal immune system of the rat gut in response to cholera toxoid.

The present observations on immunocyte populations in infected mice concur with the poor relationship between circulating antibody levels and the hosts' immune response reported by Befus (1975b) in which he showed no differences in the levels of serum or intestinal immunoglobulins between H. diminuta infected and uninfected mice using immunodiffusion studies. The level of plasma antibodies is indeed not an adequate index for ascertaining the status of intestinal tapeworm immunity, as not infrequently no correlation exists between the levels of circulating antibodies and resistance to intestinal parasitsm (Wassom et al., 1974). Evidence that intestinal parasitism influences the appearance of specific antibody-producing cells in the gut lamina propria has been reported by Curtain and Anderson (1971) for Ostertagia, Trichostrongylus and Nematodirus infections in sheep and by Smith et al. (1979) for Hyostrongylus rubidus infections in pigs. Husband, Beh and Lascelles (1979) using crystalline ovalbumin as antigen injected intraperitoneally have reported an increase in the appearance of IgA-specific immunocytes in the intestinal lamina propria of sheep and that these cells reach the intestine via the intestinal lymph and blood circulation. The

role of protective antibodies in the sera of mice infected with tapeworms has been investigated by Wassom *et al.*, (1974) for *H. citelli*, Howard, (1976b) for *H. microstoma* and Andreassen *et al.* (1978a) and Hopkins (1980) for *H. diminuta* infections. The passive transfer of immune serum generally fails to confer protection against subsequent challenge, even when large volumes are administered, unlike the situation in nematode infections (Ogilvie and Jones, 1968; Ogilvie and Love, 1974; Selby and Wakelin, 1973; Behnke and Parish, 1979b and Hagan, 1980). Although the present investigation does not provide proof of the local synthesis of specific immunoglobulins by intestinal plasma cells following antigenic stimulation in *H. citelli*, *H. diminuta* and *H. microstoma* infections, it is well known that the intestinal mucosa is a very active lymphoid tissue involved in local defense mechanisms and that the occurrence of plasma cells in the gut is dependent upon the presence of antigenic material in the lumen (Crabbe *et al.*, 1970; Crandall *et al.*, 1967b and 1974; Crandall and Crandall, 1972 and Pierce and Gowans, 1975).

The results on the detection of immunoglobulins on worm surfaces (Table 2) proved disappointing, as there was no appreciable abundance or distribution of these molecules on the teguments of neither *H. citelli* nor *H. diminuta*. The abundance of specific IgA and IgM

fluorescence on tegumental surfaces of H. microstoma worms was slightly greater than on the two aforementioned parasites; albeit the distribution was very patchy. The time of appearance of both IgA and IgM on H. citelli was later than on H. diminuta or H. microstoma, and invariably the intensity of fluorescence when detected was brighter on the latter parasites. The results vis-a-vis surface immunoglobulins on worm tegument although not as extensive as those of Befus (1977) corroborate his findings, in which he reported the binding of immunoglobulins on H. diminuta and H. microstoma. Threadgold and Befus (1977) have confirmed the occurrence of immunoglobulins and complement (C_3) on the surface of H. diminuta by ultrastructural visualisation of antibody localisation of binding-sites on the tegument. They demonstrated that these molecules (IgA, IgM, IgG and C_3) were scattered between and over the entire surface of the microtriches and that they might be attached to the outer component of the plasma membrane-glycocalyx complex of the worm tegument.

The question of relevance here is whether these immunoglobulins shown to be present on worm surfaces are specific antibodies bound to antigens? Neither the abundance nor the distribution of these molecules increased significantly with the duration of the infection. If these molecules were specific, it would be expected that a possible site for their interaction would be the

tegument, with its digestive/absorptive function probably impaired. Coleman and Fotorny (1962) and Coleman, McMorrow and Fimian (1963) stated that specific antibody binds to H. nana in infected mice. Antibodies to H. microstoma have been reported to occur systemically in infected mice (Moss, 1971 and Goodall, 1973) and Harris and Turton (1973) have also detected the presence of circulating antibodies in rats infected with H. diminuta. The significance of immunoglobulins associated with worm surfaces as described herein, is difficult to assess, partly because their specific roles still remain to be clarified. However, it is not unreasonable to presume that these molecules were directed at antigenic targets on the tegument. An alternative explanation could be that these host immunoglobulins were non-specifically absorbed on the tegument. If this was the case, it would not be improbable that the non-specifically absorbed molecules might block the antigenic targets, i.e. immunological blockade. The presence of immunoglobulins on the tapeworm tegument does not entirely support the concept of immunological blockade, since the blocking phenomenon has usually been associated only with specific IgG molecules (Kemp et al. 1977 and Sogandares-Bernal, 1976). Evidence for the presence of Fc receptors on the tapeworm tegument that specifically interact with and bind host IgG as has been reported for Schistosoma mansoni by Torpier et al. (1979) is required in order to augment the blockade phenomenon.

If it is assumed that the immunoglobulins are probably directed at antigenic targets on the tegument, i.e. specifically bound to one or more components of the glycocalyx, it is conceivable that membrane transport of metabolites (e.g. methionine - Bland, 1976b) may be impaired through the binding of antibody to the membrane. The capacity of the tapeworm tegument to turnover its surface membrane (Arme, 1976; Oaks and Lumsden, 1971 and Lumsden, 1975) as has been shown also for schistosomes (Wilson and Barnes, 1977), maybe representative of a mechanism to "slough off" these antibodies or other equally deleterious cellular components. The replacement of surface membrane is thus probably a facet of the mechanisms whereby the parasite avoids the hosts' response (Porter and Knight, 1974) in primary *H. microstoma* and *H. diminuta* infections in the mouse and rat respectively.

In conclusion, the present findings did not fully demonstrate the role of immunoglobulins at the intestinal mucosal surface in *H. citelli*, *H. diminuta* or *H. microstoma* infections in mice; thus leaving once again the participation of antibodies in tapeworm infections equivocal. Nevertheless, the observations probably reflect the compartmentalisation of humoral and cellular responses. In view of the fact that the study was limited to histological study of plasma cell responses in tapeworm infections, further studies are

required to evaluate fully, what other components (e.g. mast cells and antibodies in mucus and intestinal secretions) are instrumental in the local effector mechanisms of these immunogenic Hymenolepis sp. infections in mice.

SUMMARY

1. A brief description of the local intestinal immune system is given. The prime objective of the present work was to determine the participation of antibody-producing cells in local intestinal responses to H. citelli, H. diminuta and H. microstoma infections in mice.

2. In the intestinal lamina propria of H. citelli and H. diminuta infected mice, there was no evidence for the participation of antibody-producing cells, when compared to that of uninfected mice. There was an increase in the mean number of plasma cells in H. microstoma infected mice in comparison with control, uninfected mice.

3. The numbers of IgG_1 positive cells in both infected and uninfected mice were very small relative to cells positive for IgA and IgM.

4. The distribution of immunocytes in the intestinal lamina propria of infected and uninfected mice was essentially similar. The localisation of immunoglobulins in sections of stained intestinal tissue showed no immunoglobulins in villous epithelial cells.

4. The IgA and IgM responses of plasma cells showed no marked differences between the primary and secondary *H. citelli*, *H. diminuta* and *H. microstoma* infections in the mouse; indicating that in secondary *H. citelli*, *H. diminuta* and *H. microstoma* infections antibody-producing cells play no major role.

5. Immunofluorescence studies showed the presence of IgA and IgM immunoglobulins on the tegument of *H. citelli*, *H. diminuta* and *H. microstoma* worms. The distribution of these molecules was more abundant on *H. microstoma* than on the aforementioned parasites, and the intensity of fluorescence when detected on worm surfaces was variable. The time of appearance of both IgA and IgM was later on *H. citelli* worms than on either *H. diminuta* or *H. microstoma*.

6. The significance of these immunoglobulins on worm surfaces is discussed as to whether they are non-specifically associated with the tegument or specifically bound to antigenic targets.

7. It is suggested that local intestinal immune responses in tapeworm infections warrant further study.

General Discussion

Prior to the investigation in this thesis, work on *H. citelli* as a useful model for studies on immunity to tapeworms was scanty (see Introduction in Chapter 1, above) and therefore, the usefulness of the system in this regard needed further evaluation. Studies by Wassom *et al*. (1973 and 1974) on *H. citelli* in *Peromyscus maniculatus* demonstrated the involvement of an immunological response against the parasite. Hopkins and Stallard (1974) demonstrated the suppression of worm loss using cortisone acetate, and postulated that expulsion of the parasite in the laboratory mouse was immune-mediated. Although their results were not definitive evidence that an immune response is evoked in the mouse, they do augment an immunologically-mediated concept when considered with the results of Weinmann (1966) who reported that growth of secondary worms was retarded in comparison with that of primary worms. Evidence for the probable involvement of an immune response against the parasite was thus primordial; inevitably, in order to provide a basis for studying immunological responses against *H. citelli* infections, the growth and survival of primary and secondary worms in the mouse needed to be fully characterised. The objective of the work described in this thesis was thus born, and from the findings it has emerged that the mouse-*H. citelli* model offers

tremendous potential not only in the study of immunity to _Hymenolepis sp_. but also to give a comparative perspective of aspects of immunity to other intestinal helminths.

The objectives and findings herein were aimed at characterising the rejection of the parasite in the mouse and rat hosts, with particular reference to the growth and survival of worms. In the first section of Chapter 1, the dynamics of infection in the mouse was described. One of the salient points that emerged was the inherent variability in the system, vis-a-vis worm growth. Variation in worm growth was evident in both single and multiple worm infections in different experiments using mice of the same strain, age, sex and environmental history. The observation is very akin to that reported for the _H. diminuta_-mouse model, where Befus(1975b) suggested that the variation in worm growth could be minimised by the use of multiple (6 cysts or more) cysticercoid infections instead of single worm infections. The variation in worm growth observed in the present study in both single and multiple infections was comparable; however, as the effects of "crowding" are compounded at higher levels of infections, this necessitated caution in the interpretation of results in experiments in which growth of worms was of importance. The age of mice at infection has been demonstrated to be important in cestode work (Befus, 1975b), probably reflect-

ing the immunological and physiological maturation of the gut (Jarret, *et al.*, 1968; Dineen and Kelly, 1973 and Ogilvie and Jones, 1973); therefore throughout the work described in this thesis, the age of mice at time of infection was invariably 6 weeks ±2 days old. Six cysticercoid infections were mostly used in the work, as at this level of infection (than at the minimum of infections i.e. using single cysts) the immunological response of the mouse is potentiated (Hopkins and Stallard, 1974). The findings in Chapter 1, section 1, establishes the system in CFLP mice that have been re-derived from a defunct company (Carworth, Europe) and showed that worm growth and survival depended on the intensity of the primary infection.

In Chapter 1, section 2, it was unequivocally demonstrated that following the exposure of mice to primary *H. citelli* infections, there is evidence of a protective response (acquired immunity) against homologous challenge. The response is manifested mainly as retardation in the growth of secondary worms, and not as worm expulsion because of the difficulty in finding very stunted/destrobilated worms in challenge infections. The effectiveness of the protective response was shown to be quantitatively related to the intensity of the primary infection. Surprisingly, there was no evidence of a protective response in mice that were immunised for 7 days with six and twenty-four cysticercoid infections

as measured by the reduction in the biomass of secondary worms relative to that of control, primary worms. This observation was at variance with those recorded for H. diminuta (Befus, 1975b and Elowni, 1980) in which they showed that an immunising infection terminated after only 3 days was effective in inducing a protective response against challenge. Nevertheless, subsequent findings corroborated with those of the above authors, in that the degree of protection was shown to increase with the duration and intensity of the primary infections in mice. The response evoked in H. citelli immunised mice by homologous challenge infections, was shown to diminish with time in the absence of the primary worms. The differential immunisation recorded in mice that have been sensitised with primary infections of varying intensities is not easily quantitatively explained. The "curse" is that the functional antigens which induce different elements of the immune response have not yet been isolated and characterised. Indeed until such time when the protective immunogens are characterised, vaccination studies against Hymenolepis sp. infections may have to rely on "crude antigenic" preparations. It is however, envisaged that in time, the immunogenic molecules might prove to be surface antigens of either strobilar or scolecial origin (Christie, 1979 and Elowni, 1980); although attempts at discriminating between true surface antigens and excretory/secretory

antigens which may pass to the surface probably by diffusion, causing subsequent surface coatings would be warranted. The use of antigens secreted in culture or by intraperitoneal implantation of worms might help in this regard (Elowni, 1980).

The findings that cortisone acetate prolonged the survival of the parasite in primary infections and that growth of the worms was enhanced in comparison with worms from untreated mice, indicated that an immunologically-mediated response may have been involved. Acquired immunity was partially ablated by cortisone treatment of immune mice. It is necessary to point out that a compensatory nutritional effect (due to increased food intake by cortisone treated mice - Moss, 1972) may have also been operative in cortisone treated mice (in both primary and secondary infections) which resulted in the increased growth of worms.

In Chapter 2, a study of the nature of the interaction (cross-protection) that exists between H. diminuta, H. microstoma and H. citelli was undertaken and the results proved rewarding. Cross-immunisation between H. citelli and the aforementioned parasites was demonstrated, probably indicating that a specific immunological interaction due to the sharing of common antigens was involved. The results from a phylogenetic point of view suggest that Hymenolepis sp. may have a very homogenous antigenic make up. These findings might indicate

that antigen sharing between the aforementioned tapeworms could constitute convergent evolution towards greater compatibility with a common host (Sprent, 1959) and that cross-protective responses either from the ecological or immunological perspective are representative of a mechanism evolved to limit the abundance of competitive species.

The interaction between H. citelli and Nematospiroides dubius in NIH mice in Chapter 2, section C, was of considerable interest, in that the immunodepressive effects of the nematode was successfully used as a biological tool to protract survival of the cestode in a concurrent infection. Acquired immunity to homologous H. citelli challenge was inhibited, and the observation that the expression of an "anamnestic response" to secondary infections was not ablated by a concurrent N. dubius infection may be indicative of the nature (as yet unidentified) of the immunosuppressive mechanism(s) of the nematode. Growth of the cestode was retarded in a concurrent N. dubius infection, probably as a consequence of the inflammatory response induced by the nematode infection in the mouse. It is urged that further studies are required to monitor the histopathological/biochemical changes that occur in the inflammed intestine in order to clarify the nature of the interaction.

The successful use of the rat as a suitable model for studying immunity to tapeworms in

Chapter 3 is of significance, in augmenting the concept that loss of Hymenolepis spp. in the rat was immunologically-mediated (Hopkins, 1980). The characteristics of primary infections of varying intensities were described and it was shown that following the establishment and growth of worms in CFHB rats, survival of the parasite depended on the intensity of the infection. Acquired immunity to homologous and heterologous infections in the rat was also demonstrated. The delineation of the quantitative aspects of the response has contributed to the emerging evidence that secondary Hymenolepis spp. infections in the rat are immunogenic (Andreassen and Hopkins, 1980). The nature of the response in the rat model is similar to that observed in the mouse-H. citelli relationship in that the degree of retardation in growth of secondary worms (relative to primary worms) was quantitatively related to the intensity of the sensitising infection and that the protective response also wanes with time. Further studies in attempting to distinguish between heightened reactivity to challenge infections in the primed intestine and the demonstration of "memory" are required.

The findings in Chapter 4 on the role of local mucosal immune responses in H. citelli infections in the mouse proved disappointing, in that studies on the proliferation of class specific immunoglobulin - containing immunocytes in response to antigenic stimulation bore

no fruition. There was no increase in the numbers of plasma cells in the lamina propria of infected mice when compared with that of uninfected mice. Comparative studies with H. diminuta also proved fruitless, although with H. microstoma infections there was some evidence for IgA and IgM plasma cell involvement in infected mice, relative to control uninfected mice. The occurrence of immunoglobulins associated with the tegumental glycocalyx observed in the work, was discussed as to whether they are specifically directed at antigenic targets or non-specifically absorbed on to the tegument. In the event, the question of relevance was left equivocal; however, it was suggested that on H. microstoma worms these immunoglobulins may have a protective role in acting as a blocking component of host immunity thereby enhancing the survival of the parasite in mice. Alternatively, if it is presumed that the associated immunoglobulins were specific molecules, their site of interaction could be on the glycocalyx, thereby subsequently hindering nutrient transport (Bland, 1976b).

In conclusion, the work presented in this thesis has unequivocally established H. citelli as a parasite with tremendous potential for the study of immunity to tapeworms, comparable with that of the other related immunogenic tapeworms, H. diminuta and H. microstoma. The findings have indeed helped to

"knock the final nail in the coffin" --------- that unless there is considerable mucosal damage to the host, adult tapeworms living entirely in the intestinal lumen are not immunogenic. The stage is now reached whereby the nature of the effector mechanisms that might be instrumental in the expression of immunity against this virtually non-pathogenic parasite should be evaluated. Studies on the humoral and cellular changes that occur in the intestine of infected mice and rats and the suppression of these changes, should prove worthwhile. One profoundly hopes that work in this line shall continue and that such an "admirable" host/parasite relationship (either in the mouse or the rat) will not be neglected.

REFERENCES

ANDRE, C., VAERMAN, J.P. & HEREMANS, J.F. (1978a). Oral immunization of rats with human serum albumin: interference with intestinal absorption and tolerogenic effect. In Antigen Absorption by the gut. (Edited by W.A. Hemmings) p 65-80. M.T.P. Press Ltd., Lancaster.

ANDRE, C., ANDRE, F., DRUGUET, M. & FARGIER, M. (1978b). Response of anamnestic IgA-producing cells in the mouse gut after repeated intragastric immunization. Advances in Experimental Medicine and Biology 107, 583-591.

ANDRE, C., HEREMANS, J.F., VAERMAN, J.P. & CAMBIASO, C.L. (1975). A mechanism for the induction of immunological tolerance by antigen feeding: antigen antibody complexes. Journal of Experimental Medicine 142, 1509-1519.

ANDRE, C., LAMBERT, R., BAZIN, H. & HEREMANS, J.F. (1974). Interference of oral immunization with the intestinal absorption of heterologous albumin. European Journal of Immunology 4, 701-704.

ANDREASSEN, J. & HOPKINS, C.A. (1980). Immunologically-mediated rejection of Hymenolepis diminuta by its normal host - the rat. Journal of Parasitology (in press).

ANDREASSEN, J., HINDSBO, O. & HESSELBERG, C.A. (1974). Immunity to Hymenolepis diminuta in rats: destrobilation and expulsion in primary infections, its suppression by cortisone treatment and increased resistance to secondary infections. Proceedings of the 3rd International Congress of Parasitology, Munich 2, 1056-1057.

ANDREASSEN, J., HINDSBO, O. & RUITENBERG, E.J. (1978a). _Hymenolepis diminuta_ infections in congenitally athymic (nude) mice: worm kinetics and intestinal histopathology. _Immunology_ 34, 105-113.

ANDREASSEN, J., JESPERSEN, S. & ROEPSTORFF, A. (1978b). Tapeworm (_Hymenolepis diminuta_) infections in rats and mice. _Parasitology_ 77, xxv. (Proceedings of the British Society for Parasitology Spring Meeting).

ARME, C. (1976). Feeding. In _Ecological Aspects of Parasitology_ (Ed. C.R. Kennedy) p 75-97. North-Holland Publishing Co., Amsterdam.

ASHTON, N. & COOK, C. (1952). _In vivo_ observations of the effects of cortisone upon the blood vessels in rabbit ear chambers. _British Journal of Experimental Pathology_ 83, 445-50.

BAKER, N.F. (1955). The pathogenesis of Trichostrongyloid parasites: Some effects of _Nematospiroides dubius_ on the erythrocyte patterns and spleens of mice. _Experimental Parasitology_ 4, 526-41.

BARTLETT, A. & BALL, P.A.J. (1972). _Nematospiroides dubius_ in the mouse as a possible model of endemic human hookworm infection. _Annals of Tropical Medicine and Parasitology_ 66, 129-139.

BAZIN, H. (1977). Secretory immunoglobulins and local immunity. In _Immunity in Parasitic Diseases_. Colloques INSERM, 72, p 185-200.

BEFUS, A.D. (1975a). Secondary infections of _Hymenolepis diminuta_ in mice: effects of varying worm burdens in primary and secondary infections. _Parasitology_ 71, 61-75.

BEFUS, A.D. (1975b). Intestinal immune responses of mice to the tapeworms Hymenolepis diminuta and H. microstoma. Ph.D. Thesis, University of Glasgow.

BEFUS, A.D. (1977). Hymenolepis diminuta and H. microstoma: mouse immunoglobulins binding to the tegumental surface. Experimental Parasitology 41. 242-251.

BEFUS, A.D. & FEATHERSTON, D.W. (1974). Delayed rejection of single Hymenolepis diminuta in primary infections of young mice. Parasitology 69, 77-85.

BEFUS, A.D. & PODESTA, R.B. (1976). Intestine. In Ecological Aspects of Parasitology. (Ed. C.R. Kennedy) p 305-325. North-Holland Publishing Co., Amsterdam.

BEFUS, A.D. & THREADGOLD, L.T. (1975). Possible immunological damage to the tegument of Hymenolepis diminuta in mice and rats. Parasitology 71, 525-534.

BEFUS, A.D., O'NEILL, M. & BIENENSTOCK, J. (1978). Immediate IgA precursor cells in rabbit intestinal lamina propria. Immunology 35, 901-906.

BEHNKE, J.M. (1975). Suppression of expulsion of Aspiculuris tetraptera in hydrocortisone and methotrexate treated mice. Parasitology 71, 109-16.

BEHNKE, J.M. & PARISH, H.A. (1979a). Nematospiroides dubius: arrested development of larvae in immune mice. Experimental Parasitology 47, 116-127.

BEHNKE, J.M. & PARISH, H.A. (1979b). Expulsion of Nematospiroides dubius from the intestine of mice treated with immune serum. Parasite Immunology, 1, 13-26.

BEHNKE, J.M. & WAKELIN, D. (1977). Nematospiroides dubius: Stimulation of acquired immunity in inbred strains of mice. Journal of Helminthology 51, 167-176.

BEHNKE, J.M., BLAND, P. & WAKELIN, D. (1977). Effect of the expulsion phase of Trichinella spiralis on Hymenolepis diminuta in mice. Parasitology 75, 79-88.

BEHNKE, J.M., WAKELIN, D. & WILSON, M.M. (1978). Trichinella spiralis: delayed rejection in mice concurrently infected with Nematospiroides dubius. Experimental Parasitology 46, 121-130.

BELL, R.G., McGREGOR, D.D. & DESPOMMIER, D.D. (1979). Trichinella spiralis: Mediation of the Intestinal Component of Protective Immunity in the rat by Multiple, Phase-Specific, Antiparasitic Responses. Experimental Parasitology 47, 140-157.

BERENBAUM, M.C. (1974). Comparison of the mechanisms of action of immunosuppressive agents: In Progress in Immunology II, Vol. 5, p233-243. (L. Brent and J. Holborow - Eds.) North Holland Publishing Co., Amsterdam.

BIENENSTOCK, J. (1974). The physiology of the local immune response and the gastrointestinal tract. In Progress in Immunology II, Vol. 4, p 197-207. (L. Brent and J. Holborow - Eds.) North-Holland Publishing Co., Amsterdam.

BLACK, S.J. & INCHLEY, C.J. (1974). Characteristics of immunological memory in mice I. Separate early generation of cells mediating IgM and IgG memory to sheep erythrocytes. Journal of Experimental Medicine 140, 333-48.

BLAND, P. (1976a). Immunity to Hymenolepis diminuta: unresponsiveness of the athymic nude mouse to infection. Parasitology 72, 93-97.

BLAND, P. (1976b). The Immune Response of the Mouse to the Tapeworm Hymenolepis diminuta. Ph.D. Thesis, University of Glasgow.

BRANDTZAEG, P. (1973). Structure, Synthesis and external transfer of mucosal immunoglobulins. Annals d'Immunologie (Collection des "Annales de L'Institut Pasteur"), 124 C 417-438.

BRANDTZAEG, P. (1974). Mucosal and glandular distribution of immunoglobulin components: differential localisation of free and bound SC in secretory epithelial cells. Journal of Immunology 112, 1553-1559.

BRANDTZAEG, P. & BAKLIEN, K. (1977). Intestinal Secretion of IgA and IgM: A hypothetical model. In Immunology of the Gut. Ciba Foundation Symposium 46, p 77-113. Elsevier, Excerpta Medica, North-Holland, Amsterdam.

BROWN, A.R., CRANDALL, R.B. & CRANDALL, C.A. (1976). Increased IgG catabolism as a possible factor in the immunosuppression produced in mice infected with Nematospiroides dubius. Journal of Parasitology 62, 169-71.

BROWN, W.R., NEWCOMB, R.W. & ISHIZAKA, K. (1970). Proteolytic degradation of exocrine and serum immunoglobulins. Journal of Clinical Investigations 49, 1374-1380.

CASTRO, G.A., COTTER, M.V., FERGUSON, J.D. & GORDON, C.W. (1973). Trichinosis-physiologic factors possibly altering the course of infection. Journal of Parasitology 59, 268-276.

CEBRA, J.J., KAMAT, R., GEARHEART, P., ROBERTSON, S., & TSENG, J. (1977). The secretory IgA system of the gut. In Immunology of the Gut. Ciba Foundation Symposium 46, p 5-22. Elsevier, Excerpta Medica, North-Holland, Amsterdam.

CHANDLER, A.C. (1939). The effects of number and age of worms on development of primary and secondary infections with Hymenolepis diminuta in rats, and an investigation into the true nature of 'premunition' in tapeworm infections. American Journal of Hygiene 29, 105-114.

CHAPPELL, L.H. & PIKE, A.W. (1976a). Loss of Hymenolepis diminuta from the rat. International Journal for Parasitology 6, 333-339.

CHAPPELL, L.H. & PIKE, A.W. (1976b). Interactions between Hymenolepis diminuta and the rat. In Biochemistry of Parasites and Host-parasite Relationships (Ed. H. van den Bossche) p 379-384. Elsevier/North-Holland Press, Amsterdam.

CHOROMANSKI, L. (1978). The influence of cyclophosphamide on the development of Hymenolepis diminuta in mice. Short Communications of the 4th International Congress of Parasitology E, 32-33.

CHOWANIEC, W., WESCOTT, R.B. & CONGDON, L.L. (1972). Interaction of Nematospiroides dubius and influenza virus in mice. Experimental Parasitology 32, 33-34.

CHRISTIE, P.R. (1979). The intestinal immune response of the mouse to the tapeworm Hymenolepis diminuta. Ph.D. Thesis, University of Glasgow.

CIBA FOUNDATION SYMPOSIUM 46 (New Series) (1977). In Immunology of the Gut. p 356-380. Elsevier, Excerpta Medica, North-Holland Press, Amsterdam.

CLAMAN, H.N. (1975). How corticosteroids work. Journal of Allergy and Clinical Immunology 55, 145-151.

CLEGG, J.A. & SMITH, M.A. (1978). Prospects for the development of dead vaccines against helminths. Advances in Parasitology 16, 165-218.

COHEN, J.J. (1971). The effects of hydrocortisone on the immune response. Annals of Allergy 29, 358-361.

COHEN, S. (1976). Survival of parasites in the immunised host. In Immunology of Parasitic Infections (Ed. Cohen, S. and Sadun, E.H.) p 35-46. Blackwell Scientific Publications, Oxford.

COLEMAN, R.M. & FORTONY, N.M. (1962). In vivo isolation of Hymenolepis nana and antibody-binding sites. Nature 195, 920-921.

COLEMAN, R.M., CARTY, J.M. & GRAZIADEI, W.D. (1968). Immunogenicity and phylogenetic relationship of tapeworm antigens produced by Hymenolepis nana and Hymenolepis diminuta. Immunology 15, 297-304.

COLEMAN, R.M., McMORROW, A. & FIMIAN, W. (1963). Association of specific intestinal antibody with dwarf tapeworm. Proceedings of the Seventh International Congress on Tropical Medicine and Malaria, Rio de Janeiro, Argentina, (Abstract of the Papers), p 107.

COURTNEY, C.H. & FORRESTER, D.J. (1973). Interspecific interactions between Hymenolepis microstoma (Cestoda) and Heligmosomoides polygyrus (Nematoda) in mice. Journal of Parasitology 59, 480-483.

COX, F.E.G. (1978). Specific and non-specific immunization against parasitic infections. Nature 273, 623-626.

CRABBE, P.A., NASH, D.R., BAZIN, H., EYSSEN, H. & HEREMANS, J.F. (1970). Immunohistochemical observations on lymphoid tissues from conventional and germ-free mice. Laboratory Investigation 22, 448-457.

CRAIG, S.W. & CEBRA, J.J. (1971). Peyer's patches: an enriched source of precursors for IgA-producing immunocytes in the rabbit. Journal of Experimental Medicine 134, 188-200.

CRAIG, S.W. & CEBRA, J.J. (1975). Rabbit Peyer's patches, appendix and popliteal lymph node B lymphocytes: a comparative analysis of their membrane immunoglobulin components and plasma cell percursor potential. Journal of Immunology 114, 492-502.

CRANDALL, C.A., CRANDALL, R.B. & AREAN, V.A. (1967a). Increased resistance in mice to larval Ascaris suum infection induced by Nippostrongylus brasiliensis. Journal of Parasitology 53, 214-215.

CRANDALL, R.B. & CRANDALL, C.A. (1972). Trichinella spiralis: immunologic response to infection in mice. Experimental Parasitology 31, 378-398.

CRANDALL, R.B., CEBRA, J.J. & CRANDALL, C.A. (1967b). The relative proportions of IgG-, IgA- and IgM-containing cells in rabbit tissues during experimental trichinosis. Immunology 12, 147-58.

CRANDALL, R.B., CRANDALL, C.A. & FRANCO, J.A. (1974). Heligmosomoides polygyrus (=Nematospiroides dubius): Humoral and intestinal immunologic reaponses to infection in mice. Experimental Parasitology 35, 275-287.

CROMPTON, D.W.T. (1973). The sites occupied by some parasitic helminths in the alimentary tract of vertebrates. Biological Reviews 48, 27-83.

CURTAIN, C.C. & ANDERSON, N. (1971). Immunocytochemical localization of the ovine immunoglobulins IgA, IgG_1, IgG_{1a} and IgG_2: effect of gastro-intestinal parasitism in the sheep. Clinical Experimental Immunology 8, 151-162.

CYPESS, R.H. & ZIDIAN, J.L. (1975). Heligmosomoides polygyrus (=Nematospiroides dubius): the development of self-cure and/or protection in several strains of mice. Journal of Parasitology 61, 819-24.

CYPESS, R.M., EBERSOLE, J.L. & MOLINARI, J.A. (1977). Specific antibody levels in the intestinal perfusates of Heligmosomoides polygyrus-infected mice. International Archives of Allergy and Applied Immunology 55, 496-503.

DESPOMMIER, D. (1977). Immunity to Trichinella spiralis. The American Journal of Tropical Medicine and Hygiene 26, 68-75.

DINEEN, J.K. (1978). The nature and role of immunological control in gastrointestinal helminthiasis. In The Epidemiology and Control of Gastrointestinal Parasites in Sheep in Australia (Ed. Donald, D.A., Southcott, W.H. and Dineen, J.K.) p 121. CSIRO, Australia.

DINEEN, J.K. & KELLY, J.D. (1973). Immunological unresponsiveness of neonatal rats to infection with Nippostrongylus brasiliensis. The competence of neonatal lymphoid cells in worm expulsion. Immunology 25, 141-50.

DINEEN, J.K., GREGG, P., WINDON, R.G., DONALD, A.D. & KELLY, J.D. (1977). The role of immunologically specific and non-specific components of resistance in cross-protection to intestinal nematodes. International Journal for Parasitology 7, 211-215.

DRACOTT, B.N. & SMITH, C.E.T. (1979a). Hydrocortisone and the antibody response in mice. I. Correlations between serum cortisol levels and cell numbers in thymus, spleen, marrow and lymph nodes. Immunology 38, 429-435.

DRACOTT, B.N. & SMITH, C.E.T. (1979b). Hydrocortisone and the antibody response in mice. II. Correlations between serum antibody and PFC in thymus, spleen, marrow and lymph nodes. Immunology 38, 437-443.

EHRENFORD, F.A. (1954). The life-cycle of Nematospiroides dubius Baylis (Nematoda: Heligmosomidae). Journal of Parasitology 40, 480-1.

ELOWNI, E.E. (1980). Immunity to tapeworms: vaccination against Hymenolepis diminuta and role of the bursa of Fabricius in rejection of Raillietina cesticillus. Ph.D. Thesis, University of Glasgow.

FAUBERT, G.M. (1976). Depression of the plaque-forming cells to sheep red blood cells by the new-born larvae of Trichinella spiralis. Immunology 30, 485-489.

FELDBUSH, T.L. (1973). Antigen Modulation of the Immune Response. The Decline of Immunological Memory in the Absence of Continuing Antigenic Stimulation. Cellular Immunology 8, 435-444.

FERGUSON, A. (1972). Immunological roles of the gastrointestinal tract. Scottish Medical Journal 17, 111-8.

FERGUSON, A. (1976). Coeliac disease and gastrointestinal food allergy. In Immunological Aspects of the Liver and Gastrointestinal Tract (Ed. Ferguson, A. and MacSween, R.N.M.) p 153-202. M.T.P. Press Ltd., Lancaster.

FERGUSON, A. & PARROTT, D.M.V. (1972). The effect of antigen deprivation on thymus-dependent and thymus-independent lymphocytes in the small intestine of the mouse. Clinical Experimental Immunology 12, 477-88.

FORD, B.R. (1972). Hymenolepis citelli: Development and chemical composition in hypothermic ground squirrels. Experimental Parasitology 32, 62-70.

FREEMAN, J., HUDSON, K.M., LONGSTAFFER, J.A. & TERRY, R.D. (1973). Immunodepression in trypanosome infections. Parasitology 67: p xxiii. (Proceedings of the British Society for Parasitology Spring Meeting).

GAAFAR, S.M., DUGAS, S. & SYMENSA, R. (1973). Resistance of pigs recovered from transmissible gastroenteritis against infection with Ascaris suum. American Journal of Veterinary Research 34, 793-795.

GANGULY, R. & WALDMAN, R.H. (1979). Cell-mediated immunity on Secretory Surfaces. Advances in Experimental Medicine and Biology 114, 75-84.

GEMMELL, M.A. & JOHNSTONE, P.D. (1977). Experimental epidemiology of hydatidosis and cysticercosis. Advances in Parasitology 15, 311-369.

GOODALL, R.I. (1973). Studies on the growth, location specificity and immunobiology of some hymenolepid tapeworms. Ph.D. Thesis, University of Glasgow.

GRAY, J.S. (1973). Studies on host resistance to secondary infections of Raillietina cesticillus Molin, 1858 in the fowl. Parasitology 67, 375-82.

GREENWOOD, B.M., PLAYFAIR, J.H.L. & TORRIGIANI, G. (1971). Immunosuppression in murine malaria - I. General characteristics. Clinical and Experimental Immunology 8, 467-478.

GRUNDMANN, A.W. & FRANDSEN, J.C. (1960). Definitive host relationships of the helminth parasites of the deer-mouse, Peromyscus maniculatus, in the Bonneville Basin of Utah. Journal of Parasitology 46, 673-8.

GUY-GRAND, D., GRISCELLI, C. & VASSALLI, P. (1974). The gut-associated lymphoid system: nature and properties of large dividing cells. European Journal of Immunology 4, 435-443.

HAGAN, P. (1980). Studies on host-parasite relationship of Nematospiroides dubius in the mouse. Ph.D. Thesis, University of Glasgow.

HALL, J. (1979). Lymphocyte recirculation and the Gut: The cellular basis of humoral immunity in the intestine. Blood Cells 5, 479-492.

HALL, J., ORLANS, E., REYNOLDS, J., DEAN, C., PEPPARD, J., GYURE, L. & HOBBS, S. (1979). Occurrence of specific antibodies of the IgA class in the bile of rats. International Archives of Allergy and Applied Immunology 59, 75-84.

HARRIS, W.G. & TURTON, J.A. (1973). Antibody response to tapeworm (Hymenolepis diminuta) in the rat. Nature 246, 521-522.

HEMMINGS, W.A. (1978). The transmission of high molecular weight breakdown products of protein across the gut of suckling and adult rats. In Antigen Absorption by the Gut (Ed. W.A. Hemmings) p 37-47. MTP Press Ltd., Lancaster.

HEMMINGS, W.A. & WILLIAMS, E.W. (1978). Transport of large breakdown product of dietary protein through the gut wall. Gut 19, 715-723.

HESSELBERG, C.A. & ANDREASSEN, J. (1975). Some influences of population density on Hymenolepis diminuta in rats. Parasitology 71, 517-524.

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

HEYNEMAN, D. (1963). Host-parasite resistance patterns - Some implications from experimental studies with helminths. Annals of the New York Academy of Sciences 113, 114-29.

HOBART, M.J. & McCONNELL, I. (1978). The Immune System. Blackwell Scientific Publications, Oxford.

HOPKINS, C.A. (1970). Diurnal movement of Hymenolepis diminuta in the rat. Parasitology 60, 255-271.

HOPKINS, C.A. (1980). Immunity and Hymenolepis diminuta. In Biology of the tapeworm Hymenolepis diminuta (Arai, H.P., Ed.) p 551-614. Academic Press, New York.

HOPKINS, C.A. & ALLEN, L.M. (1979). Hymenolepis diminuta: the role of the tail in determining the position of the worm in the intestine of the rat. Parasitology 79, 401-410.

HOPKINS, C.A. & STALLARD, H.E. (1974). Immunity to intestinal tapeworms: the rejection of *Hymenolepis citelli* by mice. *Parasitology* 69, 63-76.

HOPKINS, C.A. & STALLARD, H.E. (1976). The effect of cortisone on the survival of *Hymenolepis diminuta* in mice. *Rice University Studies* 62, 145-159.

HOPKINS, C.A., GOODALL, R.I. & ZAJAC, A. (1977). The longevity of *Hymenolepis microstoma* in mice, and its immunological cross-reaction with *H. diminuta*. *Parasitology* 74, 175-184.

HOPKINS, C.A., GRANT, P.M. & STALLARD, H. (1973). The effect of oxyclozanide on *Hymenolepis microstoma* and *Hymenolepis diminuta*. *Parasitology* 66, 355-65.

HOPKINS, C.A., SUBRAMANIAN, G. & STALLARD, H.E. (1972a). The development of *Hymenolepis diminuta* in primary and secondary infections in mice. *Parasitology* 64, 401-412.

HOPKINS, C.A., SUBRAMANIAN, G. & STALLARD, H.E. (1972b). The effect of immunosuppressants on the development of *Hymenolepis diminuta* in mice. *Parasitology* 65, 111-120.

HOWARD, R.J. (1976a). The growth of secondary infections of *Hymenolepis microstoma* in mice: the effect of various primary infection regimes. *Parasitology* 72, 317-323.

HOWARD, R.J. (1976b). Aspects of the host/parasite relationship of *Hymenolepis microstoma*. Ph.D. Thesis, University of Glasgow.

HOWARD, R.J. (1977). *Hymenolepis microstoma*: a change in susceptibility to resistance with increasing age of the parasite. *Parasitology* 75, 241-249.

HOWARD, R.J., CHRISTIE, P.R., WAKELIN, D., WILSON, M.M. & BEHNKE, J.M. (1978). The effect of concurrent infection with Trichinella spiralis on Hymenolepis microstoma in mice. Parasitology 77, 273-279.

HUSBAND, A.J. (1978). An immunization model for the control of infectious enteritis. Research in Veterinary Science 25, 173-177.

HUSBAND, A.J., BEH, K.J. & LASCELLES, A.K. (1979). IgA-containing cells in the ruminant intestine following intraperitoneal and local immunization. Immunology 37, 579-601.

HUSBAND, A.J., MONIE, H.J. & GOWANS, J.L. (1977). The natural history of the cells producing IgA in the gut. In Immunology of the Gut. Ciba Foundation Symposium 46, p 29-54. Elsevier, Excerpta Medica, North-Holland, Amsterdam.

ISAAK, D.D. (1976). Analysis of the mechanisms of immune expulsion from mice of Hymenolepis diminuta and H. nana. Dissertation Abstracts International 37B, 2675.

JARRETT, E.E.E., JARRETT, W.F.H. & URQUHART, G.M. (1968). Immunological unresponsiveness to helminth parasites. I. The pattern of Nippostrongylus brasiliensis infection in young rats. Experimental Parasitology 23, 151-60.

JARRETT, W.F.H. & CRAIG-SHARP, N.C. (1963). Vaccination against parasitic disease: Reactions in vaccinated and immune hosts in Dictyocaulus viviparus infection. Journal of Parasitology 49, 177-89.

JENKINS, D.C. (1977). Nematospiroides dubius: The course of primary and challenge infections in the jird Meriones unguiculatus. Experimental Parasitology 41, 335-340.

JENKINS, S.N. & BEHNKE, J.M. (1977). Impairment of primary expulsion of Trichuris muris in mice concurrently infected with Nematospiroides dubius. Parasitology 75, 71-78.

JOHNSON, G.D. & HOLBOROW, E.J. (1973). Immunofluorescence. In "Handbook of Experimental Immunology" Vol. 1 (D.M. Weir) Ed.) p 18.1-18.20. Blackwell Scientific, Oxford.

KAZACOS, K.R. (1975). Increased resistance in the rat to Nippostrongylus brasiliensis following immunization against Trichinella spiralis. Veterinary Parasitology 1, 165-174.

KAZACOS, K.R. (1976). Increased resistance in the rat to Strongyloides ratti following immunization against Trichinella spiralis. Journal of Parasitology 62, 493-494.

KAZACOS, K.R. & THORSON, R.E. (1975). Cross-resistance between Nippostrongylus brasiliensis and Strongyloides ratti in rats. Journal of Parasitology 61, 525-529.

KEMP, W.M., MERRITT, S.C., BOGUCKI, M.S., ROSIER, J.G. & SEED, J.R. (1977). Evidence for absorption of heterospecific host immunoglobulin on the tegument of Schistosoma mansoni. Journal of Immunology 119, 1849-1854.

KHOURY, P.B., STROMBERG, B.E. & SOULSBY, E.J.L. (1977). Immune mechanisms to Ascaris suum in inbred guinea-pigs. I. Passive transfer of immunity by cells and serum. Immunology 32, 405-412.

KOCH, K.F. (1972). Fluorescence Microscopy: Instruments, Methods, Applications. Leitz Wetzlar.

LAMM, M.E. (1976). Cellular aspects of IgA. Advances in Immunology 22, 223-290.

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

LEMAITRE-COELHO, I., JACKSON, G.D.F. & VAERMAN, J.P. (1977). Rat bile as a convenient source of IgA and free secretory component. European Journal of Immunology 8, 588-590.

LEVIN, D.M., OTTESEN, E.A., REYNOLDS, H.Y. & KIRKPATRICK, C.H. (1976). Cellular immunity in Payer's patches of rats infected with Trichinella spiralis. Infection and Immunity 13, 27-30.

LITCHFORD, R.G. (1963). Observations on Hymenolepis microstoma in three laboratory hosts: Mesocricetus auriculatus, Mus musculus and Rattus norvegicus. Journal of Parasitology 49, 403-10.

LLOYD, S. (1979). Homologous and Heterologous Immunization against the Metacestodes of Taenia saginata and Taenia taeniaeformis in cattle and mice. Zeitschrift fur Parasitenkunde 60, 87-96.

LOEHR, K.A. & MEAD, R.W. (1979). A maceration technique for the study of cytological development in Hymenolepis citelli. Journal of Parasitology 65, 886-889.

LOUCH, C.D. (1962). Increased resistance to Trichinella spiralis in the laboratory rat following infection with Nippostrongylus muris. Journal of Parasitology 48, 24-26.

LOVE, R.J. & OGILVIE, B.M. (1975). Nippostrongylus brasiliensis in young rats. Lymphocytes expel larval infections but not adult worms. Clinical Experimental Immunology 21, 155-162.

LUMSDEN, R.D. (1975). Surface ultrastructure and cytochemistry of parasitic helminths. Experimental Parasitology 37, 267-339.

LUMSDEN, R.D. & KARIN, D.S. (1970). Electron microscopy of peribiliary connective tissues in mice infected with the tapeworm Hymenolepis microstoma. Journal of Parasitology 56, 1171-1183.

MATTIOLI, C.S. & TOMASI, T.B. (1973). The life span of IgA plasma cells from the mouse intestine. Journal of Experimental Medicine 138, 452-460.

McLEAN, J.M., MOSLEY, J.G. & GIBBS, A.L.C. (1974). Changes in thymus, spleen and lymph nodes during pregnancy and lactation in the rat. Journal of Anatomy 118, 223-230.

McLEOD, J.A. (1933). A parasitological survey of the genus Citellus in Manitoba. Canadian Journal of Research 9, 108-27.

MIMS, C.A. (1977). The Pathogenesis of Infectious Disease p 305. Academic Press, London.

MITCHELL, G.F. (1979). Review, Effector cells, molecules and mechanisms in host-protective immunity to parasites. Immunology 38, 209-223.

MOLINARI, J.A., EBERSOLE, J.L. & CYPESS, R.H. (1978). Specific antibody levels in the serum of Heligmosomoides polygyrus-infected mice. Parasitology 64, 233-238.

MOQBEL, R. (1976). Studies on the host-parasite relationship of Strongyloides ratti in rats. Ph.D. Thesis, University of London.

MOQBEL, R. & DENHAM, D.A. (1978). _Strongyloides ratti_: the effect of betamethasone on the course of infection in rats. _Parasitology_ 76, 289-298.

MOSS, G.D. (1971). The nature of the immune response of the mouse to the bile duct cestode _Hymenolepis microstoma_: _Parasitology_ 62, 285-294.

MOSS, G.D. (1972). The effect of cortisone acetate treatment on the growth of _Hymenolepis microstoma_ in mice. _Parasitology_ 64, 311-20.

NELSON, G.S., AMIN, M.A., SAOUD, M.F.A. & TEESDALE (1968). Studies on heterologous immunity in Schistosomiasis. I. Heterologous schistome immunity in mice. _Bulletin of the World Health Organisation_ 38, 9-17.

NORTH, R.J. (1971). The action of cortisone acetate on cell mediated immunity to infection: Suppression of host cell proliferation and alteration of cellular composition of infective foci. _Journal of Experimental Medicine_ 134, 1485-1500.

NORTH, R.J. (1972). The action of cortisone on cell-mediated immunity to infection: histogenesis of the lymphoid cell response and selective elimination of committed lymphocytes. _Cellular Immunology_ 3, 501-5.

OAKS, J.A. & LUMSDEN, R.D. (1971). Cytological studies on the absorptive surfaces of cestodes. V. Incorporation of carbohydrate-containing macromolecules into tegument membranes. _Journal of Parasitology_ 57, 1256-1268.

OGILVIE, B.M. (1965). Use of cortisone derivatives to inhibit resistance to _Nippostrongylus brasiliensis_ and to study the fate of parasites in resistant hosts. _Parasitology_ 55, 723-30.

OGILVIE, B.M. & JONES, V.E. (1968). Passive protection with cells or antiserum against Nippostrongylus brasiliensis in the rat. Parasitology 58, 939-949.

OGILVIE, B.M. & JONES, V.E. (1971). Nippostrongylus brasiliensis: a review of immunity and the host/parasite relationship in the rat. Experimental Parasitology 29, 138-177.

OGILVIE, B.M. & JONES, V.E. (1973). Immunity in the parasite relationship between helminths and hosts. Progress in Allergy 17, 93-144.

OGILVIE, B.M. & LOVE, R.J. (1974). Co-operation between antibodies and cells in immunity to a nematode parasite. Transplantation Reviews 19, 147-168.

OGILVIE, B.M. & PARROTT, D.M.V. (1977). The immunological consequences of nematode infection. In Immunology of the Gut. Ciba Foundation Symposium 46, p 183-201. Elsevier, Excerpta Medica, North-Holland, Amsterdam.

OGILVIE, B.M. & WILSON, R.J.M. (1976). Evasion of the immune response by parasites. British Medical Bulletin 32, 177-181.

OGRA, P.L. (1971). The secretory immunoglobulin system of the gastrointestinal tract. In The Secretory Immunologic System (Eds. Small, P.A., Dayton, D.H., Channock, R.M., Kaufman, H.E. & Tomasi, T.B.) p 259. U.S. Dept. of Health, Education & Welfare.

OLIVER, L. (1962). Studies on natural resistance to Taenia taeniaeformis in mice. II. The effect of cortisone. Journal of Parasitology 48, 758-62.

ORLANS, E., PEPPARD, J., REYNOLDS, J. & HALL, J.G. (1978). Rapid active transport of immunoglobulin A from blood to bile. Journal of Experimental Medicine 147, 588-592.

PARROTT, D.M.V. (1976). The gut-associated lymphoid tissues and gastrointestinal immunity. In Immunological Aspects of the Liver and Gastrointestinal Tract. (Eds. Ferguson, A. & MacSween, R.N.M.) p 1-32. M.T.P. Press Ltd., Lancaster.

PHILLIPS, R.S., SELBY, G.R. & WAKELIN, D. (1974). The effect of Plasmodium berghei and Trypanosoma brucei infections on the immune expulsion of the nematode Trichuris muris from mice. International Journal for Parasitology 4, 409-415.

PIERCE, N.F. & GOWANS, J.L. (1975). Cellular kinetics of the intestinal immune response to cholera toxoid in rats. Journal of Experimental Medicine 142, 1550-1563.

PORTER, P. & ALLEN, W.D. (1972). Classes of immunoglobulins related to immunity in the pig. Journal of American Veterinary Medical Association 160, 511-518.

PORTER, R. & KNIGHT, J. (1974). Parasites in the Immunized Host: Mechanisms of Survival. Associated Scientific Publishers, London.

PROWSE, S.J., EY, P.L. & JENKIN, C.R. (1978a). Immunity to Nematospiroides dubius: cell and immunoglobulin changes and the onset of immunity in mice. Australian Journal of Experimental Biology and Medical Science 56, 237-246.

PROWSE, S.J., MITCHELL, G.F., EY, P.L. & JENKIN, C.R. (1978b). Nematospiroides dubius: susceptibility to infection and the development of resistance in hypothymic (nude) Balb/c mice. Australian Journal of Experimental Biology and Medical Science 56, 561-570.

PROWSE, S.J., MITCHELL, G.F., EY, P.L. & JENKIN, C.R. (1979). The development of resistance in different inbred strains of mice to infection with Nematospiroides dubius. Parasite Immunology 1, 277-88.

RAUSCH, R. & TINER, J.D. (1948). Studies on the parasite helminths of the North Central States. I. Helminths of Sciuridae. American Mid Naturalist 39, 728-747.

READ, C.P. (1955). Intestinal physiology and the host-parasite relationship. In Some Physiological Aspects and Consequences of Parasitism (Ed. W.H. Cole). p 27-43. Rutgers University Press, New Brunswick.

READ, C.P. (1959). The role of carbohydrates in the biology of cestodes. VIII. Some conclusions and hypotheses. Experimental Parasitology 8, 365-382.

READ, C.P. (1967). Longevity of the tapeworm Hymenolepis diminuta. Journal of Parasitology 53, 1055-1056.

READ, C.P. & KILEJIAN, A.Z. (1969). Circadian migratory behaviour of a cestode symbiote in the rat host. Journal of Parasitology 55, 574-578.

READ, C.P. & PHIFER, K. (1959). Role of CHO in the Biology of Cestodes VII. Interaction between individual tapeworms of the same and different species. Experimental Parasitology 8, 46-50.

READ, C.P. & VOGE, M. (1954). The size attained by Hymenolepis diminuta in different host species. Journal of Parasitology 40, 88-89.

REES, G. (1967). Pathogenesis of adult cestodes. Helminthological Abstracts 36, 1-23.

RIDLEY, R.K. & MACINNES, A.J. (1968). A fast, simple method for obtaining visible hymenolepidid cysticercoids from Tribolium confusum. Journal of Parasitology 54, 662.

ROBERTS, L.S. (1961). The influence of population density on patterns and physiology of growth in Hymenolepis diminuta (cestode: Cyclophyllidea) in the definitive host. Experimental Parasitology 11, 332-371.

ROBERTS, L.S. (1966). Developmental physiology of cestodes. I. Host dietary carbohydrate and the "crowding effect" in Hymenolepis diminuta. Experimental Parasitology 18, 305-310.

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

ROITT, I. (1978). "Essential Immunology". 3rd Edition. Blackwell Scientific, Oxford.

RUDOLPHI, C.A. (1819). Entozoorum Synopsis cui Accedunt mantissa Duplex et Indices Locupletissimi, x+811 pp. Berolini (quoted from Wardle, R.A. & McLeod, J.A., 1952).

SAINTE-MARIE, G. (1962). A paraffin embedding technique for studies employing immunofluorescence. Journal of Histochemistry and Cytochemistry 10, 250-6.

SELBY, G.R. & WAKELIN, D. (1973). Transfer of immunity against Trichuris muris in the mouse by serum and cells. International Journal for Parasitology 3, 717-722.

SHEARMAN, D.J.C., PARKIN, D.M. & McLELLAND, D.B.L. (1972). The demonstration and functions of antibodies in the gastrointestinal tract. Gut 13, 483-99.

SHIMP, R.G., CRANDALL, R.B. & CRANDALL, C.A. (1975). Heligmosomoides polygyrus (=Nematospiroides dubius): Suppression of antibody response to orally administered sheep erythrocytes in infected mice. Experimental Parasitology 38, 257-69.

SINSKI, E. (1972). Preliminary studies on cross-resistance in Nippostrongylus brasiliensis and Trichinella spiralis infection of rats. Acta Parasitologica Polonica 20, 551-56.

SMITH, H.V., HERBERT, I.V. & DAVIS, A.J. (1979). The immune response of pigs to infection with the stomach worm Hyostrongylus rubidus (Hassall and Stiles, 1892). III. Effect of a primary infection on immunoglobulin-positive cell numbers. Immunology 38, 659-664.

SMITH, M.A., CLEGG, J.A. & WEBBE, G. (1976). Cross-immunity to S. mansoni and S. haematobium in hamster. Parasitology 73, 53-64.

SMYTH, J.D. (1966). Genetic aspects of Speciation in Trematodes and Cestodes: some speculations. Proceedings of the 1st International Congress of Parasitology (Rome 1964) Vol. I, 473-474 (Ed. A. Corradetti). Pergamon Press, Oxford.

SMYTH, J.D. (1969a). Parasites as biological models. Parasitology 59, 73-91.

SMYTH, J.D. (1969b). The Physiology of Cestodes. W.H. Freeman and Co., San Francisco.

SNEDECOR, G.W. & COCHRAN, W.G. (1967). Statistical Methods 6th Edition, Iowa State University Press.

SOGANDARES-BERNAL, F. (1976). Immunoglobulins attached to and in the integument of adult S. mansoni, Sambon, 1907, from first infection of CF_1 mice. Journal of Parasitology 62, 222-226.

SPRENT, J.F.A. (1959). Parasitism, immunity and evolution. In The Evolution of Living Organisms (Ed. G.W. Leeper) p 149-65. Melbourne University Press, Victoria, Australia.

STEWARD, M.W. (1971). Resistance of rabbit secretory IgA to proteolysis. Biochimica biophysica. Acta (Amsterdam) 236, 440-449.

STOKES, C.R., SOOTHILL, J.F. & TURNER, M.W. (1975). Immunological exclusion is a function of IgA. Nature 255, 745-746.

SYMONS, L.E.A. & JONES, W.O. (1970). Nematospiroides dubius, Nippostrongylus brasiliensis and Trichostrongylus colubriformis: Protein Digestion in Infected Mammals. Experimental Parasitology 27, 496-506.

SYMONS, L.E.A. & JONES, W.O. (1971). Protein Metabolism. I. Incorporation of ^{14}C-L-Leucine into Skeletal Muscle and Liver Proteins of Mice and Guinea Pig Infected with Nematospiroides dubius and Trichostrongylus colubriformis. Experimental Parasitology 29, 230-241.

TAN, B.D. & JONES, A.W. (1968). Resistance of mice to infection with the bile duct cestode Hymenolepis microstoma. Experimental Parasitology 22, 250-255.

THOMAS, H.C. & PARROTT, D.M.V. (1974). The induction of tolerance to a soluble protein antigen by oral administration. Immunology 27, 631-9.

THONG, Y.H. & FERRANTE, A. (1979). Inhibition of mitogen-induced human lymphocyte proliferative responses by tetracycline analogues. Clinical and Experimental Immunology 35, 443-446.

THREADGOLD, L.T. & BEFUS, A.D. (1977). Hymenolepis diminuta: ultrastructural localisation of immunoglobulin-binding sites on the tegument. Experimental Parasitology 43, 169-179.

TOMASI, T.B. & BIENENSTOCK, J. (1968). Secretory immunoglobulins. In Advances in Immunology 9, 1-96.

TOMASI, T.B. & GREY, H.M. (1972). Structure and function of immunoglobulin A. Progress in Allergy 16, 81-213.

TORPIER, G.A., CAPRON, A. & OUAISSI, M.A. (1979). Receptor for IgG (Fc) and human B-2 microglobulin on Schistosoma mansoni schistosomula. Nature 278, 447-449.

TURTON, J.A. (1968). The growth and development of Hymenolepis diminuta and H. nana in vitro and in vivo. Ph.D. Thesis, University of Glasgow.

TURTON, J.A. (1971). Distribution and growth of Hymenolepis diminuta in the rat, hamster and mouse. Zeitschrift fur Parasitenkunde 37, 315-329.

URQUHART, G.M. (1980). Vaccines against parasites, Symposia of the British Society for Parasitology vol. 18, p 107-114. (Ed. A.E.R. Taylor & R. Muller). Blackwell Scientific Publications, Oxford.

VOGE, M. (1956). Studies on the life history of Hymenolepis citelli (McLeod, 1933) (Cestoda: Cyclophyllidea). Journal of Parasitology 42, 485-9.

WAKELIN, D. (1969). Studies on the immunity of albino mice to Trichuris muris. The stimulation of immunity by chemically abbreviated infections. Parasitology 59, 549-55.

WAKELIN, D. (1970). The stimulation of immunity and the induction of unresponsiveness to Trichuris muris in various strains of laboratory mice. Zeitschrift fur Parasitenkunde 35, 162-168.

WAKELIN, D. (1973). The stimulation of immunity to Trichuris muris in mice exposed to low-level infections. Parasitology 66, 181-9.

WAKELIN, D. (1975a). Immune expulsion of Trichuris muris from mice during a primary infection: analysis of the components involved. Parasitology 70, 397-405.

WAKELIN, D. (1975b). Genetic control of immune responses to parasites: Immunity to Trichuris muris in inbred and random-bred strains of mice. Parasitology 71, 51-60.

WAKELIN, D. (1978a). Immunity to intestinal parasites. Nature 273, 617-620.

WAKELIN, D. (1978b). Genetic control of susceptibility and resistance to parasitic infection. Advances in Parasitology 16, 219-284.

WAKELIN, D. & LLOYD, M. (1976). Immunity to primary and challenge infections of Trichinella spiralis in mice: a re-examination of conventional parameters. Parasitology 72, 173-182.

WAKELIN, D. & SELBY, G.R. (1974). The induction of immunological tolerance to the parasitic nematode Trichuris muris in cortisone-treated mice. Immunology 26, 1-10.

WAKELIN, D. & WILSON, M.M. (1979a). T and B cells in the transfer of immunity against Trichinella spiralis in mice. Immunology 37, 103-109.

WAKELIN, D. & WILSON, M.M. (1979b). Immunity to Trichinella spiralis in irradiated mice. International Journal for Parasitology 10, 37-41.

WAKSMAN, B.H. (1979). Cellular hypersensitivity and immunity: Conceptual changes in last decade. Cellular Immunology 42, 155-169.

WALDER, G.L. (1978). Comparative studies on host-parasite interactions of Hymenolepis spp. with the hamster. Ph.D. Thesis, University of Calgary.

WALKER, W.A. & ISSELBACHER, K.J. (1972). Intestinal uptake of macromolecules: Effect of oral immunization. Science 177, 608-610.

WALKER, W.A. & ISSELBACHER, K.J. (1974). Uptake and transport of macromolecules by the intestine. Possible role in clinical disorder. Gastroenterology 67, 531-550.

WALKER, W.A., ISSELBACHER, K.J. & BLOCH, K.J. (1973). Intestinal uptake of macromolecules. II. Effect of parenteral immunization. Journal of Immunology 111, 221-226.

WALKER, W.A., ISSELBACHER, K.J. & BLOCH, K.J. (1974). The role of immunization in controlling antigen uptake from the small intestine. In The Immunoglobulin A System (Ed. J. Mestecky & A.R. Lawton), p 295-303. Plenum Press, New York.

WARDLE, R.A. & McLEOD, J.A. (1952). The Zoology of Tapeworms. University of Minnesota Press, Minneapolis.

WASSOM, D.L., DEWITT, C.W. & GRUNDMANN, A.W. (1974). Immunity to Hymenolepis citelli by Peromyscus maniculatus: genetic control and ecological implications. Journal of Parasitology 60, 47-52.

WASSOM, D.L., GUSS, V.M. & GRUNDMANN, A.W. (1973). Host resistance in a natural host-parasite system. Resistance to Hymenolepis citelli by Peromyscus maniculatus. Journal of Parasitology 59, 117-121.

WEINER, L.M. & NEELY, J. (1964). The nature of the antigenic relationship between Trichinella spiralis and Salmonella typhi. Journal of Immunology 92, 908-911.

WEINER, L.M. & PRICE, S. (1956). A study of antigenic relationships between Trichinella spiralis and Salmonella typhi. Journal of Immunology 77, 111-114.

WEINMANN, C.J. (1966). Immunity mechanisms in cestode infections. In Biology of Parasites (Ed. E.J.L. Soulsby), p 301-320. Academic Press, New York.

WEINMANN, C.J. (1970). Cestodes and Acanthocephala. In Immunity to Parasitic Animals (Ed. G.J. Jackson, R. Herman & I. Singer) Vol. 2, p 1021-1059. Appleton-Century-Crofts, New York.

WILLIAMS, J.F. (1979). Recent advances in the immunology of cestode infections. Journal of Parasitology 65, 337-349.

WILSON, R.A. & BARNES, P.E. (1977). The formation and turnover of the membranocalyx on the tegument of S. mansoni. Parasitology 74, 61-71.

WORLD HEALTH ORGANISATION (1976). Special Program for Research and Training in Tropical Diseases. Malaria (by W.H. Wernsdorfer) TDR/WP/76.6.

WRIGHT, W.H. (1972). A consideration of the economic impact of Schistosomiasis. Bulletin of World Health Organisation 47, 559-566.