INTESTINAL IMMUNE RESPONSES OF MICE TO THE TAPEWORMS *HYMENOLEPIS DIMINUTA* AND *H. MICROSTOMA*

THESIS

for the

Degree of Doctor of Philosophy

by

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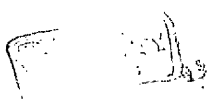

PREFACE

This thesis is the result of work conducted at the Wellcome Laboratories for Experimental Parasitology from September, 1972 until June, 1975. Included as portions of the thesis are three manuscripts: one published in 1974 under joint authorship with Dr. D.W. Featherston (SECTION 2), one presently in press with single authorship (SECTION 3), and one recently submitted for publication with Dr. L.T. Threadgold as joint author (SECTION 6). Both Dr. Featherston and Dr. Threadgold have provided statements (see pp. iii and iv) confirming that my contribution to the respective manuscripts was significant.

Throughout the thesis the style of Parasitology has been followed as closely as possible, because the three manuscripts were submitted to this journal. The inclusion of the manuscripts has made the numbering of figures and tables more complex than is usual but in all cases it is clear which illustration is being discussed.

A.D.B.

Glasgow
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How does one thank your loving wife for sharing the work so patiently and enthusiastically? Perhaps, its completion!

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SUMMARY

Previous investigations suggested that adult cestodes do not evoke host immune responses such as those directed against intestinal nematodes, but this is not so and the recent demonstration that Hymenolepis diminuta is expelled from mice by an immunological mechanism provides an excellent model system with which to investigate these responses. H. microstoma provides an interesting comparison with H. diminuta as, in the mouse, the former survives well despite host immune responses.

Following the initial administration of one (1c) or six (6c) cysticercoids to mice, ≥80% of H. diminuta can be recovered 8 to 10 days later, but thereafter, worm rejection commences. Prior to rejection, worms grow rapidly. Herein the term 'rejection' includes both destrobilation (loss of the strobila) and expulsion, although only some worms destrobilate prior to being expelled. For objective analyses of the dynamics of infection the following criteria are established: 1) mean survival time - the first day on which ≥50% of the worms administered had been lost or destrobilated, 2) destrobilated worm - a worm <0.2 mg dry weight on day 10 or thereafter, and 3) biomass - the total dry weight of all worms recovered on a given day from a group of mice (worms <0.1 included as 0.1 mg).

The mean survival time of 1c primary infections in 6 week old CFLP and NIH male mice can vary from 12 to 18 days but normally was 14-16 days, whereas with 6c

infections the mean survival time was invariably 12 days. Worms grow more slowly in 6c than in 1c infections, a well known phenomenon called 'crowding' and previously attributed solely to inter-worm competition for nutrient. However, it may be largely due to potentiated host immune responses in the multiple (6c), more immunogenic, infections.

In young, relatively immunologically immature mice (2-4 weeks old at infection), rejection of 1c infections occurs, but is delayed in comparison with infections in older, relatively immunologically mature, mice (5-7 weeks old). Biomass, which combines both worm survival and growth, shows clearly the relative immunological unresponsiveness of young mice to H. diminuta. The unresponsiveness is due to a quantitative rather than an absolute immunological deficiency and the increasing responsiveness of mice to H. diminuta with age correlates well with the maturation of intestinal lymphoid tissue. Mice >7 weeks old may be more responsive than the 2-7 weeks old mice studied herein.

Following the natural termination of a primary infection, an enhanced response occurs to secondary infection which is expressed as stunting of the worms. The severity of stunting is directly related to the intensity of the primary and secondary infections. Furthermore, when the primary infection is terminated after 3 days by anthelmintic only limited stunting of secondary worms can be detected, but following a primary infection terminated after 12 days stunting of secondary worms is severe.

As protective immunity to H. diminuta is expressed in the lumen of the intestine, a précis of intestinal immune responses is given and double immunodiffusion, single radial immunodiffusion and direct anti-globulin immunofluorescence were used to study the intestinal immune responses of infected mice. No anti-H. diminuta antibodies were detected in the sera or intestinal contents of infected or resistant mice and no consistent differences were detected between infected and uninfected mice in the levels of IgA, IgG_1, IgG_2 and IgM in the sera or intestinal contents. However, all these immunoglobulins occur on the tegument of both H. diminuta and H. microstoma and are probably specific antibodies combining with the antigens which are in the tegument. The third component of complement (C_3) was detected fixed to the tegument of H. diminuta but not to H. microstoma, although this result is equivocal. The morphology and occurrence of darkened areas in the tegument of H. diminuta are described and it is concluded that they are sites of immunological damage to the worm perhaps induced by the tegument bound antibody and C_3. The results are discussed in relation to protective immunity and survival mechanisms of intestinal helminths.

The thesis provides essential information for the further characterization of immunity to H. diminuta and strongly urges that knowledge of protective immunity to adult cestodes will be of considerable medical significance.

ABBREVIATIONS

AB	Autophagic-like body
B	Thymus independent
BL	Basal lamina
C	Crypt of Lieberkühn
CC	Cytoplasmic connection
CM	Circular muscle
C_3	Complement component three (β_1C fraction of serum)
DA	Darkened area
DN	Duration natural
DID	Double immunodiffusion
DSB	Discoidal secretory body
E	Epithelium
EC	Epithelial cell
Exp	Experiment
FITC	Fluorescein isothiocyanate
G	Golgi complex
GAM	Goat anti-mouse
GER	Granular endoplasmic reticulum
GALT	Gut-associated lymphoid tissue
HBSS	Hanks' balanced salt solution
H. dim	Hymenolepis diminuta
H. mic	Hymenolepis microstoma
Ig(s)	Immunoglobulin(s)
IM	Interstitial material
ILP	Intercryptal lamina propria
J	Joining (chain)
L	Lipid droplet
LM	Longitudinal muscle

Lu	Lumen
LAC	Laboratory Animals Centre
M	Mitochondrion
Me	Medulla (Peyer's patch)
MSE	Measuring & Scientific Equipment
MTC	Multitubular complex
MLNC	Mesenteric lymph node cells
N	Nucleus
NA	Not available
NKP	North Kent Plastic
PBS	Phosphate buffered saline
PCA	Passive cutaneous anaphylaxis
RAM	Rabbit anti-mouse
RAR	Rabbit anti-rat
S	Svedberg unit
S'	Sham
SC	Secretory component
Se	Serosa
SM	Submucosa
Su	Subepithelium
SAR	Swine anti-rabbit
s-IgA	Secretory IgA
SRBC	Sheep red blood cells
SRID	Single radial immunodiffusion
T	Thymus dependent
V	Vacuole
V_1	Villus
VLP	Villous lamina propria
ZD_3	Zanil day three
ZD_{12}	Zanil day twelve
1C	One cysticercoid

6C	Six cysticercoids
3D	Three days
12D	Twelve days

GENERAL INTRODUCTION

Tapeworms are a parasitic group of flatworms which have been the subject of much study because man and his domestic animals are definitive hosts for some and intermediate hosts for other species. The basic cestode life cycle (reviewed by Freeman, 1973) is almost without exception characterized by adult development with sexual maturation in the gut or associated diverticulum of a vertebrate, and development of the metacestode wholly, or in part, outside the gut of one or more intermediate host.

For a variety of medical and economic reasons a large number of tapeworms, including species of the genera Diphyllobothrium, Dipylidium, Echinococcus, Hymenolepis, Moniezia, Multiceps, Stilesia and Taenia, are important to man. As adults they are rarely serious pathogens (Rees, 1967), although many people find them aesthetically undesirable. However, the metacestodes can be extremely pathogenic (Smyth & Heath, 1970) depending upon which parenteral site they inhabit and what size they attain. Therefore, many attempts have been made to elucidate aspects of tapeworm-host relationships. The adult stage has been studied at least as much as the potentially more pathogenic metacestode because control methods can be directed against either stage in the life cycle.

In addition to the medical and economic importance of tapeworms, they pose an inexhaustible list of interesting biological problems. Perhaps the fundamental problem with widespread implications, is that of endosymbiosis. What are the strategies adopted by

endosymbionts and their hosts which provide for the survival of each species? The acquisition of nutrients by the adults is a relatively unique problem for cestodes as they lack a gut. Their tegument, which has an elaborate structure (Lee, 1966), has both digestive and absorptive functions (Smyth, 1972) and has been compared with the intestinal epithelium (Mettrick & Podesta, 1974). How far this comparison can be carried remains to be determined. Furthermore, cestodes show a degree of specificity for both the host and the site occupied within the host. The latter is often a more strict specificity than the former, which perhaps should be more correctly termed host range rather than specificity. Much has been written about possible factors controlling host range. Clearly ecological factors are important as, given the simplest situation, suitable contact between host and parasite is essential for infection. Once the worm is in the host, factors such as amino acid ratios and characteristics of the exocrino-enteric circulation have been implicated in determining host range (Read, Rothman & Simmons, 1963). Little importance has been attributed to the host immune response in influencing the host range of adult cestodes, but recent observations on infection of atypical hosts suggests that immunity can be important (Hopkins, Subramanian & Stallard, 1972 a & b; Wassom, Guss & Grundmann, 1973; Wassom, DeWitt & Grundmann, 1974; Hopkins & Stallard, 1974).

Indeed, a priori one would expect immune responses against a large mass of genetically distinct tissue to be important, and the obvious question is how do tapeworms survive despite host immunity? However, as

discussed more fully in SECTION 1, early studies suggested that unless there is considerable mucosal damage to the host, adult tapeworms are not immunogenic. On the other hand, immune responses to metacestodes are well known (Gemmell & Macnamara, 1972; Leid & Williams, 1974 a & b). Now that it is recognized that adult cestodes are immunogenic, there is no reason to assume that protective immune responses against them are similar to protective immune responses against parenteral metacestodes, or that the mechanisms of protective immunity against adult cestodes are identical to those against the extensively studied nematodes (see below). These considerations, together with the fact that a field of investigation, such as immunity to helminths, normally progresses by the apparent demonstration of a phenomenon and its subsequent corroboration or refutation by other workers using similar and dissimilar systems (Kuhn, 1970), establish the necessity of studying model systems of immunity to adult cestodes.

Therefore, the primary objective of the work described herein was to characterize more fully the recently established model system of Hymenolepis diminuta infection in the laboratory mouse (Hopkins et al. 1972 a & b). An historical introduction to the system and the fundamental dynamics of infection are given in SECTION 1. SECTION 2 compares the dynamics of infection in young and older mice. The dynamics of worm growth and survival in secondary infections following primary infections of various intensities and durations are given in SECTIONS 3 and 4. As an adult, H. diminuta occurs solely in the intestinal lumen and causes no obvious mucosal damage, hence it

would seem ideally suited for study of intestinal immune responses. SECTION 5 provides a précis of intestinal immune responses which is followed by the results of immunodiffusion and immunofluorescent studies on the intestine of normal mice and mice infected with H. diminuta or, for comparison, with the less immunogenic H. microstoma. SECTION 6 describes possible immunologically induced damage to H. diminuta. In each SECTION the specific objectives of the experiments are outlined in more detail than here. The body of the thesis is completed by a statement of conclusions and suggestions for further approaches to the characterization of the system.

GENERAL MATERIALS AND METHODS

This section contains descriptions of the materials and methods which apply widely throughout the thesis; descriptions of specific techniques used only in portions of this study are given in appropriate sections.

1. Animals and their maintenance

a) mice and rats

For the majority of experiments to be described male mice were purchased from commercial sources but male and female mice bred in university laboratories were used occasionally. In an effort to define and standardize the quality and immunological history of laboratory animals the Medical Research Council, Laboratory Animals Centre (LAC), Carshalton, Surrey, grades mice from various suppliers according to a scale of five categories given in The Accreditation and Recognition Scheme for Suppliers of Laboratory Animals, 1974 (Manual Series #1, second edition).

The outbred strain CFLP purchased from Anglia Laboratory Animals, Huntingdon (formerly Carworth Europe) was used most often; these mice varied from 1-4 star categories but all were tapeworm-free; although category 1 mice can theoretically possess tapeworm infections, such infections did not occur. The categories 1-3 star do not exclude the possibility of infections with the pinworms Aspiculuris tetraptera and Syphacia obvelata and such infections were encountered, especially in mice maintained in the laboratory for some time. Tapeworm-free NIH and C_3H (inbred) male mice which at that time had

not been categorized by LAC were purchased from Anglia Laboratory Animals and Scientific Agribusiness Consultants (International) Ltd., Braintree, Essex, respectively. For other experiments CFLP and CD-1 mice bred in our laboratories or CD-1 or hairless mice from the Department of Microbiology laboratories were used. When rats were required for experiments, CFY or CFHB male or female rats from our laboratories were used. The age, sex and source of mice and rats will be given for each experiment in the appropriate sections.

For all experiments using more than 20-25 mice the animals were caged in groups of seven in polythene cages 48 x 15 x 13 cm (M3 cage, North Kent Plastic (NKP) Cages Ltd., Dartford, Kent) or in metal cages 36 x 15 x 11 cm (Bowman Co. Ltd., London). In experiments where fewer mice were used they generally were caged in groups of five in M2 polythene cages 33 x 15 x 13 cm (NKP Cages). Rats were caged in groups of up to 10 in RC1 cages 56 x 38 x 18 cm (NKP Cages). Sawdust litter was changed twice weekly.

Tap water, normally without additives, was provided ad libitum from 300 ml (mice) or 600 ml (rats) polythene water bottles. In experiments where the immunodepressant cortisone acetate was administered to some mice, all mice received drinking water containing 3 g/l of Terramycin powder (Pfizer Ltd., Sandwich, England). Assuming a mouse drinks 5 ml of water daily this would provide approximately 25 mg/kg of the antibiotic, oxytetracycline hydrochloride, to each animal. Ad libitum supplies of, initially, Diet 41 (Primrose and Son) but later Diet 41B (Oxoid) were provided to mice and rats.

The animal rooms were maintained by thermostatic control at 20-22°C; lighting was not strictly controlled.

b) Hymenolepis diminuta and H. microstoma

Technical staff maintained supplies of Hymenolepis spp. for experimental purposes as outlined below.

The strains of H. diminuta and H. microstoma were acquired from Rice University, Houston, Texas in 1963 and 1964 respectively and since then have been passaged repeatedly through flour beetles, Tribolium confusum, and either rats (H. diminuta) or mice (H. microstoma). The flour beetles are kept in stock jars in unlighted incubators at 25-28°C and fed wholewheat flour. The rodent definitive hosts are maintained as described in the preceding section.

To collect tapeworm eggs for the infection of beetles the adult tapeworms are removed from the intestines of rats or mice into water at room temperature. The terminal gravid proglottids containing eggs with fully formed oncospheres are collected and homogenized in water in a blender (Measuring & Scientific Equipment Ltd., (MSE) Crawley, Sussex) for 90 s by turning the starting knob through 180° (rough estimate of speed of blender). The milky homogenate is placed in a round bottom crystallizing dish where the eggs settle to the bottom and then the supernatant, with tissue which has not settled with the eggs, is removed by suction. The eggs are resuspended in water and the procedure repeated. The button of eggs is dried by blotting with tissue paper and then given to beetles which have been starved for 2-5 days. After about 24 h most of the eggs are ingested and

the beetles are given wholewheat flour.

By 14 days after having been given eggs the beetles contain, on average, about 10 (H. diminuta) or 25 (H. microstoma) cysticercoids each. Monthly the jars containing infected beetles are cleaned to remove larvae and pupae so that no uninfected adults, which will 'dilute' the population of infected beetles, are recruited by reproduction. The stock mice and rats are each given six cysticercoids of the appropriate species as described below.

2. Infection of mice and rats

Cysticercoids used varied in age from 2 weeks to 7 months but normally were <3 months old when used for infections. When <50 cysticercoids were required for an experiment they were dissected from flour beetles in modified Hanks' balanced salt solution (HBSS), Hopkins & Stallard (1974), however, when >50 cysticercoids were needed they were collected by an homogenization procedure similar to that of Ridley & MacInnis (1968). The desired number of beetles were placed in HBSS at room temperature and then homogenized in a MSE blender for 30 s (same speed as described above for the collection of tapeworm eggs). The homogenate was poured into petri dishes and the cysticercoids were pipetted into clean HBSS at room temperature. This collection procedure was advantageous as large numbers of cysticercoids could be acquired rapidly and easily, but care must be taken not to damage cysticercoids, and hence reduce their infectivity by homogenizing for too long or at too high a

speed.

Within 5-120 min of collection from beetles the cysticercoids were administered by stomach tube to mice or rats which had been anaesthetized with anaesthetic ether (MacFarlan Smith Ltd., Edinburgh). If large numbers of animals were to be infected on a given day cysticercoids were collected on a number of occasions. The stomach tube apparatus consisted of a 5 ml disposable syringe full of HBSS, and a needle onto which 1.0 mm o.d. (mice) or 1.2 mm o.d. (rats) polythene tubing (Portex Ltd., Hythe, Kent) was attached. Cysticercoids were sucked into the end of the tubing which was then inserted orally down the oesophagus into the stomach. The abdominal wall normally was palpated to ensure that the stomach tube was properly placed and then about 0.3 ml of HBSS was evacuated from the syringe down the tube, flushing the cysticercoids into the stomach. Normally all the mice in a cage were infected together, but cages of mice from various groups in the experiment were infected alternately rather than all cages of one group followed by all of another. Throughout the thesis the day of infection is day 0.

3. Anthelmintic treatment of mice

In experiments on secondary infections of *H. diminuta* it was necessary to ensure that no worms from the primary infections remained, hence the anthelmintic oxyclozanide, 'Zanil' (I.C.I.) was administered to these mice by stomach tube (Hopkins, Grant & Stallard, 1973). To determine the correct dilution of Zanil in water for a

dosage of 150 mg/kg of the drug in 0.5 ml, mice from one or two cages were weighed and the mean weight used as representative of all the mice in the experiment.

This anthelmintic treatment was used in two types of experiments: a) where virtually all the primary worms were rejected naturally; Zanil given day 21 after infection (SECTION 3), and b) where the primary infection was prematurely abbreviated; Zanil given on either day 3, 5 or 12 after infection (SECTION 4).

4. Chemical immunodepression of mice

In some experiments the immunodepressant and anti-inflammatory agent Cortisyl (cortisone acetate, 25 mg/ml, Roussel Laboratories Ltd., London) was administered intramuscularly to the mice. Commencing on day 0 each animal received 0.04 ml every 48 h in alternate hind legs. As stated above all mice in experiments using cortisone were given oxytetracycline in their drinking water.

5. Recovery of H. diminuta from mice

Where data on worm growth and percentage recovery were collected, mouse weight in grams was recorded. Prior to autopsy mice were killed by cervical dislocation or, if serum was to be collected, by exsanguination under ether anaesthesia. For worm recovery the abdomen was opened and the small intestine, severed at the pylorus and ileal-caecal junction, was removed and its length recorded. The intestine was divided into 10 cm

lengths and each was slit longitudinally in HBSS, the contents shaken free and looked at by eye for worms. If no worms were visible the gut and contents were searched for small worms using X 6 magnification of a dissecting microscope. To find small worms which had remained attached to the intestinal mucosa the sections of intestine were incubated in HBSS at 37°C in small (51 x 18 mm) petri dishes for 1-6 h. At irregular intervals these dishes were searched with X 6 or X 12 magnification for worms which had detached from the mucosa. If after these procedures worm recovery was not 100% of those administered, it was assumed that worms not found had been expelled from the mouse, or were never there.

Occasionally scoleces or complete worms were found in the caecum or large intestine of a mouse. Such worms were considered expelled from the mouse as they were being lost and would not re-enter the small intestine or remain in the host longer than a few hours.

Worms were washed in clean HBSS, blotted on filter paper to remove any adhering intestinal contents and excess HBSS, and dried at 98-100°C in aluminium foil cups for 24-48 h. Worm weight to the nearest tenth of a mg was determined using a Stanton Unimatic balance (Stanton Instruments Ltd., London) by subtracting the weight of the empty foil cup from the weight of the foil cup containing the worm. In single worm infections worms were weighed individually but in multiple worm infections worms from a mouse were weighed collectively and the mean weight per worm determined.

SECTION 1

HISTORICAL INTRODUCTION TO *HYMENOLEPIS DIMINUTA* AND A DESCRIPTION OF THE SYSTEM

INTRODUCTION

Adult *Hymenolepis diminuta* are in nature found in the small intestine of rats. In 1937 the late Asa C. Chandler introduced *H. diminuta* to experimental studies by isolating it from a wild Norwegian rat in Houston, Texas. Descendants from this original isolation are distributed in laboratories throughout the world and are maintained by cyclical passage from intermediate host beetles (*Tenebrio molitor* and *Tribolium* spp.) to rats. Studies on the biology of *H. diminuta* were initiated by Chandler and his student the late Clark P. Read, and his students and associates, have made *H. diminuta* the most extensively studied tapeworm. Read (1955, p.27) stated that *H. diminuta* is "an admirable model since it is obligately bound to a cornucopian existence in the gut and does not incite a complicating immunological response in the host".

Prior to this statement there had been numerous studies on protective immunity to helminths and examples of worm expulsion by acquired immune responses were common (*e.g.* reviews by Taliaferro, 1940 *a* & *b*; Chandler, 1953; Larsh, 1951). The evidence that *H. diminuta* was not immunogenic in the rat was given by

Chandler (1939) in a paper which characterized the establishment and growth of H. diminuta in primary and secondary infections. The recovery and growth of worms in secondary infections was inversely proportionate to the number of primary worms present but when the primary worms were removed by carbon tetrachloride or surgery the rate of growth of secondary worms was no different than in rats similarly treated but not given primary worms. These results strongly suggested that (p.112) "premunition (reduced establishment and stunting) observed in young rats harbouring a primary infection with Hymenolepis diminuta is due entirely to a crowding effect, and not to immunity in the ordinary sense" (explanation in parenthesis mine). This conclusion has been widely quoted (e.g. Read, 1955; Heyneman, 1962 a & b, 1963; Weinmann, 1966, 1970; Smyth, 1969) and was the basis together with the work of Hunninen (1935), Hearin (1941), Larsh (1951), and Heyneman (1962 a & b) on H. nana, for Heyneman's (1963, p.124) statement that "direct mucosal contact is required for intestinal immunity and that worms or developmental stages restricted to the intestinal lumen lack sufficient contact to induce such a reaction". The concept that adult tapeworms which do not cause mucosal damage are non-immunogenic occurs in parasitology text-books (Chandler, 1955, pp.24-25; Cheng, 1964, pp.349-350; Smyth, 1969, pp.219-220) and was even used in a discussion at the Third International Congress of Parasitology, 1974, by a well known helminth immunologist despite evidence to the contrary (Weinmann, 1966, 1970; see below also).

That mucosal damage is necessary for macromolecular uptake from the lumen of the gut seems

naive now that there is abundant evidence to the contrary (Bernstein & Ovary, 1968; Rothberg, Kraft & Michalek, 1973, 1974; Walker & Isselbacher, 1974; Walker, Isselbacher & Bloch, 1974; Warshaw, Walker & Isselbacher, 1974; Thomas & Parrott, 1974). The presence of circulating antibody against H. diminuta in infected rats (Coleman, Carty & Graziadei, 1968; Harris & Turton, 1973) in the absence of obvious mucosal damage confirms that macromolecular antigen uptake occurs and, therefore contrary to Read's (1955) statement, there is an immune response to H. diminuta in the rat. The important question whose answer has widespread implications in helminth, protozoan and microbial diseases and most importantly in neoplasms, becomes: how does H. diminuta survive in rats despite the immune response of the host?

One approach to solving this question is to study the response of other hosts, such as the mouse, to H. diminuta. Initial attempts to infect mice with H. diminuta were designed to study the effect of host size and species on the size attained by tapeworms (Read & Voge, 1954). In a preliminary experiment these authors recovered only one worm from 12 "grey mice" each given a single cysticercoid 20 days earlier. In a subsequent experiment 24 "albino mice, 60 to 73 days old" were given opium intraperitoneally 30 minutes before receiving a cysticercoid; 12 similar mice each received a cysticercoid but no opium. None of these control mice had worms day 20 post infection but all the opium treated mice were infected. As opium slows peristalsis and hence increases intestinal emptying time it was suggested that H. diminuta occurs seldomly in mice because

intestinal emptying is too rapid for excystation and establishment of the young worms. Read (1955) provided further support for this hypothesis by pretreating cysticercoids with pepsin-HCl, pH 1.6, prior to administration. Recoveries from 5 week old "albino Swiss mice" given four cysticercoids each were 17/20 in the pretreated group and 4/20 in the controls day 8 after infection. The pepsin-HCl pretreatment was said to hasten excystation increasing infectivity. Heyneman (1962 b), however, using Heston/A strain and Webster C white mice recovered 40-80% of the worms 14-26 days after administration of 10 apparently untreated (Heyneman did not say) cysticercoids to each mouse. Further support for Read's hypothesis came from the results of Goodchild & Moore (1963); only 12.5% of untreated cysticercoids were recovered but 80% of cysticercoids pretreated with "0.5% pepsin in 0.85% saline" were recovered. Apart from the results of Heyneman, these early studies with H. diminuta in mice suggested that intestinal motility was important in establishment but once worms were established their survival was good.

However, using male Swiss albino mice 6-15 weeks old given morphine sulphate to slow intestinal emptying Weinmann (1966) concluded, from 1 and 10 cysticercoid infections, that in primary infections "almost all worms persisted for at least 7 days but few survived 10 days in multiple infections or 12 days in single worm infections". Following three oral infections with a single cysticercoid at 10 day intervals, no H. diminuta were recovered (day 7) from a challenge infection given 4 weeks after the last immunizing infection; control mice were infected, however.

Turton (1968) recovered, 12-72 hours after infection, 83% of 80-130 cysticercoids (not pretreated) administered to Porton mice and he concluded that in mice there is "no refractoriness to initial infection" with H. diminuta. By days 6-7, however, recovery declined to 47% and the worms on these days were shorter than on days 4 and 5. Pretreatment of cysticercoids with 1% pepsin did not influence the establishment or survival of H. diminuta in A strain mice (Turton, 1968). Using CFLP mice Turton (1971) confirmed his earlier results on the establishment, survival and growth of H. diminuta and the lack of influence of pepsin. He showed that in single cysticercoid infections worms increased in length until day 9 but by day 11 some small worms consisting of "scolex and 'neck' region only" were recovered. Such destrobilated worms occurred on days 13 and 15 also but by day 21 no worms remained. Turton (1971) concluded, regarding the destrobilation and loss of H. diminuta from mice, that "further research is required to elucidate possible causes".

Hopkins, Subramanian & Stallard (1972 a) confirmed the results of Turton (1968; 1971) on the establishment, growth, destrobilation and loss of H. diminuta from CFLP and Porton mice. In single worm infections of 6 ± 1 week old mice loss commenced by day 10 and after day 15 few large worms remained, although some destrobilated worms were recovered as late as day 38 post infection. Destrobilated worms did not regrow in the same hosts but when surgically transplanted into the intestine of naive mice or rats they quickly recommenced normal growth. In secondary infections of two worms 22

days after a primary of two worms there was a small reduction in the initial recovery of worms and, more notably, considerable stunting of growth in comparison to worms in control primary infections. From these results on surgical transplants and secondary infections Hopkins et al. (1972 a) concluded that destrobilation and loss of H. diminuta from mice is immunologically based. The immunodepressants, cortisone acetate, sodium methotrexate and horse anti-mouse lymphocyte serum either suppressed or delayed this destrobilation and loss (Hopkins, Subramanian & Stallard, 1972 b) conclusively demonstrating the immunological basis of the phenomenon.

Goodall (1973) provided further information on characteristics of H. diminuta infection in mice. He reported that previous infection with H. microstoma reduced worm recovery 8 and 9 days after infection with H. diminuta. Serological studies showed cross reactions between antigens of H. diminuta and H. microstoma and H. citelli but in infected mice the presence of circulating antibody against H. diminuta was at best unpredictable and normally undetectable by double immunodiffusion. The particulate antigens Freund's complete adjuvant, di-ethyl stilboesterol or sheep red blood cells administered intraperitoneally to mice at appropriate times delayed the rejection of H. diminuta. Goodall attributed this delay to antigenic competitions between the particulate and worm antigens. Rejection was delayed also in mice which were lactating (Goodall, 1973), a result which agrees with those from other host-parasite relationships (for reviews see Kelly, 1973; Ogilvie & Jones, 1973).

Although studies of the infection of mice were begun to supplement our understanding of the rat - H. diminuta relationship, infection of the mouse is of interest in its own right. What are the immunological mechanisms which lead to the rejection of the worms? The original question, why does H. diminuta survive in the rat, is nevertheless still important. Understanding how rejection occurs in one situation should help elucidate why it does not in another. These questions are relevant to other organisms which inhabit the intestine.

The objective of this SECTION of the thesis is to describe the dynamics of infection of mice with H. diminuta in my hands. Characteristics such as the percentage recovery, growth and time of rejection of worms varied considerably during the study and are described here. Factors such as host age and number of worms administered also are mentioned but will be considered in detail elsewhere. Understanding the characteristics of the system and their variability is essential before evaluating the results and subsequently the conclusions of other SECTIONS.

MATERIALS AND METHODS

This SECTION of the thesis requires no description of materials and methods in addition to that provided in the GENERAL MATERIALS AND METHODS. Throughout the SECTION the numbers of the experiments are given to show the sequence in which they were conducted.

RESULTS

1. A representative one cysticercoid primary infection

As there is no such thing as a typical infection, one which is considered representative is presented below. Category 2 star CFLP male mice purchased from Anglia Laboratory Animals were infected when 40 days old and subsequently 10 were autopsied every second day from days 8 to 20 post infection (Exp. 8).

The dry weight of every worm recovered (⊛) and the mean dry weight (x) of the worms which had not destrobilated are shown in Fig. 1 - 1. Destrobilated worms are defined as those which on day 10 or thereafter are <0.2 mg dry weight; prior to day 10 such small worms are not considered destrobilated but simply to have grown slowly. On days 8 and 10 respectively 90 and 100% of the worms administered were recovered but by day 12 worm loss had commenced. No undestrobilated worms were recovered after day 16. To provide a relatively objective means of comparing the rate of worm destrobilation and loss in various experiments the mean survival time was used which is defined as the first day in which ≥50% of the worms administered had been lost or destrobilated. In the experiment shown (Fig. 1 - 1) mean survival time was 14 days.

The mean dry weight of worms which had not destrobilated increased from days 8 to 16. On each day, however, there was a considerable range in worm size (e.g. day 8, 0.1 - 2.4 mg; day 14, 4.7 - 39.2 mg). The worms

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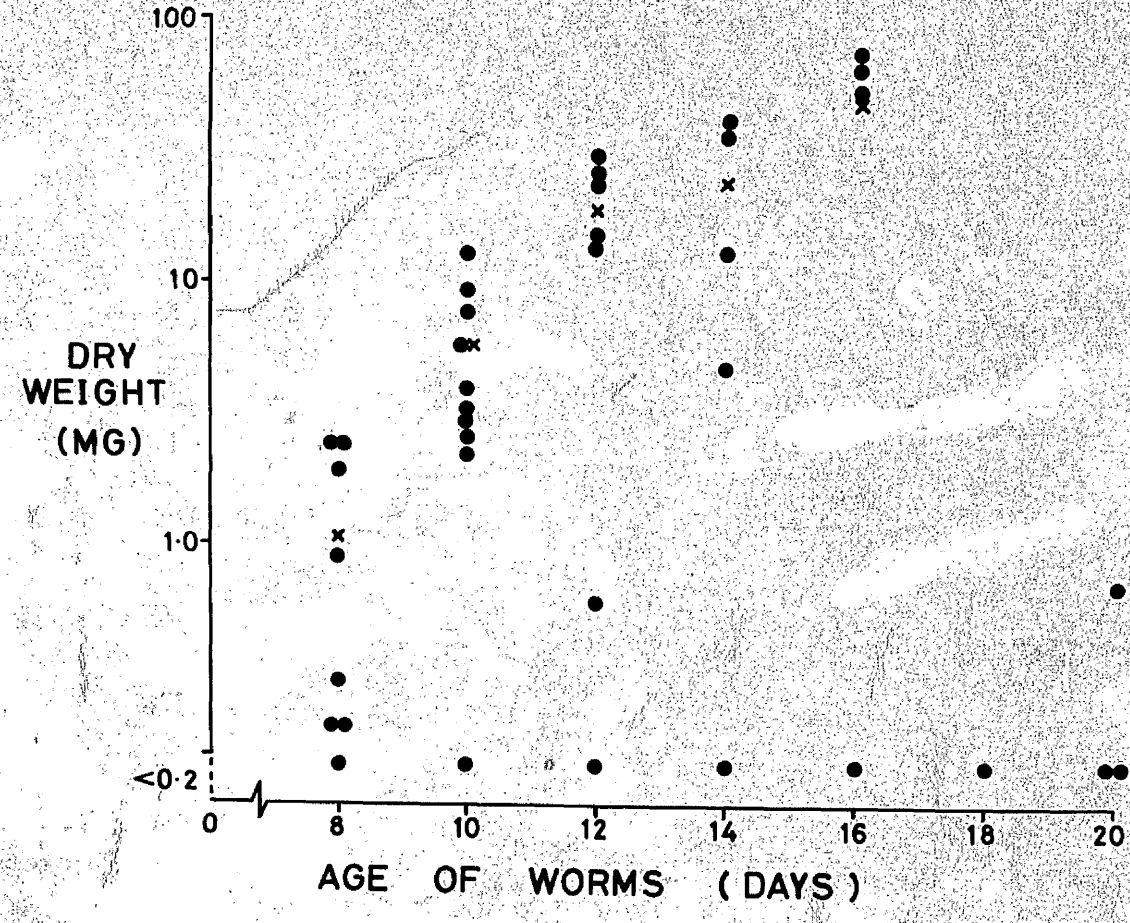
Figure 1 - 1

Growth and survival of single Hymenolepis diminuta in CFLP male mice (Exp. 8). Each solid point is the weight of a worm in a mouse, after day 8 worms <0.2 mg are destrobilated; on each day the mean weight of worms, not destrobilated, is given by x. n = 10 mice/day.


100
10
1·0
<0·2
DRY
WEIGHT
(MG)
0
8
10
12
14
16
18
20
AGE OF WORMS (DAYS)

which weighed 0.6 (day 12) and 0.7 mg (day 20) seem particularly out of place in Fig. 1 - 1 as they are smaller than expected for growing worms on these days but too large to be considered destrobilated by the above definition. These worms will be discussed further below. Destrobilated worms from single worm primary infections are relatively easily recognized as unlike other worms they are short (<1 cm), comprised generally of only a scolex and neck, with no segments (see Pl. 1D, SECTION 6). Often the posterior of a destrobilated worm is darkened and has a terminal 'nipple-like' protrusion; these two characteristics are not invariably present, however. One destrobilated worm was recovered on each of days 10-18 and two on day 20 (Fig. 1 - 1).

2. 'Atypical' one cysticercoid primary infection

The above section describes the recovery, growth and rejection of single H. diminuta in a representative experiment. In this section variations encountered in the characteristics of infections are described. The examples chosen for presentation generally represent the extremes in the range of variability and hence delimit the system in my hands. Except where indicated, experiments with CFLP male mice 6 weeks old at infection are described.

a) initial worm recoveries

In all but 2 of about 20 experiments where worm survival was studied closely, at least 80% of worms administered were recovered from mice autopsied prior to the beginning of rejection (days 8-10). These two

experiments (7; 16) which were similar in design, each having two groups of mice infected 7 days apart, had lower recoveries. In Exp. 7 each group (n = 40) had recoveries of 55%; cysticercoids administered to each group were 7 months old and from the same source. Recoveries in Exp. 16 were 70% from one group (n = 50) and 67.5% from the other (n = 40). Both groups had received 3 month old cysticercoids from the same source. One experiment (11) comparing 4 and 5 week old CFLP male mice had recoveries of 60% (n = 50) days 8 and 10 post infection. Fortunately, low initial worm recoveries were uncommon; they make interpretation of the results difficult.

b) mean survival time and rejection (destrobilation and expulsion)

The mean survival time of single H. diminuta in 6 week old CFLP male mice varied from 12-16 days. Destrobilated worms appear as early as day 10 and thereafter can occur on any day studied (e.g. Fig. 1 - 1; Table 1 - 1). In this study the latest a destrobilated worm was recovered from a primary single cysticercoid infection was day 30 post infection. Generally the number of destrobilated worms recovered on a given day was small (Table 1 - 1) but large recoveries of destrobilated worms occurred in some experiments (see Fig. 1, SECTION 2, 5 week old mice). In two experiments comparing the dynamics of infection in 4 and 5 week old CFLP male mice (9; 11) 34% (18/55) of the worms recovered days 12-20 from the younger mice were destrobilated but 60% (36/60) were destrobilated in the 5

Table 1 - 1

Comparison of the recovery and growth of Hymenolepis diminuta from single worm infections of 6 week old CFLP male mice in two experiments; n = 10 mice/day/experiment.

Age of worms (days)	Exp. 5	Exp. 19
8	10 (0) 0.88*	10 (0) 2.10
10	9 (0) 4.22	9 (1) 8.28
12	8 (1) 15.09	9 (1) 26.31
14	1 (1) -	8 (0) 60.36
16	1 (0) 95.3	7 (2) 55.82
18	3 (3) -	2 (1) 1.0
20	1 (0) 0.2	1 (0) 73.4

* The three numbers given for each experiment on a day are: total number of worms recovered; in parenthesis, the number of destrobilated worms; the mean dry weight (mg) of worms not destrobilated.

week old mice ($X^2 = 2.95$; $0.10 > P > 0.05$; 2 x 2 contingency table). Recoveries of destrobilated worms also were high on days 12-20 in an experiment with 5 week old CFLP and NIH male mice; 68% (21/31) in CFLP and 54% (20/37) in NIH. This atypical recovery of large numbers of destrobilated worms occurred in all experiments with mice 5 weeks old at infection but occurred only occasionally in experiments using mice of other ages.

c) worm growth

The variation in size of individual worms on a given day is well known (Fig. 1 - 1) and an example of the variation which occurs in the mean dry weights of the worms from different experiments is shown in Table 1 - 1. Prior to rejection the mean weights in both Exp. 5 and 19 increased but in 19 the weights were consistently greater than in 5. Prior to rejection in Exp. 5 the maximum mean weight was 15 mg whereas in 19 it was 60 mg. Solitary large worms were recovered after rejection of most worms in each experiment and it was common to find occasional large worms after the rejection of most worms in many other experiments.

d) biomass

Worm survival and growth are variable and affected by host immunity (Hopkins et al. 1972 a & b; this thesis), hence a parameter reflecting both worm numbers and size would express well the dynamics of infection. Worm biomass, defined as the total dry weight of all worms recovered on a given day from a group of mice (worms <0.1 included as 0.1 mg), is such a parameter (see SECTION 2).

The differences between experiments 5 and 19 in worm survival and growth (Table 1 - 1) are expressed graphically as biomass in Fig. 1 - 2. In both experiments biomass increased while most of the worms survived and continued to grow, but when rejection neared completion the biomass declined. On all days the biomass in Exp. 19 was greater than in Exp. 5; this was most pronounced on day 14 when the respective values were 482.9 and 0.1 mg. The high values on days 16 (Exp. 5) and 20 (Exp. 19) were due to the solitary large worms recovered on these days (see above and Table 1 - 1). It is not unusual to get such recoveries which make figures using biomass look irregular. In the examples chosen the differences are sufficiently great that the irregularities are not difficult to explain but within some experiments this unfortunately was not true.

3. Primary infections with six cysticercoids

The dynamics of infection with six cysticercoids (6c) are compared with those of one cysticercoid (1c) infections in SECTION 3 and hence will not be described in detail here. In summary, however, infections with 6c result in rejection which commences sooner and occurs more quickly than in 1c infections. With 6c infections growth of individual worms is slower than in 1c infections. This density-dependent 'crowding' phenomenon is well known in cestode infections.

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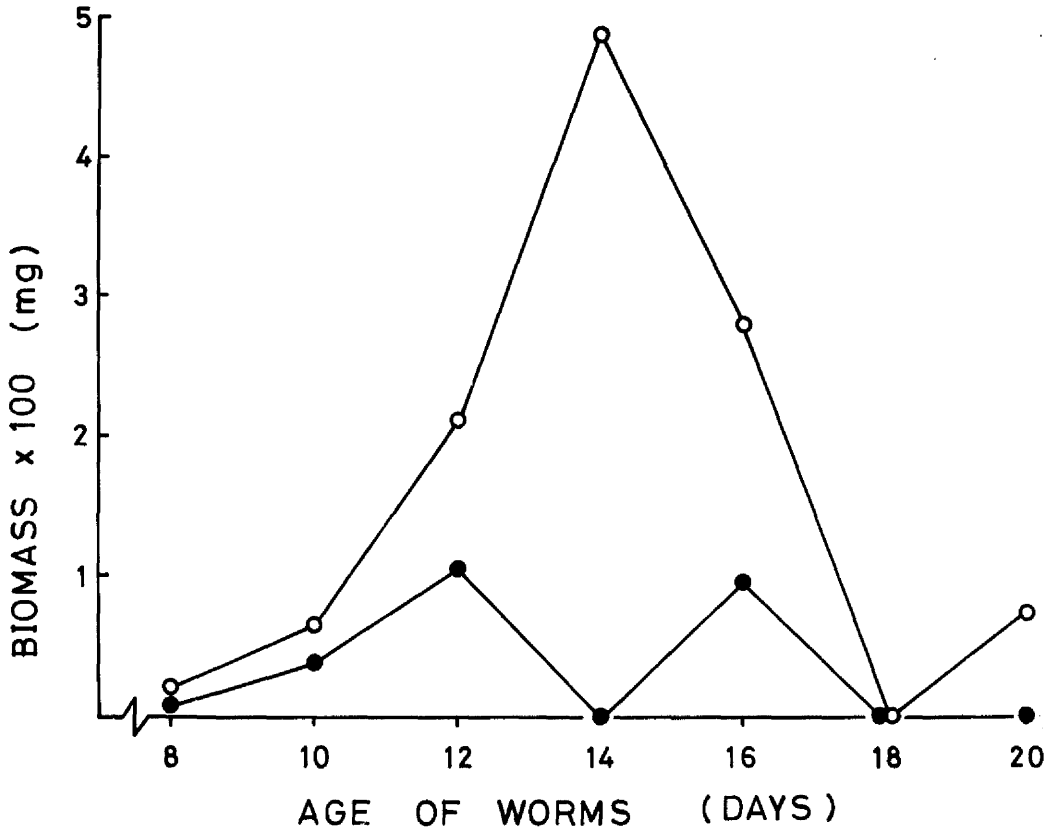
Figure 1 - 2

Biomass of Hymenolepis diminuta from single worm infections of CFLP male mice; Exp. 19 0, Exp. 5 0. n = 10 mice/day/Exp.


BIOMASS x 100 (mg)
5
4
3
2
1
8
10
12
14
16
18
20
AGE OF WORMS (DAYS)

DISCUSSION

The results show that H. diminuta establishes well in CFLP and NIH mice 6 ± 1 week old at infection, grows for sometime but then, unlike in rats, is rejected within 3 weeks. Contrary to the findings of Read & Voge (1954), Read (1955) and Goodchild & Moore (1963) agents which decrease intestinal motility or hasten excystation of the worms are not required for infection of mice. Nevertheless, the results of these authors pose some interesting questions on survival of H. diminuta in mice but until their experiments are repeated controlling for variables such as mouse age, strain and immunological history no conclusions can be reached. Although there are certain differences, discussed below, the characteristics of infections obtained herein are similar to those of Turton (1968; 1971) and Hopkins et al. (1972 a & b). The major purpose of this chapter is not to show that the results of Turton and Hopkins et al. can be confirmed but to establish accurately the dynamics of the system in my hands, to show its variability and to provide definitions which are essential to make objective comparisons between groups of results.

Worm recoveries days 8-10 post infection were normally ≥80% but in two experiments, each with two groups of mice infected 7 days apart, recoveries were 55-70% on these days. These poor recoveries could not be due to careless infection or recovery techniques on a

particular day as it is unlikely that this would occur in the separate groups of each experiment treated 7 days apart. The source of cysticercoids could have been important as in each experiment the cysticercoids used to infect one group of mice were from the same batch of beetles as the cysticercoids used to infect the other mice. For example the cysticercoids in one experiment were 7 months old and perhaps infectivity is reduced with aging. Gerris & De Rycke (1972) established, however, that for cysticercoids of H. microstoma up to about 5 months old there is no reduction in infectivity with increasing age. Although Evans (1973, p.39) showed that the time required for in vitro excystation increases with the age of cysticercoids, he concluded that it is unlikely that age has any effect upon the infectivity of cysticercoids of Hymenolepis spp. (p.64). It would seem then that the use of old cysticercoids is probably not the cause of the poor recoveries reported above especially as in the other experiment the cysticercoids used were 3 months old. There is room, nevertheless, for study on the infectivity of cysticercoids older than 5-6 months.

Another common factor in the two groups of each experiment with a low recovery is that the mice were of the same age and environmental history. Perhaps some factor in their environment made them relatively insusceptible to infection with H. diminuta. It is obvious that the reason for the low recoveries is unknown. Fortunately low recoveries are unusual and not, as some other characteristics of infection discussed below, subject to considerable variation.

Worm size on each day in an experiment and in different experiments was perhaps the most variable characteristic. The intestinal environment is extremely complex (Mettrick & Podesta, 1974) and it has been assumed for many years that a variety of physico-chemical components affect cestode growth. There is, however, little evidence to support this assumption and much controversy. Perhaps most studied is the influence of alterations in host dietary constituents; it seems generally agreed that carbohydrate levels, but not protein or lipid, influence worm growth (for reviews see Smyth, 1969; Chappell & Read, 1973). Diets from various commercial suppliers do not differ in composition to the extent which those prepared for dietary experiments differed and, assuming commercial diets with adequate carbohydrate and vitamin composition are used, it seems unlikely that variation in worm size can be attributed wholly to dietary factors. That no consistent differences occurred when one commercial diet used in this study was replaced by another supports this contention (see SECTION 2). However, any steps towards standardization and quality control of commercial diets by organizations such as the M.R.C. Laboratory Animals Centre will contribute further to the definition and standardization of laboratory animals, ultimately enhancing our understanding of the causes of variability in many biological systems, not just in the growth of tapeworms.

Apart from the influence of dietary constituents we know little of what influences cestode growth. Some recent work with perfusion studies in

vivo using closed loops of the entire small intestine hold considerable potential for studying the influence of many components (Podesta & Mettrick, 1974 a, b & c).

As well as being influenced by environmental variations, variability in size will be affected by differences in the responses of the worms and of the mice. An excellent example of differing responses of worms is the recent demonstration by Smyth & Davies (1974) of sheep and horse strains of Echinococcus granulosus with different physiological, nutritional or metabolic requirements. It seems likely that such differences are genetic and, on a less dramatic scale, genetic differences between individual H. diminuta, controlling such features as tegumental efficiency in a broad sense, could influence worm growth. Similarly genetic differences between mice which would affect nutrient availability to the worms could affect worm growth. Demonstrations by Wassom, DeWitt & Grundmann (1974) and Wakelin (1975 a) showing that the mechanism of immunological rejection of helminths can be genetically controlled renews interest in the genetics of host resistance. There has been much interest in the genetic control of immune responsiveness recently (for reviews see Benacerraf, 1974; Benacerraf & Dorf, 1974) and perhaps genetic variability in responsiveness of mice or susceptibility of worms will explain the variation in worm size.

As concluded previously regarding worm recovery, the reasons for variability in worm size are unknown although many possibilities exist. Presently the variability must be accepted and experiments

designed with it in mind. There is, however, the problem of how to present results with such variation. Ideally the weights of all worms should be presented as in Fig. 1 - 1 but often this would require too many or too cluttered figures. Therefore, some measure of central tendency and variation is required. When worm numbers (n) are decreasing because of rejection, measures of variation such as standard deviation or standard error become enormous and are meaningless. In this thesis results are presented in various ways (e.g. weights of all worms or just mean weights) depending upon the type of experiment and what is to be demonstrated from the results.

Rejection defined herein has two components: 1) destrobilation and 2) subsequent worm loss. In some cases worm loss occurs without prior destrobilation (intact worms have been found in the fecal mass of the large intestine) but the proportion of worms lost without destrobilating is unknown. Also, the half-life of a destrobilated scolex is unknown: some survive for considerable periods as evidenced by recoveries of destrobilated worms days 30 (this study) and 38 (Hopkins et al. 1972 a) post infection; but many must be expelled quickly as recoveries of destrobilated worms normally are low except in 5 week old mice (see ADDENDUM SECTION 2).

As stated above (RESULTS) destrobilated worms in single worm primary infections are relatively easily distinguished by their size and morphology but it is not always possible to be certain whether a worm is destrobilated or just stunted (see SECTION 3). In H.

diminuta destrobilation normally occurs in the neck region and is an abrupt loss of the strobila. In Raillietina cesticillus, however, destrobilation occurs progressively until by 70 days post infection all worms have lost part or all of their strobilae (Gray, 1972). Whether progressive destrobilation ever occurs in H. diminuta infections of mice is uncertain but recovery of unexpectedly small, but strobilated, worms (RESULTS) on some days suggests that it may occur. Nevertheless, it is not possible to identify confidently such worms as destrobilated. In the past destrobilated worms have been described as being 0.43-1.73 mm long (Turton, 1971), 1-2 mm long and <<0.1 mg (Hopkins et al. 1972 a) and <2 cm and <0.4 mg dry weight (Hopkins et al. 1972 b). An objective definition is essential and throughout this thesis destrobilated worms in primary infections are those <0.2 mg dry weight day 10 or thereafter post infection. Some worms which have partially destrobilated will not be included by this definition but their numbers are considered insignificant.

Typically the mean survival time of single H. diminuta in CFLP male mice 6 weeks old at infection was 14 days but varied from 12 to 16 days. Whether this survival time differs from that recorded previously (Hopkins et al. 1972 a) is uncertain (see SECTION 2). There is no doubt that increased survival time was more common in the latter part of the present study than earlier (compare Fig. 1 SECTION 2 and Fig. 1 SECTION 3). Mean survival time is less variable when 6c infections are used (SECTION 3).

In conclusion infections of mice with H. diminuta are variable in many characteristics but the variation can be reduced by using multiple worm infections. The variation must be considered when experiments are designed and appropriate controls must always be included. The definitions established above are essential for relatively objective comparisons between groups.

SUMMARY

1. An historical introduction to the use of Hymenolepis diminuta infections of mice as a model for studying immunity to adult cestodes is given. It is stated that this chapter is meant to describe the system and the methods used to express results.

2. A representative one cysticercoid infection of 6 week old CFLP male mice is presented showing recovery, growth and rejection of worms. Destrobilation and mean survival time are defined.

3. Other experiments are presented which show the variation of the system and possible causes are discussed. Biomass is defined and used as a parameter for comparison between groups as it reflects both survival and growth of worms.

SECTION 2

RELATIVE UNRESPONSIVENESS OF YOUNG MICE IN THE REJECTION OF *HYMENOLEPIS DIMINUTA*

PREFACE

Many neonatal mammals are relatively unresponsive to antigenic stimulation because, although the immune system begins to differentiate in the foetus, at birth it is not completely functional (reviews by Sterzl & Silverstein, 1967; Solomon, 1971; Fahey, 1974). Some authors have claimed that immunological responsiveness, once acquired by the young, is of the same relative magnitude as in adults (quoted by Arrenbrecht, 1973) whereas, other authors have shown a gradually increasing responsiveness (Carter & Rector, 1972; Rector & Carter, 1973). Neonatal unresponsiveness may be due to: a) the presence or absence of factors which respectively inhibit or stimulate immunocompetent cells or b) a quantitative deficiency of such cells (Arrenbrecht, 1973). If unresponsiveness is due to a qualitative factor, as (a) above, then one might expect a sudden acquisition of full responsiveness, for example, if a large number of inactive cells are suddenly activated. On the other hand if the unresponsiveness results from a quantitative deficiency in immunocompetent cells then the acquisition of responsiveness should

reflect the multiplication of such cells. As immune responses are complex, involving interactions between a number of cell types such as macrophages, thymus-dependent (T cells) and thymus-independent cells (B cells) (Miller & Rajewsky, 1974) there may be one or more quantitatively deficient populations responsible for the unresponsiveness. In the simplest situation where one cell population would be responsive, the acquisition of responsiveness would parallel the log increase of these cells by mitosis. If more than one cell type is lacking however, increasing responsiveness would reflect not only the log increases in the populations but also the increased probability of their interaction (Arrenbrecht, 1973).

Associated with neonatal unresponsiveness is an increased susceptibility to many helminthiases of which nematode infections have been most extensively studied (for reviews see Urquhart, 1970; Jarrett & Urquhart, 1971; Ogilvie & Jones, 1971, 1973; Murray, 1972 b). In Nippostrongylus brasiliensis the immunological mechanism, which expels the worms from rats within 12-18 days, is fully functional by the time the rats are between 6 and 9 weeks old, but can be stimulated in younger rats by using larger infections than are necessary in adults. Expulsion stimulated by large infections in the young occurs more slowly than in adults and leaves a larger residual population of worms. This strongly suggests that the immunological deficiency is a quantitative rather than a qualitative deficiency and in fact there seems to be no example of a sudden acquisition of responsiveness to nematode infections as one would

expect if the unresponsiveness was due to a qualitative immunological deficiency.

In contrast to nematode infections, there are no well substantiated reports of neonatal unresponsiveness to intestinal tapeworms. Hence, the present investigation was to determine if neonatal unresponsiveness to Hymenolepis diminuta occurs in mice and if so, whether the acquisition of responsiveness is sudden or gradual. The experiments are presented and discussed in the following published paper. An ADDENDUM with further discussion, particularly of some points from recent publications, follows the paper.

Parasitology (1974), 69, 77–85
With 4 figures in the text

Delayed rejection of single *Hymenolepis diminuta* in primary infections of young mice

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SUMMARY

In CFLP male tapeworm-free mice, from 2 to 7 weeks old at infection, at least 80 % of single *Hymenolepis diminuta* establish and grow but then are rejected; day of rejection defined as the first day when ⩽ 50 % of the worms were recovered. Young mice, 2 to 4 weeks old, usually reject their worms during days 16–20 while older mice, 5 to 7 weeks old, reject them during days 12–14. Biomass (total dry weight of all worms recovered on a given day from a group of mice) varied markedly with host age and was consistently greatest in 4-week-old mice. The position of the worm in the intestine did not vary with host age. The quality of mice (categorized by the Medical Research Council Laboratory Animals Centre) did not appear to affect the course of a primary, single *H. diminuta* infection although, undoubtedly, the mice had varied immunological histories. Variations in time of rejection and biomass of worms recovered are accounted for by both immunological and physiological mechanisms.

INTRODUCTION

Chandler (1939) concluded that the cestode *Hymenolepis diminuta* is not immunogenic in the laboratory rat. However, Hopkins, Subramanian & Stallard (1972*a*) have shown that in CFLP and Porton mice, 6 ± 1 week old at exposure, over 90 % of *H. diminuta* administered established and grew for about 2 weeks when destrobilation usually occurred, and subsequently the scoleces were expelled. In secondary infections of mice, fewer worms were recovered than from primary infections and these were stunted. Immunodepressant drugs such as cortisone acetate, sodium methotrexate and antilymphocyte serum prevented or delayed rejection of primary infections (Hopkins *et al.* 1972*b*). Therefore these authors concluded that in mice of the two strains used *H. diminuta* is rejected by an immunological mechanism.

In the most extensively studied helminth model of immunity, *Nippostrongylus brasiliensis*, adult rats reject the nematode after about 2 weeks (for reviews see Jarrett & Urquhart, 1971; Ogilvie & Jones, 1971; Murray, 1972). Young rats (< 6 weeks old at infection), however, do not reject *N. brasiliensis* when the worm burden is < 200. With a worm burden of > 200–250 rejection occurs, but at a

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slower rate than in older rats, and ceases when the burden has fallen to about 200 (Jarrett, Jarrett & Urquhart, 1968).

The aim of the present study was to determine whether mice of various ages show differences in responsiveness to *H. diminuta* as rats do to *N. brasiliensis*. Unlike *N. brasiliensis*, which shows little growth in the intestine of the definitive host, *H. diminuta* increases rapidly in size from <1 to 100–400 mm, occupying much of the length of the small intestine of mice prior to rejection. It is well known that an immune response by the host may reduce the number of helminths, stunt their growth or alter their position in the intestine (Ogilvie & Jones, 1971); therefore all of these parameters were measured.

MATERIALS AND METHODS

Tapeworm-free CFLP male mice, categorized 1, 2 or 4 star by the Medical Research Council Laboratory Animals Centre, Carshalton, Surrey (see Register of Accredited Breeders and Recognized Suppliers, 1973, for description of categories), were purchased from Carworth Europe. Mice were maintained in the laboratory for 1–5 days prior to exposure to *H. diminuta* at the desired age in weeks (± 2 days); they were weaned when 18–21 days. In the first experiments with mice of each age Diet 41 (Primrose and Son) was provided *ad libitum*, but in the replicate experiments Diet 41 B (Oxoid) was used. The change in diet did not appear to affect the course of infection of *H. diminuta*.

The strain of *H. diminuta* used was described by Hopkins *et al.* (1972*a*). Cysticercoids, either dissected from beetles or collected by the homogenization procedure of Ridley & MacInnis (1968), were administered by stomach tube while mice were under ether anaesthesia. Worms were recovered and their dry weights determined using the method of Hopkins *et al.* (1972*a*), with the exception that in the present study the small intestines of mice were divided into 10 cm instead of 5 cm sections. Unless otherwise stated the age of mice given refers to the age at exposure to single cysticercoids of *H. diminuta*.

In each experiment two or three groups of mice of different ages were infected and usually 10 mice from each group were autopsied on appropriate days. Experiments with each age group were repeated at least once and the results of two experiments (representing usually a total of 20 mice per day) were pooled for presentation. The results given are representative of those obtained.

Worms were placed into one of two categories: $\leqslant 0{\cdot}1$ or $> 0{\cdot}1$ mg dry weight. After day 8 post infection the former were considered to be rejected worms, *i.e.* worms which had destrobilated. On day 8 some worms $\leqslant 0{\cdot}1$ mg were found; these were not destrobilated worms but worms which had grown slowly.

RESULTS

Number of worms

In mice of all ages tested, at least 80 % of the cysticercoids administered were recovered as worms on days 8 and 10 post infection, but by day 20, few if any of the worms remained (Fig. 1). However, from days 10 to 18, mice 2–4 weeks old

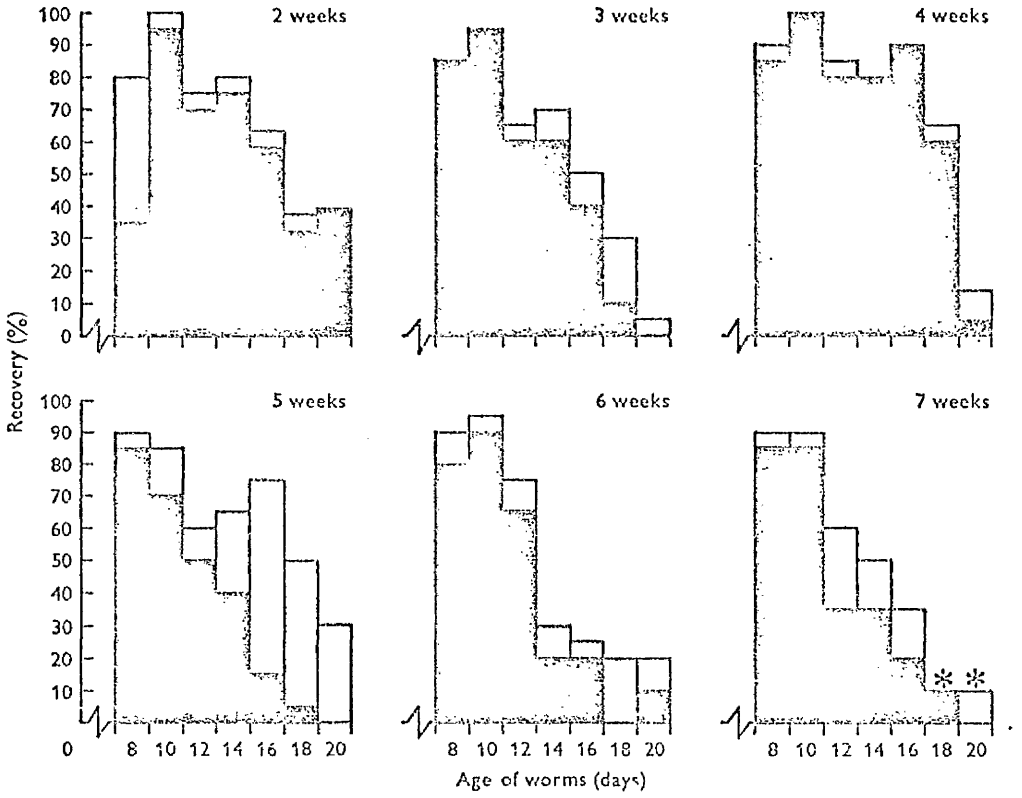

Fig. 1. Percentage recovery of *Hymenolepis diminuta* from 2- to 7-week-old CFLP male mice each given a single cysticercoid. Solid portions of bars represent worms $> 0{\cdot}1$ mg dry weight; open portions, worms $\leqslant 0{\cdot}1$ mg. Each day 18–23 mice (n) were autopsied except for $^{*}n = 10$.

had more worms $> 0{\cdot}1$ mg than had mice 5–7 weeks old. Consistently on these days, mice 4 weeks old had more worms than mice of any other age. In mice 2 weeks old, 39 % (all $> 0{\cdot}1$ mg) of the worms remained on day 20.

If the day of rejection is defined as the first day when $\leqslant 50$ % of the worms administered were recovered (including only worms $> 0{\cdot}1$ mg), then rejection in mice 2–4 weeks old occurred during days 16–20, and in mice 5–7 weeks old during days 12–14 (Fig. 1).

Mean dry weight of worms

The mean dry weight of single *H. diminuta* recovered (after day 8 including only worms $> 0{\cdot}1$ mg) increased from day 8–16, thereafter, with the exception of 2-week-old mice, it fell to zero when the worms were rejected (Fig. 2). In mice 3, 5, 6 and 7 weeks old the mean dry weights of worms recovered were similar, but worms from 4-week-old mice were consistently heavier on days 8–18 than worms from other mice. As some worms in 2-week-old mice persist until at least day 20 (Fig. 1), the mean dry weight of worms did not fall to zero in these mice.

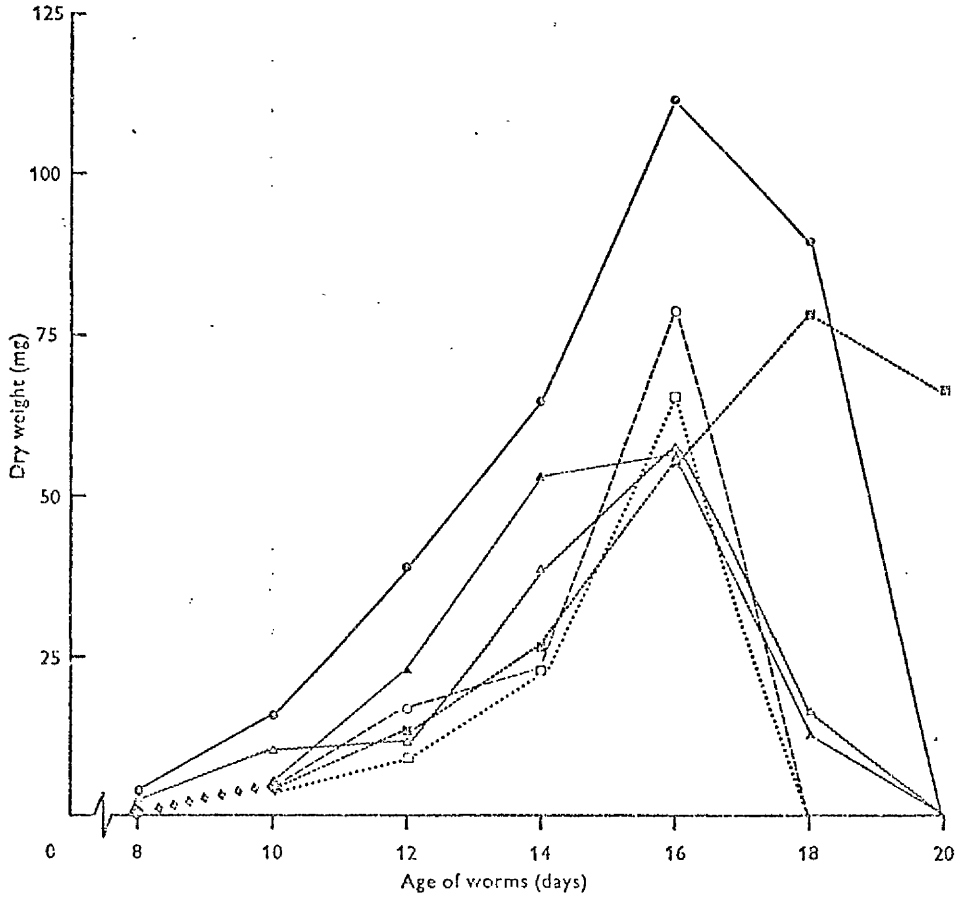

Fig. 2. Mean dry weight of *Hymenolepis diminuta* from single worm infections of CFLP male mice 2–7 weeks old (except for day 8 only worms $>0{\cdot}1$ mg included; usually $n = 18$–23 mice, but $n = 10$ on days 18 and 20, 7-week-old mice). ▣- - - -▣, 2 weeks; △- - - -△, 3 weeks; ○——○, 4 weeks; □ . . . . □, 5 weeks; ○– – – –○, 6 weeks; △wwww△, 7 weeks; ◇◇◇◇, 2-, 3-, 5- and 6-week-old mice.

Biomass of worms

Worm biomass, defined as the total dry weight of all worms recovered on a given day from mice of a group, reflects both number of worms recovered and their dry weights (worms $\leqslant 0{\cdot}1$ mg are included as 0·1 mg).

In groups of mice 5, 6 and 7 weeks old, worm biomass was 175 ± 100 mg on day 12 and was relatively constant until day 16, as growth of worms remaining in some mice approximately compensated for rejection of worms from other mice (Fig. 3). After day 16 worm biomass in these mice declined to zero. Worm biomass in 4-week-old mice did not plateau by day 12 but increased to over 2000 mg on day 16 and declined to zero by day 20. For 3-week-old mice, worm biomass increased almost as rapidly as in 4-week-old mice until day 14 after which it declined. In 2-week-old mice the increase in biomass was slower than in 3- and

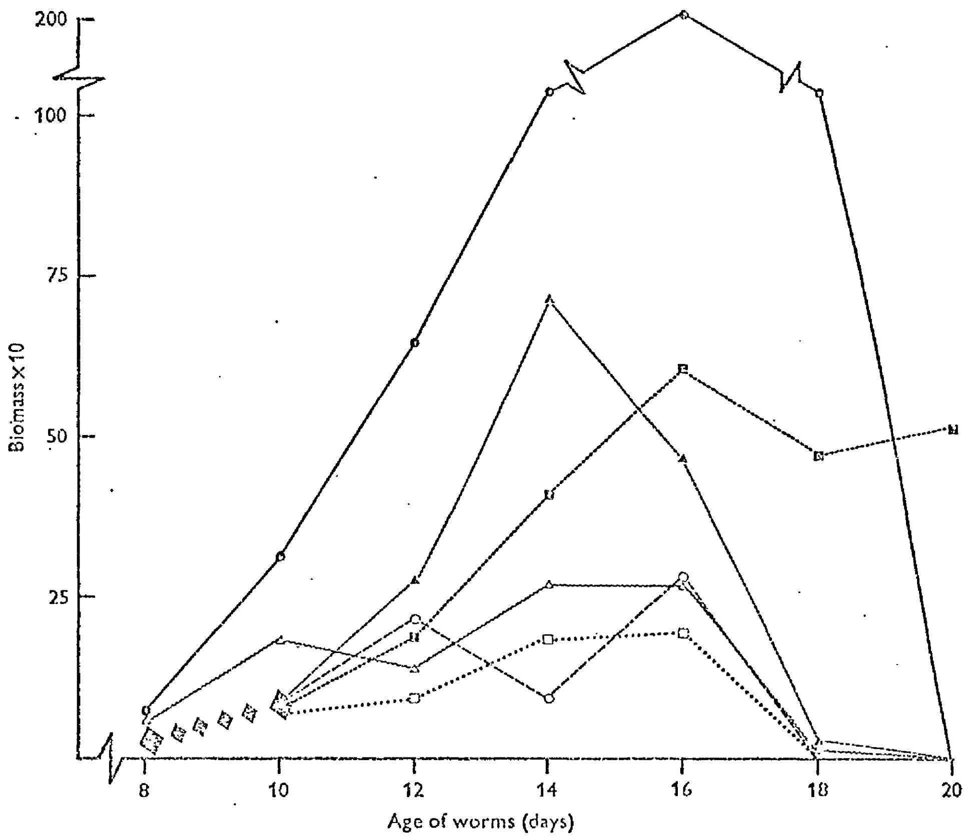

Fig. 3. Biomass of *Hymenolepis diminuta* from single worm infections in CFLP male mice 2–7 weeks old (usually n = 18–23 mice; but n = 10 on days 18 and 20, 7-week-old mice). ⊠- - - - -⊠, 2 weeks; ▲- - - - -▲, 3 weeks; ◎——◎, 4 weeks; □. . . . .□, 5 weeks; ○— —○, 6 weeks; △∿∿∿△, 7 weeks; ◈◈◈◈, 2-, 3-, 5- and 6-week-old mice.

4-week-old mice but reached a plateau by day 16 at about 500–600 mg and did not decline to zero.

Position of worms in small intestines

On day 8 worms generally were attached 30–35 % along the small intestine but by days 10–12 the scoleces had moved forward to a region 10–15 % from the pylorus, where they remained for variable periods of time before being expelled. As worms consistently remained later in mice 2–4 weeks old than in mice 5–7 weeks old, only results of one representative experiment with young mice (4 weeks old) and one with older mice (6 weeks old) are presented (Fig. 4). The vertical lines do not indicate actual length of worm but merely the 10 cm sections of the intestine in which the scoleces and terminal segments occurred.

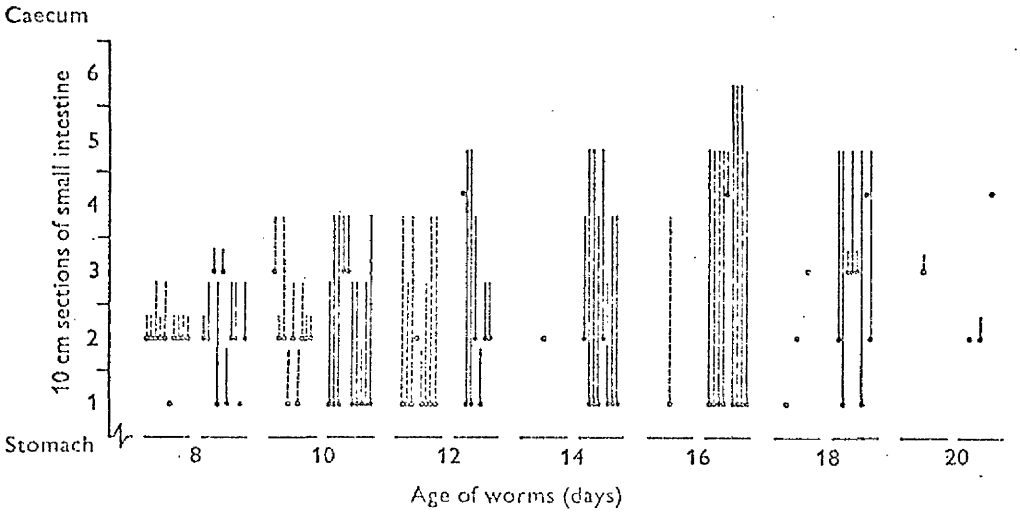

Fig. 4. Change in position of *Hymenolepis diminuta* in the small intestines of CFLP male mice 4 and 6 weeks old when infected with a single cysticercoid. Vertical lines show the 10 cm sections of the intestine in which scoleces (●—, 4-week-old; ○- - -, 6-week-old) and terminal segments occurred but do not indicate the actual length of worms [worms ≤0·1 mg dry weight shown as scoleces (● or ○) only; mice killed 1000–1600 h; intestinal lengths 38–53 cm].

Effect of mouse quality

Over the 2 years of the above experiments the quality of mice supplied (and hence their immunological history) altered irregularly from category 1 to 2 or 4 star (see Materials and Methods). Hence two experiments were conducted to determine whether the course of a primary infection with single *H. diminuta* differed in 1- and 4-star CFLP male mice.

In the first experiment the 4-star 4-week-old mice rejected their worms on day 14, whereas the 1-star 4-week-old mice had 60% (6 of 10; only worms >0·1 mg included) of their worms remaining. The latter were rejected on day 16. As rejection on day 14 was relatively early for 4-week-old mice, a third group (4-star, 6-week-old mice) was included in the second experiment in addition to the 1-star and 4-star, 4-week-old groups. Both 4-week-old groups rejected their worms on day 18 while the 6-week-old mice rejected theirs earlier, *i.e.* on day 16, confirming the earlier rejection by older mice. This experiment also confirmed the rapid growth of worms in 4-week-old mice of both category 1 and 4 star.

It must be concluded, at least from these experiments, that the course of a primary infection with single *H. diminuta* does not differ in 1- and 4-star CFLP male mice.

DISCUSSION

The results show that in mice from 2 to 7 weeks old at infection with single *H. diminuta* a basically similar course of infection occurs; at least 80% of the

worms establish, grow for 12–18 days and then are rejected. However, mice 2–4 weeks old usually rejected their worms during days 16–20 whereas in mice 5–7 weeks old rejection occurred earlier, *i.e.* usually days 12–14.

Whether this rejection period of 12–14 days is slower than that recorded by Hopkins *et al.* (1972*a*) is not certain. In their summary a rejection period of 10 ± 1 day is quoted but this appears to represent the beginning of rejection, and at least in some experiments (*e.g.* their Figs. 2, 4) rejection took nearer 12–13 days (using the criterion of rejection adopted in this paper, *i.e.* $\leqslant 50\,\%$ of the worms remaining, including only worms $> 0{\cdot}1$ mg). The possibility, however, that the rejection period is delayed relative to earlier reports is supported by the result of our final mouse quality experiment (and another recent experiment) in which late rejections (day 16) may represent a change in the CFLP mice or in *H. diminuta*. Despite this equivocal result with time of rejection, in all experiments where two age groups ran concurrently, mice $\geqslant 5$ weeks old rejected *H. diminuta* more quickly than mice $\leqslant 4$ weeks old.

Age of mice at infection, however, had no effect on the position of worms, which in all mice moved forward and remained attached 10–15 % down the intestine until expelled. The results presented in Fig. 4 suggest that movement forward occurs earlier in young mice; if this is so, the most likely explanation is that as worms in these mice grow rapidly they move forward sooner than worms which grow slower.

As Hopkins *et al.* (1972*a*, *b*) concluded that rejection of *H. diminuta* by mice is immunologically based, the delayed rejection and greater growth of worms in young mice suggests that there must be some immunological deficiency in CFLP male mice < 5 weeks old at infection. An essentially similar situation occurs with *Nippostrongylus brasiliensis* to which young rats (< 6 weeks old) are relatively unresponsive. Jarrett & Urquhart (1971) suggested that since to initiate expulsion young rats require more worms (more antigen) than do adults, their unresponsiveness may be caused by qualitative or quantitative deficiency in antibody production, some cellular factor, or an effector mechanism involved in antibody passage into the gut lumen. It is not possible from results with *H. diminuta* to say whether young mice require more worm tissue before initiating a protective immune response than do older mice, or that simply in younger mice development of the response, once initiated, occurs more slowly.

Young mice are not totally unresponsive to many antigens. Mice of a variety of strains can produce antibodies against sheep red blood cells injected within the first week of life (Auerbach, 1972). Also, the cell-mediated response causing the regression of tumours induced by murine sarcoma virus in eight strains of mice matures from days 12 to 21 after birth (Gazdar, Beitzel & Talal, 1971). Nevertheless, lymphoid tissues are immature in neonatal mice and reach adult conditions only after several weeks. Crabbé, Nash, Bazin, Eyssen & Heremans (1970) showed that the binding of fluorescein-labelled anti-immunoglobulin sera to cells of gut-associated lymphoid tissues in C_3H mice increased with age until 4–6 weeks after birth. Also, Ferguson & Parrott (1972) demonstrated that in CBA and BALB/c mice, Peyer's patches are present from birth but all these accumulations

of lymphoid tissue contain germinal centres only after 5 weeks. Villous intraepithelial lymphocytes, and lymphocytes and plasma cells in the lamina propria, first appear in the third week after birth and increase in number thereafter; numbers of the former reaching a plateau by 6 weeks. In addition to increases in the number of lymphocytes in gut-associated lymphoid tissue, there is a qualitative change from a predominately thymus-dependent lymphocyte population (Chanana, Schaedeli, Hess & Cottier, 1973) in the first week of life, to a situation in the mature small intestine where the majority of the lymphocytes are thymus-independent (Ferguson & Parrott, 1972). A good correlation exists between the rejection pattern of *H. diminuta* in mice of various ages and the maturation of gut-associated lymphoid tissue. Since small populations of various lymphoid cells are present in young mice and increase with age, perhaps the immunological deficiency in mice < 5 weeks old is quantitative.

Dineen & Kelly (1973) transferred mesenteric lymph node cells to study the unresponsiveness of young rats to *N. brasiliensis* and concluded: 'lymphocytes from neonatal donors were functionally deficient or impaired'. However, these authors apparently did not consider that in young rats, as in young mice, probably there are fewer lymphocytes in the mesenteric lymph nodes than in those of adults. Although they transferred the same number of lymph node cells from young and old animals, it is likely that the proportion of these cells which was lymphocytes was greater from the older animals. Hence, perhaps not enough lymphocytes, as distinct from lymph node cells, were transferred from young animals.

In addition to immunological changes as mice mature, there are physiological changes in the intestine which may affect the growth and survival of *H. diminuta*. Mice generally are weaned from days 18–21, and associated with the transition from a liquid to a solid diet the secretion of gastric acid and many enzymes reaches adult proportions (Brambell, 1970). Intestinal permeability is altered as the absorption of intact maternal immunoglobulins ceases on days 14–16 (Brambell, 1970). Also, there are qualitative and quantitative changes in the microflora in the third week of life (Savage, 1970). The patterns of growth and survival of *H. diminuta* in mice of various ages may involve a combination of the physiological and immunological maturation of the intestine.

Since in 2-week-old mice 39 % of the worms remained on day 20, but in all other mice only 5 % or less (only worms $> 0{\cdot}1$ mg included) remained, there is sufficient maturation of gut-associated lymphoid tissue to reject virtually all *H. diminuta* when mice are infected at 3 weeks of age. In mice infected at $\geqslant 5$ weeks rejection occurs at the maximum rate. The poor growth of worms in 2-week-old mice in comparison with worms in 3- and 4-week-old mice may reflect relatively poor physiological conditions in the intestines of the former mice. Although physiological conditions may be favourable, poor growth of worms in 5- to 7-week-old mice probably reflects the mature state of gut-associated lymphoid tissue in older mice. Since in 3-week-old mice growth is initially rapid, it appears that physiological conditions in the intestine are favourable in these mice, and the apparent earlier rejection of *H. diminuta* by 3 week-old than by 2- and 4-week-old mice cannot be explained. In 4-week-old mice the maximum growth and long survival

of *H. diminuta* may involve the combination of good physiological conditions for growth and the relatively immature state of gut-associated lymphoid tissue.

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REFERENCES

AUERBACH, R. (1972). Studies on the development of immunity: the response to sheep red blood cells. In *Current Topics in Developmental Biology*, vol. 7 (ed. A. A. Moscona and A. Monroy), pp. 257–80. New York, London: Academic Press.

BRAMBELL, F. W. R. (1970). The transmission of passive immunity from mother to young. In *Frontiers of Biology*, vol. 18 (ed. A. Neuberger and E. L. Tatum). Amsterdam, London: North-Holland Publishing Company.

CHANANA, A. D., SCHAEDELI, J., HESS, M. W. & COTTIER, H. (1973). Predominance of theta-positive lymphocytes in gut-associated and peripheral lymphoid tissues of newborn mice. *The Journal of Immunology* 110, 283–5.

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

CRABBÉ, 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, 448–57.

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.

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 and Experimental Immunology* 12, 477–88.

GAZDAR, A. F., BEITZEL, W. & TALAL, N. (1971). The age related responses of New Zealand mice to murine sarcoma virus. *Clinical and Experimental Immunology* 8, 501–9.

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

HOPKINS, C. A., SUBRAMANIAN, G. & STALLARD, H. (1972*b*). The effect of immunosuppressants on the development of *Hymenolepis diminuta* in mice. *Parasitology* 65, 111–20.

JARRETT, E. E. E. & URQUHART, G. M. (1971). The immune response to nematode infections. In *International Review of Tropical Medicine*, vol. 4 (ed. A. W. Woodruff and D. R. Lincicome), pp. 53–96. New York, London: Academic Press.

JARRETT, E. E. E., JARRETT, W. F. H. & URQUHART, G. M. (1968). Immunological unresponsiveness to helminth parasites: 1. The pattern of *Nippostrongylus brasiliensis* infection in young rats. *Experimental Parasitology* 23, 151–60.

MURRAY, M. (1972). Immediate hypersensitivity effector mechanisms. II. *In vivo* reactions. In *Immunity to Animal Parasites* (ed. E. J. L. Soulsby), pp. 155–90. New York, London: Academic Press.

OGILVIE, B. M. & JONES, V. E. (1971). *Nippostrongylus brasiliensis*: A review of immunity and the host/parasite relationship in the rat. *Experimental Parasitology* 29, 138–77.

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

SAVAGE, D. C. (1970). Associations of indigenous microorganisms with gastrointestinal mucosal epithelia. *The American Journal of Clinical Nutrition* 23, 1495–501.

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ADDENDUM

It is clear from the preceding publication that there is no absolute unresponsiveness to H. diminuta, at least in mice as young as 2 weeks old at infection. There is, however, a relative unresponsiveness in that worm survival is prolonged in young mice (2-4 weeks old) and growth, probably for a combination of immunological and physiological reasons, is greatest in 4 week old mice. Mice 5-7 weeks old are maximally responsive (of the ages studied) and reject their worms most quickly.

A worm is considered rejected when it is destrobilated or expelled (SECTION 1), and from Fig. 1 (present SECTION) and evidence presented elsewhere (e.g. SECTION 1) it appears that in most cases once a worm destrobilates it is expelled quickly. In 5 week old mice, however, a large number of destrobilated worms was recovered (Fig. 1, present SECTION, and SECTION 1) suggesting that in these mice worms once destrobilated survived longer than in other mice. Apart from attributing these high recoveries of destrobilated worms to chance, a possible explanation is that the protective mechanism of the host which induces destrobilation is different from the one causing expulsion. Accordingly in immunologically immature mice $\leq$4 weeks old at infection the two mechanisms are essentially in synchrony, as in mice relatively immunologically mature ($\geq$6 weeks old), albeit 2-6 days later than in the older mice. The two mechanisms are asynchronous in 5 week old

mice because, although the mechanism of destrobilation has matured and occurs early as in older mice, the expulsion mechanism remains relatively immature. This is an interesting hypothesis but the recovery of destrobilated worms is difficult and the high recoveries, almost exclusively in 5 week old mice, require confirmation by an independent worker.

In the DISCUSSION of the paper it is shown that the acquisition of maximum responsiveness by mice correlates well with published reports of the maturation of gut-associated lymphoid tissue (GALT). Since submission of the manuscript for publication Glaister (1973 a) has determined the numbers of intraepithelial lymphocytes (thelio-lymphocytes, Fichtelius, 1968) per 10^3 epithelial cells in the small intestine of C_3H mice 1 day to 10 weeks old. The number of thelio-lymphocytes is small in 2 week old mice but by 4 weeks increases significantly and continues to do so, apparently at a steady rate, until 10 weeks (the oldest conventional mice studied). The functions of thelio-lymphocytes are not understood but they have resemblances to both lymphocytes and mast cells (Bienenstock, 1974) and migrate to and from the lamina propria (Glaister, 1973 b) and perhaps into the lumen as well, as suggested by Glaister (1973 b) and Waksman (1973). Bienenstock (1974, p.198) suggested that these cells "may act as specific passively sensitized sentinel cells at the mucosal surface", hence it is reasonable to assume that they play some role in intestinal immunity and therefore maybe involved in the response to H. diminuta.

The increase in thelio-lymphocyte populations provides further correlation between the rejection of H. diminuta and the maturation of GALT and suggests further that if older mice were studied, increased responsiveness above that observed in 5-7 week old mice would occur. Certainly the magnitude of the anti-sheep red blood cell (SRBC) antibody response (Bosma, Makinodan & Walburg, 1967; Rector & Carter, 1973) and the sensitivity to various doses of SRBC increases, beyond the age of sexual maturity (5-6 weeks), until mice are about 12 weeks old. Given this prolonged increasing responsiveness to SRBC it would not be surprising to find a similar prolonged increase in responsiveness to helminth antigens, contrary to statements of Ogilvie & Jones (1973) and Ogilvie & Love (1974) which imply that it is surprising to have prolonged relative unresponsiveness to helminth infections. In fact there is some evidence that older mice may be more responsive to H. diminuta as in experiments studying secondary infections (SECTION 3) some 9 week old mice were given primary infections as controls and seemed to produce smaller worms and rejected them more quickly than would be expected in 5-7 week old mice. Further, using the less immunogenic tapeworm, H. citelli, Hopkins & Stallard (1974) showed that mice infected at 7 or more weeks of age are more responsive (i.e. they expel the worm faster) than mice infected when younger. However, until well designed experiments are conducted comparing the growth and survival of H. diminuta in mice 5-7 weeks old with that in older mice, whether increased immunological responsiveness to H. diminuta occurs in older mice has some support but remains speculative.

If one was to consider doing such an experiment with mice of various ages a word of caution may be helpful. Unlike the work of Bosma et al. (1967) and Rector & Carter (1973) where antibody titres to SRBC could be easily determined and compared to show increased responsiveness in older mice, the death or survival of H. diminuta seems a rather crude measure of the various immune responses elicited by the mouse against both protective and nonprotective antigens released by the worm. One must design experiments which have a reasonable probability of producing conclusive results given the variability of the experimental system. With H. diminuta infections of mice, the only measures of the immune responses are worm survival and growth, and as single worm infections show considerable variation, age experiments with older mice may not provide conclusive results. Multiple worm infections reduce the variation (SECTIONS 1; 3) but the increased immunogenicity of such infections would probably minimize the differences between the responses of mice of various ages and again lead to inconclusive results. In retrospect it seems likely that had the studies on mice 2-7 weeks old been done with, say, 6c infections there would have been no detectable differences in the responses to H. diminuta. This is in accordance with the observations of Jarrett, Jarrett & Urquhart (1968) that when <250 N. brasiliensis establish in young rats expulsion does not occur but with heavier infections it does, hence, the latter minimizes the differences between young and old rats.

The components of the rat's immune response which are involved in the expulsion of N. brasiliensis

have been studied more than those of any other helminth. Presently it is accepted that, either directly or indirectly antibody damages the worms (reviewed by Ogilvie & Jones, 1973; see SECTION 6 also). Such damaged worms (about 10 days post infection) are susceptible to another component of the host response which is radiosensitive (Jones & Ogilvie, 1971) and can be adoptively transferred by mesenteric lymph node cells (MLNC) (Keller & Keist, 1972; Dineen, Ogilvie & Kelly, 1973). Kelly, Dineen & Love (1973) demonstrated that a third component is essential in expulsion and Dineen & Kelly (1973 a) showed that this can be supplied by bone marrow. The authors argued that the third component probably is not lymphoid but myeloid in nature and presumably involves the action of biogenic amines. Since the mid 1960's workers in Glasgow have proposed that biogenic amines are involved in the expulsion of N. brasiliensis especially as a mechanism for the leak of antibody into the intestine (see Murray, 1972 b). Recently Rothwell and co-workers in Sydney have provided considerable evidence that the amines histamine and 5-hydroxytryptamine participate in the expulsion of the nematode Trichostrongylus colubriformis from guinea pigs although probably not solely by causing leak of antibody into the intestine (Rothwell, Prichard & Love, 1974). Factors present in ram semen, presumably prostaglandins, have been implicated recently in the expulsion of N. brasiliensis (Dineen, Kelly, Goodrich & Smith, 1974).

Given then that the expulsion of helminths is a complex process, what is the deficiency in the mechanism in neonatal hosts? Reaginic (IgE) antibodies

so characteristic of infections of adult rats with N. brasiliensis (Ogilvie, 1964, 1970) are produced by neonatally infected rats (Jarrett, Jarrett & Urquhart, 1966; Ogilvie & Jones, 1967; Jarrett, Urquhart & Douthwaite, 1969) and protective antibody occurs in the serum of neonatal rats given more than 800 larvae at infection (Jarrett et al. 1969). The anaphylactic reaction which facilitates the translocation of antibody into the intestine can be induced in neonatal rats (Jarrett et al. 1966; Ogilvie & Jones, 1967) and the local increase in the numbers of mast cells which occurs in adult rats (Jarrett, Jarrett, Miller & Urquhart, 1968) has been reported in neonatal rats also (Jarrett et al. 1969). Therefore, it has been argued that the antibody response of young rats to the nematode is essentially normal.

Keller & Keist (1972) transferred the ability to expel N. brasiliensis to young rats with MLNC from naive adults and Dineen & Kelly (1973 b) induced expulsion in neonatal rats by transfer of immune adult MLNC but could not transfer resistance to adult recipients using MLNC from infected young rats. Hence, as quoted in the DISCUSSION above, these latter authors concluded that lymphocytes from young rats are functionally deficient or impaired. Thus the deficiency in the mechanism of expulsion seemed to be in the cell-mediated component. Love & Ogilvie (1974) submitted that the persistence of infection is not simply a deficiency of immune lymphocytes as, when such cells are provided to young rats harbouring adult worms, expulsion does not occur. In support, Schneider (1973) showed

that mice are capable of cell-mediated responses (delayed hypersensitivity to dinitrofluorobenzene) within 2 days of birth. Despite much effort, therefore, the mechanism which is deficient in neonates is not known.

Has the correct question been asked? It seems that workers are looking for an absolute deficiency in at least one component of the expulsion mechanism whereas the evidence from the effects of various intensities of infection (E.Jarrett et al. 1968) demonstrates that even the youngest rats possess all the components for expulsion. They simply are less sensitive to antigen than adults. Therefore, as stated previously (Murray, 1972 b; DISCUSSION above) the deficiency must be quantitative rather than absolute or qualitative. Indeed there is abundant evidence of gradually increasing responsiveness of young animals as the immune system completes its differentiation in response to antigenic stimulation (e.g. Sterzl & Silverstein, 1967, p.369; Nossal & Ada, 1971, pp.137-9; Keller, 1971; Arrenbrecht, 1973; Rector & Carter, 1973). In any stimulation of a young animal one component must be limiting the immune response, probably one of the initial components. If this component is enhanced experimentally, another, not completely mature, probably will be limiting. Arrenbrecht (1973) argued that the limiting component was an insufficient T cell population, but Rector & Carter (1973) could not enhance the increase in the number of antibody forming cells to SRBC in young recipient mice by adding T cells and concluded, in opposition to Arrenbrecht, that a T cell deficiency was not the cause of relative unresponsiveness in young mice. They

proposed that maturation of the ill-defined 'antigen processing system' is the major factor in the increasing responsiveness of young mice.

As rejection of N. brasiliensis involves many components the ultimate expulsion of worms represents a summation of all components. The mechanism would be incomplete if, as in young rats, one or more of the components was quantitatively inadequate such that the essential sum is not reached. This view was expounded by Murray (1972 b, pp.184-5) where he described 'effector units' produced in baby rats but in insufficient quantity (i.e. the sum, x units, required to expel worms is not reached in low intensity infections of young rats). Reconsidering previous work with this suggestion in mind it is clear that statements like, the antibody response to N. brasiliensis in young rats is essentially normal (Ogilvie & Jones, 1973; Love & Ogilvie, 1974), are misleading. Protective antibody can be produced by young rats but only in high intensity infections (Jarrett et al. 1969). The appearance of reaginic antibody is not normal but delayed and mean titres are lower and responses of individuals more variable than in adults (Ogilvie & Jones, 1967). Mast cells, thought to be responsible for massive leakage of antibody into the gut lumen by their release of biogenic amines increase in number in young rats as in adults. The number of mast cells in the intestine of uninfected young rats is about half the number in uninfected adults, and the increase due to infection in young rats may be insufficient to cause expulsion (Murray, 1972 b). Also, the inability of the

lymphocytes from young rats to transfer immunity to adults (Keller & Keist, 1972; Dineen & Kelly, 1973 b) probably is that there are too few lymphocytes (see DISCUSSION). All this evidence supports Murray's hypothesis that effector units are present in the young but in insufficient quantity for expulsion. By increasing the intensity of infection the effector units are potentiated and reach sufficient quantity for expulsion to occur.

One factor that is not often considered fully in young and adults is that the worms make continued efforts to survive in the face of the host's immune responses (Ogilvie, 1969, 1972, 1974 b; Jenkins, 1972). Under conditions of attack by deficient effector units, as seems to be the case in young rats, the worms must have a greater probability of adapting successfully to the immune responses (Jenkins, 1974 a). This more successful adaptation would help explain limited expulsion and higher residual populations in young rats and also the finding (Love & Ogilvie, 1974) that when immune MLNC are transferred to young rats harbouring adult N. brasiliensis expulsion does not occur. Transfer of such cells simultaneously with larval infection causes expulsion (Dineen & Kelly, 1973 b; Love & Ogilvie, 1974).

In summary mice are relatively unresponsive to H. diminuta when young and become more responsive with age. The pattern of survival and growth of this tapeworm in mice 2-7 weeks old at infection is attributed to a combination of immunological and physiological

maturation of the intestine. These results and those with N. brasiliensis strongly suggest that the immunological deficiency in the young is a quantitative deficiency in many if not all components of the rejection mechanism. The young are responsive but require stimulation of greater intensity and duration. This is supported by many immunological studies on the ontogeny of responses and the maturation of the lymphoid system. It is suggested that immuno-parasitologists neglect their own evidence and that from studies of neonatal immunology, and continue to search for qualitative deficiencies in the young.

SECTION 3

SECONDARY INFECTIONS OF *HYMENOLEPIS DIMINUTA* IN MICE: EFFECTS OF VARYING WORM BURDENS IN PRIMARY AND SECONDARY INFECTIONS

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Secondary infections of *Hymenolepis diminuta* in mice: effects of varying worm burdens in primary and secondary infections

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SUMMARY

In one (1c) and six (6c) cysticercoid primary infections of *Hymenolepis diminuta* in NIH (inbred) and CFLP (outbred) male mice 6 ± 1 week old > 85% of the worms established but were rejected (destrobilated or expelled) subsequently. Rejection occurs more quickly in 6c infections than in 1c infections. Considerable worm growth occurs in 1c and 6c primary infections but worms from 6c infections weighed less than worms from 1c infections on all days studied.

Expulsion of *H. diminuta* does not occur more rapidly in secondary infections than in primary infections; loss of 6c secondary worms occurs at the same rate as 6c primary worms but 1c secondary worms survive longer than 1c primary worms. Although worms are not lost more quickly in secondary than in primary infections, they are affected at an early age by the immune response which stunts their growth. Increasing the intensity of primary and secondary infections increases the severity of stunting of secondary worms.

The results are discussed and it is suggested that immune responses to *Hymenolepis* spp. in rodents are common but that thresholds of worm numbers exist below which appreciable worm loss does not occur. Stunting due to crowding, which generally is attributed to inter-worm competition, may be in part immunologically mediated. For future immunological studies attempting to induce secondary responses to *H. diminuta* in mice, worm growth, not survival, is the criterion to evaluate.

INTRODUCTION

It is well known that in the laboratory rat the tapeworm *Hymenolepis diminuta* can grow to over 100 cm in length and survive for the life of the host. From his work on primary and secondary infections with various numbers of worms and particularly the result that 'when primary worms were removed..., no effect of the prior infection on a re-infection could be observed', Chandler (1939) suggested that effects of crowding other than immunity were the basis of resistance in infections with *H. diminuta* in rats. As well as affecting establishment and survival, crowding causes stunting of the worms and is generally accepted as being due to inter-worm competition for nutrient, probably carbohydrate (Read & Simmons, 1963).

Recently, Hopkins, Subramanian & Stallard (1972*a*, *b*) have shown, however,

that in primary infections of one worm in mice, *H. diminuta* establishes and grows to a maximum of almost 70 cm after about 2 weeks but is then rejected immunologically. In contrast to nematode systems where immunological rejection simply means expulsion of the worms from the host, rejection of *H. diminuta* often, but not always, involves destrobilation of the worm leaving only the scolex and 'neck' region which is then expelled. In secondary infections of mice with *H. diminuta* there was a slightly reduced recovery of worms 6–9 days after infection and the worms were severely stunted. Whether considerable growth, destrobilation or expulsion of worms occurs in secondary infections was not determined. Befus & Featherston (1974) showed that the mechanism of destrobilation and expulsion in single worm primary *H. diminuta* infections is partially deficient in young, immunologically immature mice.

Ogilvie & Jones (1973) suggested that this system would provide an excellent opportunity systemically to analyse immunity to intestinal tapeworms. Prior to detailed analysis of the cellular and serological components of protective immunity to *H. diminuta* in mice, however, it is essential that the dynamics of secondary infections are known so that experiments required to separate components of the immune response can be properly designed. For future immunological studies of *H. diminuta* infections of mice the intensity of infection which minimizes possible physiological effects due to crowding but produces a detectable secondary immunological response must be determined. Should worm survival (as in nematode systems) or worm growth, which is dramatic but often varies considerably from host to host (Chandler, 1939; Hopkins *et al.* 1972*a*), be used to measure acquired immunity in secondary infections and thus to determine whether resistance can be transferred successfully by cells or serum? Is there evidence that the so-called crowding effect may be in part immunologically mediated?

Given these problems the present investigation was begun to characterize single and multiple primary and secondary infections in terms of survival and growth of *H. diminuta* in outbred (CFLP) and inbred (NIH) strains of mice.

MATERIALS AND METHODS

CFLP (outbred) and NIH (inbred) male mice were purchased from Anglia Laboratory Animals (formerly Carworth Europe) and used when 6 ± 1 weeks old. Mice of both strains were tapeworm-free; the M.R.C. Laboratory Animal Centre graded the CFLP 4 star but the NIH had never been graded. The maintenance and infection of mice was described previously (Befus & Featherston, 1974). If large worms were not immediately obvious at autopsy, 10 cm sections of intestine were examined carefully using the procedure of Hopkins *et al.* (1972*a*) to reveal small worms which otherwise could be overlooked easily.

Mice received a primary infection of one cysticercoid (1 c), six cysticercoids (6 c) or were sham infected (anaesthetized and injected with Hanks' saline by stomach tube but given no cysticercoids). In all experiments 10–15 mice from both 1 c and 6 c groups were killed on days 8 or 9 post infection to determine what portion of the primary worms had established; the recoveries ranged from 87 to 100%. The 1 c

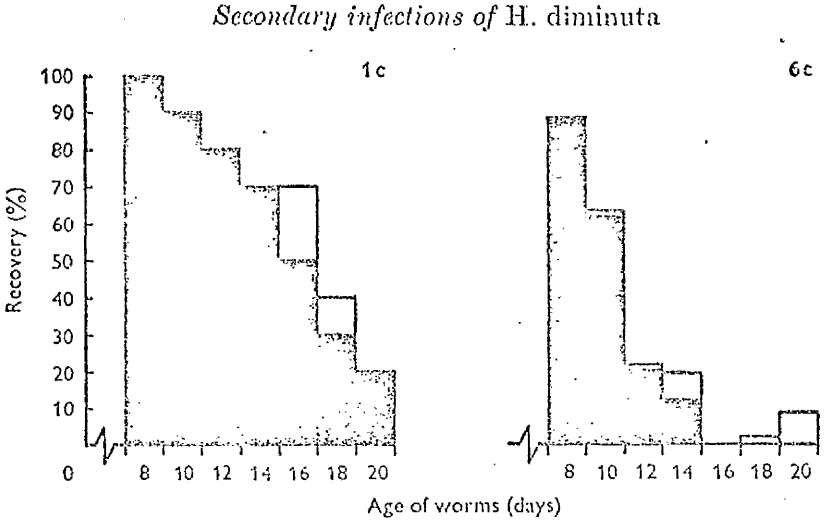

Fig. 1. Recovery (%) of *Hymenolepis diminuta* from primary infection of CFLP ♂ mice given, A, one (1 c) or B, six (6 c) cysticercoids. Solid portions of bars represent worms > 0·1 mg dry weight; open portions destrobilated worms (≤ 0·1 mg); $n = 10$ mice/group/day.

and 6 c primary infections in two experiments were studied closely with autopsies of 10 mice/group every second day from day 8 to 20. To ensure that no primary worms remained prior to secondary infection, the anthelmintic oxyclozanide ('Zanil' I.C.I.) was given on day 21 (150 mg/kg) to each mouse including sham mice, as described by Hopkins, Grant & Stallard (1973). Cysticercoids for secondary infections were administered 5 days later, day 26 after the primary infection, and mice were autopsied 8–26 days later.

In primary infections the worms grow, generally exceeding 0·1 mg dry weight by days 8 or 9, but by day 10 destrobilation commences (Hopkins *et al.* 1972*a*). Therefore, herein all worms ≤ 0·1 mg on day 10 or thereafter of a primary infection are considered destrobilated: prior to this such small worms are considered to have have grown slowly (Befus & Featherston, 1974). This arbitrary definition of destrobilated worms in primary infections cannot be used for secondary worms which grow slowly, if at all (see below), and are difficult to distinguish morphologically from destrobilated worms from primary infections. Hence, in secondary infections it is not possible to distinguish severely stunted worms from destrobilated worms (if the latter exist at all).

RESULTS

Primary infections

Primary infections of one cysticercoid (1 c) were compared with infections with six cysticercoids (6 c) of *H. diminuta* using CFLP mice in one experiment and NIH in another. Although different strains of mice were used, the establishment, growth and rejection of *H. diminuta* in both experiments were fundamentally similar. Differences of detail are recorded below but only the CFLP results are given in Figs. 1 and 3.

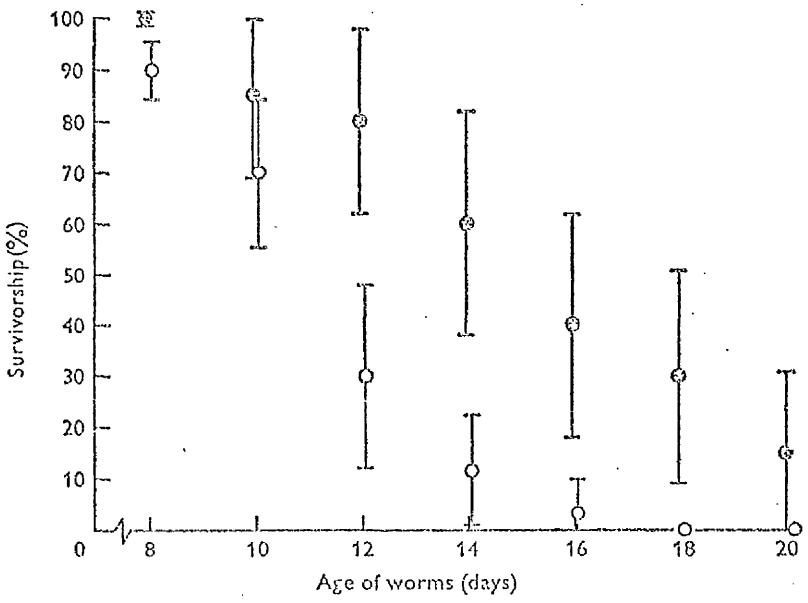

Fig. 2. Mean survivorship (± the 95 % confidence limits) of *Hymenolepis diminuta* from 5 mice given one (⊖) or six (○) cysticercoids; n = 20 mice/group/day.

(*a*) *Worm recovery*

All the worms recovered, including those destrobilated (open portions of bars), are shown for 1 c and 6 c infections of CFLP mice (Fig. 1). On day 8 post infection 100 % of the 1 c and 88 % of the 6 c worms were recovered but by day 20 most worms were rejected (destrobilated or expelled). To provide an objective means of comparing the time of rejection in 1 c and 6 c primary infections the first day on which (and consistently thereafter) ≥ 50 % of the worms administered to a group of mice had been destrobilated or lost is called the mean survival time(previously called 'day of rejection'; Befus & Featherston, 1974). Using this measure of the time of rejection, mean survival in CFLP mice was 12 days for 6 c and 16 days for 1 c infections. In NIH mice the mean survival in 6 c was 12 days and in 1 c infections was 14 days. These results confirm previous experiments in which *H. diminuta* was rejected more quickly from multiple infections than from 1 c infections in mice (*Hopkins, personal communication*).

The results from the experiments with CFLP and NIH mice are pooled in Fig. 2 which shows the mean percentage of surviving worms and the 95 % confidence limits. Destrobilated worms are not included as they have been affected by the immune responses of the hosts and are considered rejected. The rejection of worms from 6 c infections occurs more rapidly than from 1 c infections and in general the variation, expressed as the confidence limits, is smaller in the multiple infection. Mean survival time (as defined above) for 6 c infection is 12 days whereas for 1 c infections it is 16 days, but as shown by the limits could vary from 14 to 18 days. On days 18 and 20 in the multiple infection there were no large worms surviving but on these and other days destrobilated worms were found (see Fig. 1 and below).

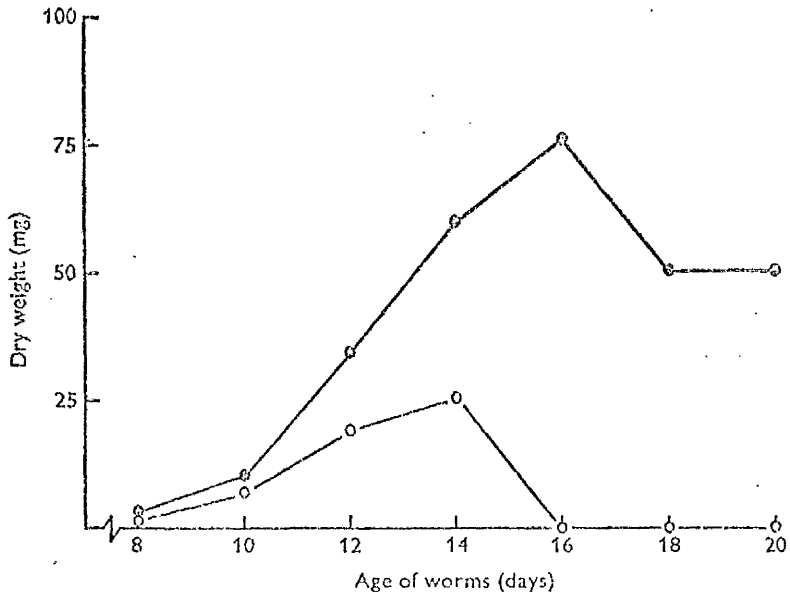

Fig. 3. Mean dry weights of *Hymenolepis diminuta* from primary infections of CFLP ♂ mice given one (◎—◎) or six (○--○) cysticercoids (except for day 8 only worms > 0·1 mg included); n = 10 mice/group/day.

(*b*) *Worm growth*

In 1 c infections of CFLP mice the worms which had not destrobilated had a greater mean dry weight (10 mice/group) than those from 6 c infections on all days studied (Fig. 3). In the 1 c infections the mean dry weight increased to a maximum of 76 mg by day 16 and in the few surviving worms on days 18 and 20 (see Fig. 1) it remained at 51 mg. The mean dry weight of strobilated worms in 6 c infections reached 25 mg by day 14 but thereafter only destrobilated worms were found (Fig. 3). Worms from 1 c infections in NIH mice reached a maximum of only 49 mg (day 18) and in 6 c infections only 17 mg (day 16). There is considerable variation in growth of worms in different experiments. However, the above results support those of an experiment comparing the two strains (unpublished), in which worms from NIH mice were consistently smaller than those from CFLP mice.

Worm biomass (total dry weight of all worms in a group of mice on a given day, worms $\leqslant$ 0·1 considered to be 0·1 mg, Befus & Featherston, 1974) combines both numbers and mean dry weights of all surviving worms and therefore perhaps best illustrates differences between groups. Biomass on days 8 and 10 (CFLP) and 8, 10 and 12 (NIH) was greater in 6 c than in 1 c infections as although individual worms were smaller in 6 c infections (Fig. 3) the larger number of worms more than compensated for their smaller size. However, the earlier and more complete rejection of worms in the 6 c than in the 1 c infections, led thereafter to the biomass in the 1 c infections becoming greater and remaining so until the experiment ended.

Table 1. *Numbers of* Hymenolepis diminuta *recovered from* 1c *challenge infections of NIH mice given a* 1c, 6c *or sham primary infection*

Day of infection	Primary infection		
	1c	6c	Sham
8	8 (6)	8 (4)	9 (5)
9	9 (8)	7 (6)	9 (1)
13	6 (5)	8 (6)	3 (1)
15	5 (5)	7 (6)	3 (3)
17	7 (7)	4 (3)	3 (3)
20	8 (8)	2 (2)	7 (7)†

Numbers in parentheses are numbers of worms ≤ 0·1 mg dry weight; in sham mice after day 9 such worms are destrobilated; in 1c and 6c mice destrobilated worms, if they exist, cannot be distinguished from stunted worms; n = 10 mice/group, †n = 9.

(c) *Worm position*

The position of single *H. diminuta* in the small intestine in primary infections of mice has been reported (Turton, 1971; Hopkins *et al.* 1972*a*; Befus & Featherston, 1974) but the position in multiple infections of mice has not. As in 1c infections, most scoleces of 6c primary worms were attached in the second 10 cm section of the intestine on day 8. However, as rejection in 6c occurs sooner than in 1c primary infections, by days 10–12 there was no forward movement of the mean scolex position in 6c like that found in 1c infections; although some worms moved anteriorly many others, which presumably were being rejected, were attached in the third and fourth 10 cm sections of the intestine.

In the experiment with NIH mice a relatively large number of destrobilated worms were recovered on days 18 (15/60) and 20 (12/60) and whereas on day 14 the mean scolex position was 75% along the intestine, on the two later days the mean position was 19% from the pylorus suggesting an anterior localization of destrobilated worms. This could not be substantiated by the results from the CFLP mice as the few destrobilated worms recovered (Fig. 1) were not localized anteriorly.

Secondary infections

(a) *Worm recovery*

A secondary infection of 1c was administered to NIH mice given a 1c, 6c or sham (see Materials and Methods) primary infection. On the 8th day 80–90% of these worms were recovered from the groups (Table 1): recoveries were within the normal range for primary infections (Hopkins *et al.* 1972*a*, *b*; Befus & Featherston, 1974). By day 13 in mice given a sham primary infection only 20% of the mice were infected with large worms. Thereafter only destrobilated worms were recovered, with an unusually large number on day 20. These results should not be compared directly with those of the 1c primary infections above as the present mice were infected at 9 weeks of age rather than at 6 weeks as above; age at infection is important (Befus & Featherston, 1974), at least within 7 weeks of birth, and probably for longer (personal observation). In mice given a 6c primary infection the recovery of 1c secondary worms was 70% on day 15 but fell to 20%

Secondary infections of H. diminuta

Table 2. *Numbers of* Hymenolepis diminuta *recovered from* 6c *challenge infections of CFLP mice given a* 1c, 6c *or sham primary infection*

Day of infection	Primary infection		
	1c	6c	Sham
8	39 (28)†	36 (33)	44 (10)
10	10 (9)	21 (19)	21 (5)
12	9 (6)	11 (10)	16 (3)
15	1 (1)	10 (10)	5 (4)
22	4 (4)	1 (1)	6 (4)

Numbers in parentheses are numbers of worms ≤ 0·1 mg dry weight; in sham mice after day 9 such worms are destrobilated; in 1c and 6c mice destrobilated worms, if they exist, cannot be distinguished from stunted worms; *n* = 10 mice/group; †*n* = 9.

by day 20. In mice given a 1c primary infection the 1c secondary worm recovery ranged from 50 to 70 % on days 13, 15 and 17 and was 80 % on day 20. As stated previously destrobilated worms cannot be recognized in secondary infections as, unlike primary worms, secondary worms do not grow much (see below) and few exceed 0·1 mg dry weight. Therefore, only expulsion of at least 50 % of the secondary worms, and not destrobilation as well, can be used to determine the mean survival time. The mean survival time of 1c secondary worms in mice previously given a 6c primary infection was 17 days. In mice earlier given a 1c primary infection the mean survival time of 1c secondary worms was > 20 days (Table 1).

CFLP mice given 1c, 6c or sham primary infections were challenged with a 6c secondary infection. From the sham mice 73 % of the worms were recovered on day 8 and from the mice given 1c and 6c primary infections the recoveries of secondary worms were 72 % and 60 % respectively (Table 2). In all three groups the mean survival time was 10 days. As stated above for the 1c infections the results from mice given a sham primary infection should not be compared directly with the results of 6c primary infections presented above because of differences in the ages at the mice at infection.

The results with 1c and 6c secondary infections suggested that, as in primary infections, worm rejection occurs more quickly in 6c infections but the experiments had been done with different strains of mice. Therefore, 310 CFLP mice were divided into six groups, three (1c, 6c and sham primary infections) given 1c secondary infections and three identical groups given 6c secondary infections. This experiment confirmed that initial recovery of secondary worms is not appreciably different from recovery of control primary worms (Fig. 4A, B). The results also establish that even by day 26 only 20–30 % of the established 1c secondary worms are expelled from mice given a 1c primary infection. However, the results do not confirm earlier results (Table 1) that the majority of 1c secondary worms are expelled after a 6c primary infection, as on day 26 the recovery of secondary worms was identical in mice given either a 1c or 6c primary infection. The results of the three groups given 6c secondary infections corroborate those shown above (Table 2) that there is rapid expulsion of 6c secondary worms.

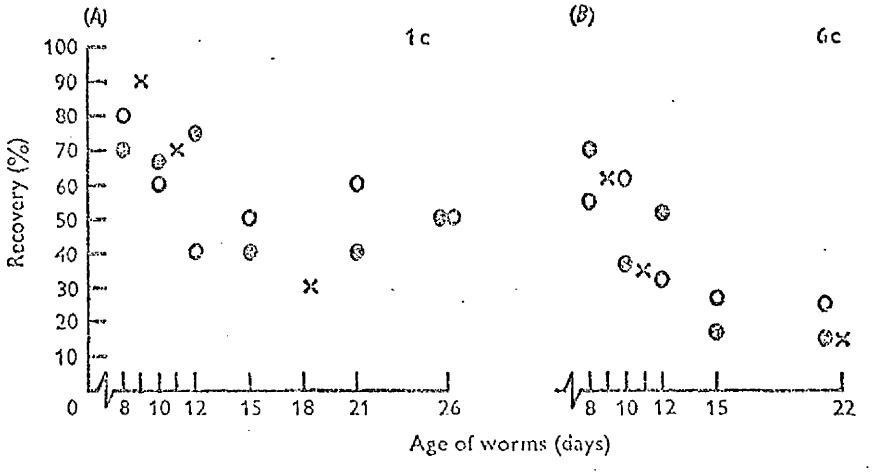

Fig. 4. Recovery (%) of *Hymenolepis diminuta* from challenge infections of CFLP ♂ mice given A, one (1 c) or B, six (6 c) cysticercoids. Mice given a sham (×) or a one (⊛) or six (○) cysticercoid primary infection; $n = 10$ except in 3 cases where $n = 9$, 9 and 8 respectively.

(*b*) *Worm growth*

There were no major differences between the weights of secondary worms in mice given primary infections of 1 c or 6 c hence these groups were combined for presentation. Only the results of the last of the experiments discussed above are given; they are representative.

Only 23 % (15/66) of the 1 c secondary worms recovered from 117 mice exceeded 0·1 mg and 47 % of these larger worms were found on day 8. This contrasted sharply with worms recovered from the 30 sham mice where considerable growth occurred and 79 % (15/19) exceeded 0·1 mg. The proportion of worms > 0·1 mg in the sham mice was significantly different from the proportion in the experimental mice ($\chi^2 = 18{\cdot}0$, $P < 0{\cdot}01$, 2×2 contingency table). Most worms from 1 c secondary infections do not grow much whereas in control primary infections considerable growth occurs (Fig. 5). However, some secondary worms grow and may survive for a considerable time.

To illustrate the limited growth of worms in 6 c secondary infections, the mean worm weight in each mouse is shown (Fig. 6). In determining these weights small worms obviously < 0·1 mg were not weighed but considered to be 0·1 mg each. These results with 6 c secondary infections confirm those of 1 c secondary infections that little growth occurs compared with that in worms from control mice. Whereas on each day in the 1 c secondary infections at least one mouse has a worm weight considerably larger than the others (Fig. 5), in the 6 c secondary infections only one mouse (day 10) had a worm weight that was disproportionately high, as shown by the large standard error on that day (Fig. 6).

In addition to being weighed the secondary worms were categorized as segmented or unsegmented. Using this qualitative criterion, no differences occurred between the 1 c secondary worms in mice given 1 c, 6 c or sham primary infections as virtually all worms were segmented on day 8. However, in mice given 6 c

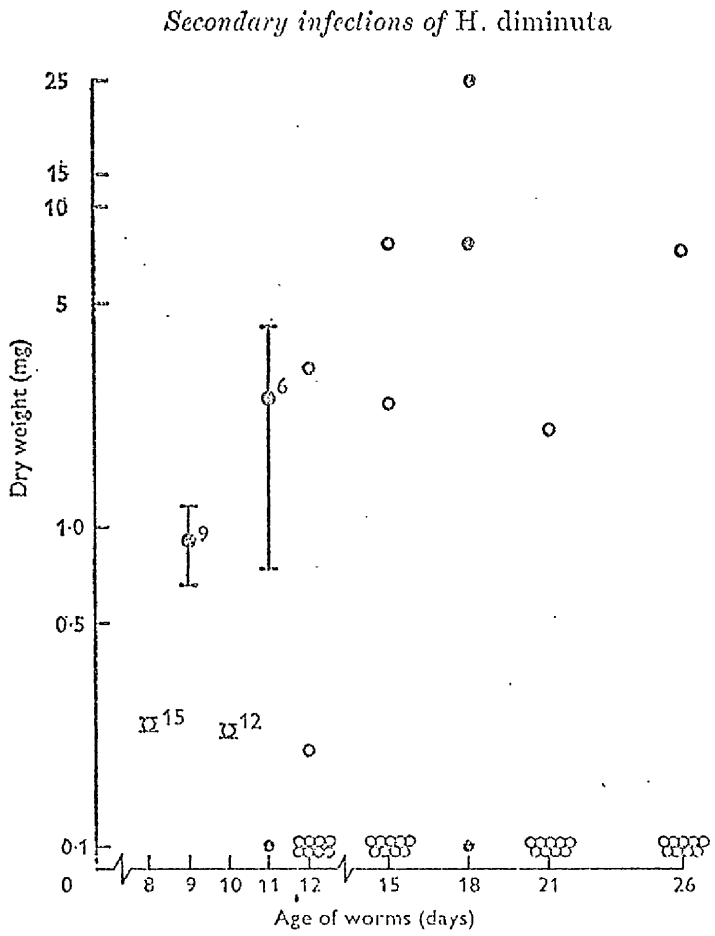

Fig. 5. Dry weight of *Hymenolepis diminuta* from one cysticercoid challenge infections of CFLP ♂ mice given one or six cysticercoid (○, n = 18–20 mice) or sham (⊙, n = 10 mice) primary infections; worms ≤ 0·1 included as 0·1 mg. On days 8–11 the mean weights (± standard errors) are shown, thereafter, the weights of all worms are given; superscripts adjacent to the means are the number of worms.

secondary infections there were differences which were related to the intensity of the primary infections. On days 8 and 9, > 90% of 'secondary' worms from sham primary infections were segmented but only 50% (19/38) and 67% (26/39), and 15% (5/33) and 14% (5/36) of secondary worms from mice given 1 c and 6 c primary infections respectively were segmented on day 8 in two experiments. The proportion of segmented 6 c secondary worms from mice given 1 c primary infections was significantly different from the proportion in the mice given 6 c primary infections in both experiments ($\chi^2 = 8{\cdot}1$, $P < 0{\cdot}01$; $\chi^2 = 19{\cdot}4$, $P < 0{\cdot}01$; 2×2 contingency tables). These results show that segmentation of worms in secondary infections is influenced by the intensity of the primary and secondary infections.

(c) *Worm position*

Generally in secondary infections on day 8 the mean scolex position was in the second 10 cm section of the intestine as in primary infections. Thereafter no general

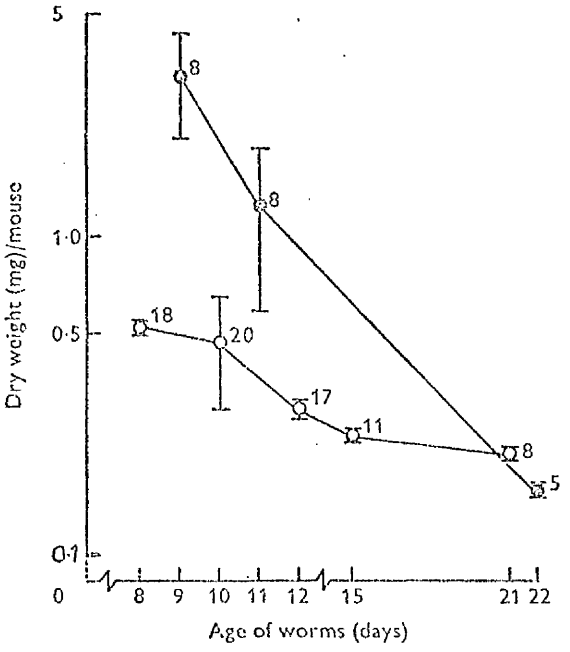

Fig. 6. Mean (± standard error) total dry weight per CFLP ♂ mouse of *Hymenolepis diminuta* from challenge infections of six cysticercoids. The mice were given one or six (○, n = 19–20 mice, but n = 15 day 21) or sham (⊗, n = 9–10 mice) primary infections; worms ≤ 0·1 included as 0·1 mg. Superscripts adjacent to the means are numbers of mice with worms.

migration of the worms was noted in either 1 c or 6 c secondary infections, and in the latter particularly, considerable scatter of worm position occurred.

DISCUSSION

In 6 c primary infections of *H. diminuta* in mice rejection occurs sooner than in 1 c primary infections and worms do not grow as large (Figs. 1, 2, 3). As rejection is immunologically based (Hopkins *et al.* 1972*a*, *b*), this earlier rejection in 6 c primary infections probably reflects a greater stimulation of host protective immune responses. The small size of worms from 6 c primary infections is due to a density dependent phenomenon which, as discussed below, may be in part immunologically mediated.

Stunting of *H. diminuta* in crowded infections of rats has been shown by many authors and is generally accepted as being due to competition for a limited resource, probably carbohydrate (Read & Simmons, 1963). In addition to stunting due to crowding, Roberts & Mong (1968) showed that in rats given 50 c considerable worm loss occurred by 17 weeks, a result confirmed by Harris & Turton (1973) using rats given 25 c and autopsied over 14 weeks. However, with 5 c no loss occurred and these latter authors argued that as serum antibody titres in 5 c and 25 c infections were similar, worm loss from the latter is not immunologically mediated but is probably due to competition through crowding. Their argument neglects that serum

antibody levels seldom correlate with protection, which with some tapeworm infections probably is multiphasic, as for example, the nematode *Nippostrongylus brasiliensis* where at least two components in addition to antibody are required for worm expulsion (Kelly, Dineen & Love, 1973).

With *H. microstoma* in mice stunting occurs in crowded infections (Dvorak, Jones & Kuhlman, 1961; Jones & Tan, 1971; Moss, 1971) and also there is a gradual loss of worms from 10c primary infections over 34 weeks (Goodall, 1973). Cortisone, an immunodepressant, causes crowded worms to grow larger (Moss, 1972), a result which suggests that stunting in crowded infections may be in part immunological in nature. However, as Moss (1972) discussed, the possibility that cortisone has a physiological effect cannot be discounted. Goodall (1973) argued that the gradual worm loss from primary infections is immunologically mediated as there are high antibody titres (Moss, 1971; Goodall, 1973) and a strong immunological response to secondary infection (Tan & Jones, 1967, 1968; Goodall, 1973). Further support for the hypothesis of immunological action in *Hymenolepis* spp. infections is given by results of Hopkins & Stallard (1974) with *H. citelli* in mice. In 1c infections gradual loss occurs but with 3c and 6c rejection begins sooner and is more precipitous. Cortisone prevents rejection of worms from 6c infections and increases their growth. The authors proposed that with *H. citelli* in mice there is a threshold of antigenic stimulation above which rejection occurs. Such thresholds may exist with other tapeworm infections, *e.g.* 5c and 25c infections of *H. diminuta* in rats (Harris & Turton, 1973).

The above studies with *H. microstoma* and *H. citelli* suggest that, contrary to the statement of Harris & Turton (1973), the loss of *H. diminuta* from heavy infections in rats and even stunting in crowded infections may have immunological components. For example, assuming that antibody from rats interacts with *H. diminuta*, an assumption which seems reasonable as immunoglobulin covers *H. diminuta* and *H. microstoma* in mice (Befus, 1974), a possible effect would be to depress the digestive-absorptive functions of the tegument. As multiple infections stimulate protective immune responses to a greater degree than 1c infections, this immunological depression of digestive-absorptive functions of the tegument would be greater in multiple infections thus potentiating the stunting due to inter-worm competition. It seems that the major evidence contradicting immunological action in the *H. diminuta*-rat system and which has been widely quoted is that of Chandler (1939). Recently, however, Andreassen, Hindsbo & Hesselberg (1974) with 100c infections of *H. diminuta* in the rat have shown destrobilation and expulsion of primary worms, suppression of this rejection by cortisone, and increased host resistance in secondary infections.

Until rejection began in the 6c primary infections there were no differences in the position in the intestines of mice of 1c or 6c *H. diminuta*. Anterior migrations into the duodenums of rats occur with certain populations of *N. brasiliensis* (Jenkins, 1973, 1974; Connan, 1974) and with destrobilated *H. diminuta* (Andreassen *et al.* 1974), and Jenkins (1974) has suggested that the duodenum is an immunologically privileged site. Whether the anterior migration to 19% (days 18 and 20) from 75% from the pylorus (day 14) of destrobilated *H. diminuta* in mice

in one of two experiments reported here is comparable is not known. In secondary infections no anterior localization occurred.

The results of *H. diminuta* recovery from 1 c and 6 c secondary infections in mice present difficulties in interpretation. Hopkins *et al.* (1972*a*) showed about a 20% reduction in recovery of 2 c secondary worms in comparison to controls. With *H. microstoma*, Tan & Jones (1967, 1968) showed 15–60% reduction in recovery of secondary worms after immunization with irradiated worms whereas Goodall (1973) showed about 80% reduction in recovery of secondary worms after natural or anthelmintic termination of primary infections. Gray (1973) showed a statistically significant reduction in recovery of secondary *Raillietina cesticillus* in female but not male fowl. However, the results in the present communication show, in comparison with controls, only a reduction of 10% (a difference of one worm from 10 mice) in three of four groups given 1 c secondaries and killed on days 8 or 9 (Table 1; Fig. 4A). It is impossible to decide whether this represents a reduced establishment or only a reduced recovery because of the difficulty of finding stunted secondary worms. This problem is compounded in 6 c secondary infections where not only are there more small worms to recover, but they are even more severely stunted than 1 c secondary worms as few are segmented. As severe stunting occurs commonly in secondary tapeworm infections (Hopkins *et al.* 1972*a*; Gray, 1973; Tan & Jones, 1967, 1968) statements of considerable reduction in establishment must be viewed sceptically unless supported by careful examination for small worms.

Whereas some doubt exists, therefore, whether worm establishment is reduced in secondary infections, it is clear that the loss of 6 c secondary worms occurs at a rate which is indistinguishable from that of 6 c primary worms from mice of the same age (Table 2; Fig. 4B). This is surprising as a more rapid expulsion was expected in the secondary infection as occurs in secondary infections with nematodes (*e.g.* Jarrett & Urquhart, 1971). In contrast to 6 c secondary infections, the loss of worms from 1 c secondary infections was normally small and occurred more slowly than the loss of 1 c primary worms from mice of the same age (Table 1; Fig. 4A). However, the small number of control mice killed in one experiment (Fig. 4A) makes statistical comparisons difficult. Why secondary worms survive for a long time is unknown but perhaps, as they are severely stunted in 1 c infections, they provide insufficient antigenic stimulation to trigger the host expulsion mechanism whereas in 6 c infections the mechanism is stimulated.

Little worm growth occurs in either 1 c or 6 c secondary infections (Figs. 5, 6) but in the former on each day studied at least one mouse had a worm weight considerably larger than the others; this variability did not occur in 6 c secondary infections. Variation in responsiveness of individuals is common (*e.g.* Hopkins & Stallard, 1974) and in some cases is genetically controlled (Wakelin, 1974; Wassom, DeWitt & Grundmann, 1974). Such variation could be explained by different thresholds of antigenic stimulation required to initiate a protective response in each mouse. The reduced variability in 6 c infections may mean that they provide sufficient antigenic stimulation to exceed the thresholds of all mice.

Other than reduced variability in 6 c infections, differences could not be detected

between 1 c and 6 c secondary infections using weight as a criterion, as most worms were ⩽ 0·1 mg. However, these small worms can include worms which have: (*a*) never segmented or grown, (*b*) segmented but not grown much and (*c*) segmented and grown considerably but have destrobilated. Are all small secondary worms severely stunted (*a* and *b*) or are some destrobilated (*c*)? Whether small worms have never grown or have grown but then destrobilated is important in determining the action of the immune response. For example, Gray (1973) stated that all *R. cesticillus* in secondary infections were destrobilated 14 days after infection, but he did not demonstrate that strobilation had occurred. On day 8 most 1 c secondary worms were segmented and 47 % exceeded 0·1 mg but in 6 c secondary infections in two experiments only 50 and 67 % (1 c primary infection) and 14 and 15 % (6 c primary infection) of the worms were segmented and few worms were > 0·1 mg. These results suggest that the severity of stunting of secondary worms increases with increasing intensity of primary and secondary infections. The possibility that in 6 c secondary infections segmentation and then destrobilation occur prior to day 8 seems unlikely; a more probable explanation is that the immune response of sensitized mice stunts the growth of worms from its onset. Stunting could be due to an immunologically mediated depression of the digestive-absorptive functions of the tegument (as suggested above for stunting in crowded primary infections) resulting in insufficient nutrient uptake. Although most 1 c and 6 c secondary worms ⩽ 0·1 mg are probably stunted, the segmentation and growth of some 1 c secondary worms suggests that a few of the small worms, particularly those recovered later in the infections, may be destrobilated.

It is well established that antigen dose is important in determining the type of immune response produced, ranging from low dose tolerance through increasing responsiveness (*e.g.* Hanna & Peters, 1971) to high dose tolerance. The effect of the immune response on *H. diminuta* increases as the intensity of primary and secondary infections increases which probably is due to sensitization of more immunocompetent cells in heavier infections. If the hypothesis that quantitative aspects influence the response to *H. diminuta* is correct, then not only the amount of antigen but also factors such as size of immunocompetent cell populations and duration of the sensitizing infection will be important. The suggestion that relative unresponsiveness of neonatal mice to *H. diminuta* is due to a quantitative deficiency in immunocompetent cell populations in gut-associated lymphoid tissue (Befus & Featherston, 1974) agrees with this hypothesis.

To identify the underlying mechanisms of the immune response to *H. diminuta*, serum and cell transfers and studies on the reconstitution of the immune response in irradiated mice will make considerable contributions. The present study provides essential information on secondary infections showing that for experiments where one attempts to induce resistance in naïve mice, worm weight, not survival, is the criterion to evaluate. Although 1 c infections are technically easier and eliminate the possibility of difficulties due to inter-worm competition, 6 c infections provide more predictable results; a characteristic which is essential in an already variable system.

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REFERENCES

ANDREASSEN, J., HINDSBO, O. & HESSELBERG, C. A. (1974). Immunity to *Hymenolepis diminuta* in rats: destrobilation and expulsion in primary infections, its suppression by cortisone treatment and increased resistance to secondary infections. *Proceedings, Third International Congress of Parasitology* 2. 1056–7; personal communication.

BEFUS, A. D. (1974). The immunoglobulin coat on *Hymenolepis* spp. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 68. 273.

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

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

CONNAN, R. M. (1974). The distribution of *Nippostrongylus brasiliensis* in prolonged infections in lactating and neonatally infected rats. *Parasitology* 68. 347–54.

DVORAK, J. A., JONES, A. W. & KUHLMAN, H. H. (1961). Studies on the biology of *Hymenolepis microstoma* (Dujardin, 1845). *The Journal of Parasitology* 47, 833–8.

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

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

HANNA, M. G., Jr. & PETERS, L. C. (1971). Requirement for continuous antigenic stimulation in the development and differentiation of antibody-forming cells: effect of antigen dose. *Immunology* 20, 707–18.

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

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

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

HOPKINS, C. A., SUBRAMANIAN, G. & STALLARD, H. (1972*b*). The effect of immunosuppressants on the development of *Hymenolepis diminuta* in mice. *Parasitology* 65, 111–20.

JARRETT, E. E. E. & URQUHART, G. M. (1971). The immune response to nematode infections. In *International Review of Tropical Medicine*, vol. 4 (ed. A. W. Woodruff and D. R. Lincicome), pp. 53–96. New York, London: Academic Press.

JENKINS, D. C. (1973). Observations on the distribution of an immune-adapted population of *Nippostrongylus brasiliensis* within the small intestine of rats given repeated small challenge infections. *Zeitschrift für Parasitenkunde* 41. 73–82.

JENKINS, D. C. (1974). *Nippostrongylus brasiliensis*: the distribution of primary worm populations within the small intestine of neonatal rats. *Parasitology* 68, 339–45.

JONES, A. W. & TAN, B. D. (1971). Effect of crowding upon growth and fecundity in the mouse bile duct tapeworm, *Hymenolepis microstoma*. *The Journal of Parasitology* 57, 88–93.

KELLY, J. D., DINEEN, J. K. & LOVE, R. J. (1973). Expulsion of *Nippostrongylus brasiliensis* from the intestine of rats: evidence for a third component in the rejection mechanism. *International Archives of Allergy and Applied Immunology* 45, 767–79.

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

Moss, G. D. (1972). The effect of cortisone acetate treatment on the growth of *Hymenolepis microstoma* in mice. *Parasitology* 64, 311–20.

Ogilvie, B. M. & Jones, V. E. (1973). Immunity in the parasitic relationship between helminths and hosts. In *Progress in Allergy*, vol. 17 (ed. P. Kallos, B. H. Waksman and A. de Week), pp. 93–144. Basel, München, Paris, London, New York, Sydney: S. Karger.

Read, C. P. & Simmons, J. E., Jr. (1963). Biochemistry and physiology of tapeworms. *Physiological Reviews* 43, 263–305.

Roberts, L. S. & Mong, F. H. (1968). Developmental physiology of cestodes. III. Development of *Hymenolepis diminuta* in superinfections. *The Journal of Parasitology* 54, 55–62.

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

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

Turton, J. A. (1971). Distribution and growth of *Hymenolepis diminuta* in the rat, hamster and mouse. *Zeitschrift für Parasitenkunde* 37, 315–29.

Wakelin, D. (1974). Genetic control of the immune response of mice to the nematode *Trichuris muris*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 68, 277.

Wassom, D. L., DeWitt, C. W. & Grundmann, A. W. (1974). Immunity to *Hymenolepis citelli* by *Peromyscus maniculatus*: genetic control and ecological implications. *The Journal of Parasitology* 60, 47–52.

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SECTION 4

SECONDARY INFECTIONS OF HYMENOLEPIS DIMINUTA IN MICE: EFFECTS OF VARYING THE DURATION OF THE PRIMARY, IMMUNIZING INFECTION

INTRODUCTION

The preceding publication (SECTION 3) and the findings of Hopkins, Subramanian & Stallard (1972 a) unequivocally establish that following the natural termination of Hymenolepis diminuta infections by mice there is immunological memory. The effect of this memory upon worms in challenge infections is influenced by the intensity of the immunizing and challenge infections, i.e. it is more pronounced in six cysticercoid (6c) than in one cysticercoid (1c) infections. In addition to the importance of the intensity of infection in the primary and secondary responses to H. diminuta by mice, it was suggested from the results of 1c infections in neonatal mice that the number of immunocompetent cells in the host is important (SECTION 2).

To provide further information about quantitative aspects of H. diminuta infections in mice (see DISCUSSION, SECTION 3), the duration of the immunizing infection was varied to determine its effect on the expression of memory. Does the effect of memory on worms in challenge infections increase gradually with

the duration of the immunizing infection, as shown by Wakelin (1969) for Trichuris muris infections in mice and by Rose (1974) for Eimeria maxima infections in fowl, or is H. diminuta nonimmunogenic until, say, day 5 at which time the immunogen(s) suddenly is/are recognized by the mice as suggested by Goodall (1973, p.146)? Given this question the present experiments were designed using a immunizing infection of 6c which was chemically abbreviated after various times and a 1c challenge infection. No MATERIALS AND METHODS are provided herein as adequate description appears in the GENERAL MATERIALS AND METHODS.

RESULTS

The results of only two experiments (Exp. 2; 20) will be described in detail. Two other experiments were conducted (Exp. 7; 16) but, as discussed in SECTION 1, the worm recoveries in these were unsatisfactorily low and therefore the results are not presented.

Experimental design:

Exp. 2:

mice - 4 star, 46 day old, CFLP males

day 0 - (4 days after arrival from supplier)
- infect groups 1A, B & C with six H. diminuta (experimental mice)
- sham infect groups 2A & B (sham mice)

day 3 - give groups 1A and 2A 150 mg/kg Zanil

day 8 - kill group 1C (10 mice) to determine if the infection on day 0 was successful; 52/60 (87%) recovery, hence a success

day 12 - give groups 1B and 2B Zanil (150 mg/kg)

day 17 - give mice in Zanil day 3 (ZD3) groups (1A and 2A) one H. diminuta each

day 26 - give mice in Zanil day 12 (ZD12) groups (1B and 2B) one H. diminuta each

kill 10 mice* in ZD3 and ZD12 groups 8, 9, 10, 12 and 15 days after their single worm infections; * day 15 the number of mice killed varied from 10 to 12.

Exp. 20:

mice - 4 star, 41 day old, CFLP males

day 0 - (8 days after arrival from supplier)

- infect groups 1A, B & C with six H. diminuta (experimental mice)
- sham infect groups 2A & B (sham mice)

day 3 - give groups 1A and 2A 150 mg/kg Zanil

day 8 - kill group 1C (15 mice) to establish if the infection on day 0 was successful; 81/90 (90%) recovery, hence a success

day 12 - give groups 1B and 2B 150 mg/kg Zanil

day 17 - give mice in ZD3 groups one H. diminuta

day 26 - give mice in ZD12 groups one H. diminuta

kill 20 mice in ZD3 and ZD12 groups on 11, 16 and 19, and 12-21 mice on days 22 (ZD3) and 23 (ZD12) after their single worm infections.

1. Exp. 2

The worm recovery (numbers near each point), and growth (mean dry weight, ± standard error; day 10 and thereafter only worms >0.2 mg included) are shown in Fig. 4 - 1a for experimental (●) and sham (○) ZD3 mice. The mean survival time in the experimental mice was 12 days whereas in the sham mice it was >15 days. Apart from the unexpected result on day 9 (due to the abnormally low mean dry weight in the sham group), the mean dry weights were consistently smaller in the experimental than in the sham mice, although the differences were not significant (t test). With the ZD12 mice (Fig. 4 - 1b) the mean survival time in the experimental mice was shorter (day 12) than in the sham mice (>day 15) as with the ZD3 mice. Furthermore as above, the mean dry weights were consistently smaller in the experimental than in the sham mice; the differences

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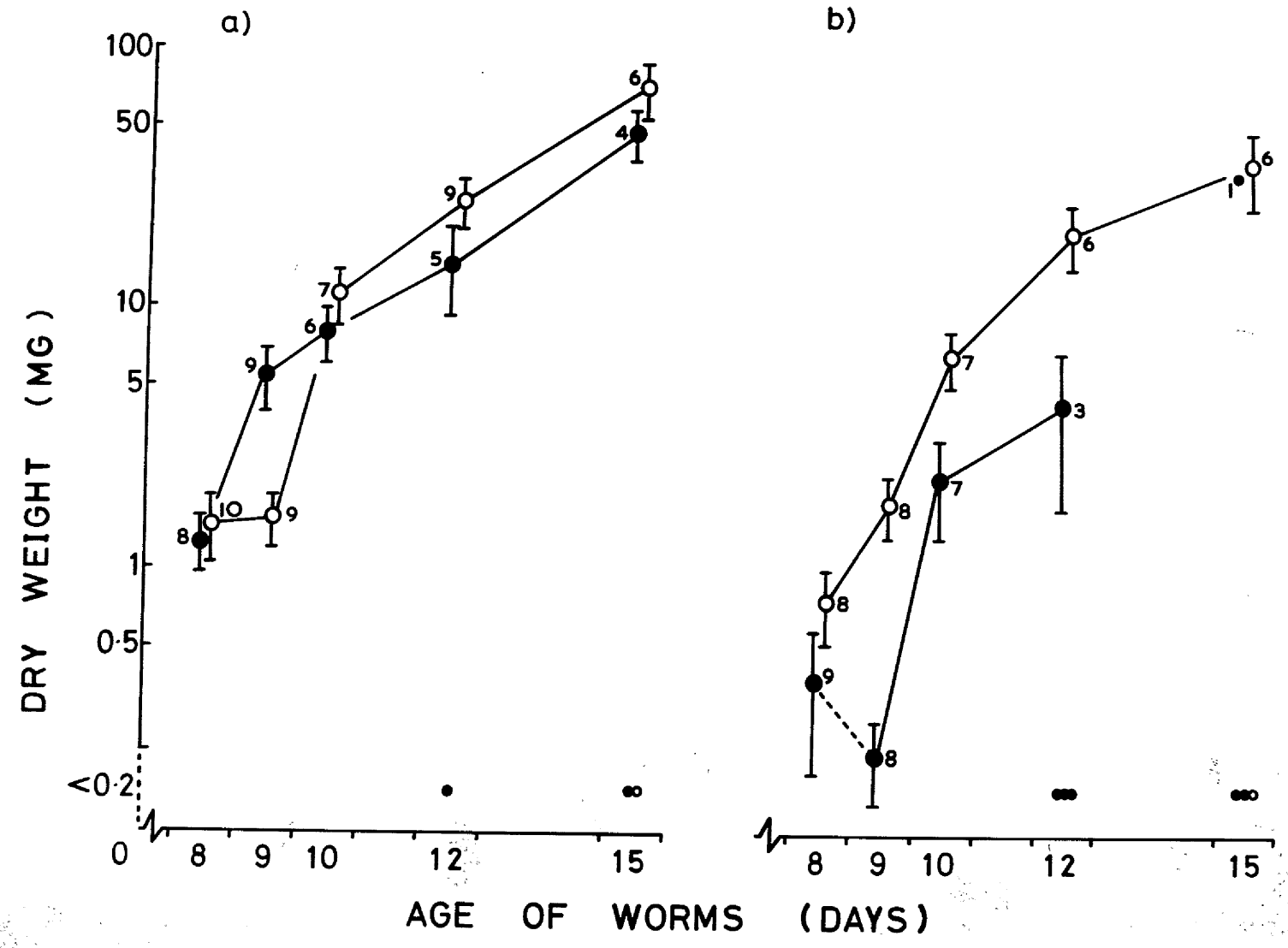
Figure 4 - 1

The growth and survival of single Hymenolepis diminuta in challenge infections of mice which received an immunizing infection for a) 3 or b) 12 days (Exp. 2). The large points with bars and adjacent numbers show the mean dry weights, ± standard errors and the number of undestrobilated worms; the small points without bars represent individual worms; ● worms from mice which were immunized, for stated period, with six H. diminuta, 0 worms from mice given sham immunization; n = 10-12 mice/group/day.


a)
b)
100
50
10
5
1
0·5
<0·2
0
DRY WEIGHT (MG)
8
9
10
12
15
AGE OF WORMS (DAYS)

were significant at the 5% level on all days after day 8 (t test).

Using the parameter, biomass (defined in SECTIONS 1 and 2), which combines the results of both numbers and mean weights, the total worm mass was considerably reduced in mice which had a primary immunizing infection of only 3 days duration (Fig. 4 - 2a). However, the biomass in these experimental ZD3 mice increased with time, albeit at a slower rate than in sham mice. In mice which had an immunizing infection of 12 days (Fig. 4 - 2b) the biomass was reduced in comparison to in the sham mice, but in contrast to the experimental ZD3 mice had not increased appreciably by day 15.

2. Exp. 20

The results show that in both the ZD3 and the ZD12 groups, worm recovery on day 11 was $\geq$80% but that in both the experimental and sham ZD3 mice the mean survival time was 10 days, whereas in the ZD12 mice it was 16 days (Fig. 4 - 3a & b). On days 22 (ZD3) and 23 (ZD12) 29% (5/17) to 37% (7/19) large, undestrobilated worms were recovered. In accordance with the results of Exp. 2 there were no significant differences between the mean worm dry weights in either experimental or sham ZD3 mice up to day 16, but in ZD12 mice on day 11 the mean weight in sham mice was significantly lower than in the experimentals. Differences which occurred in mean weights after these days are difficult to evaluate as there was considerable variation in worm recovery and size.

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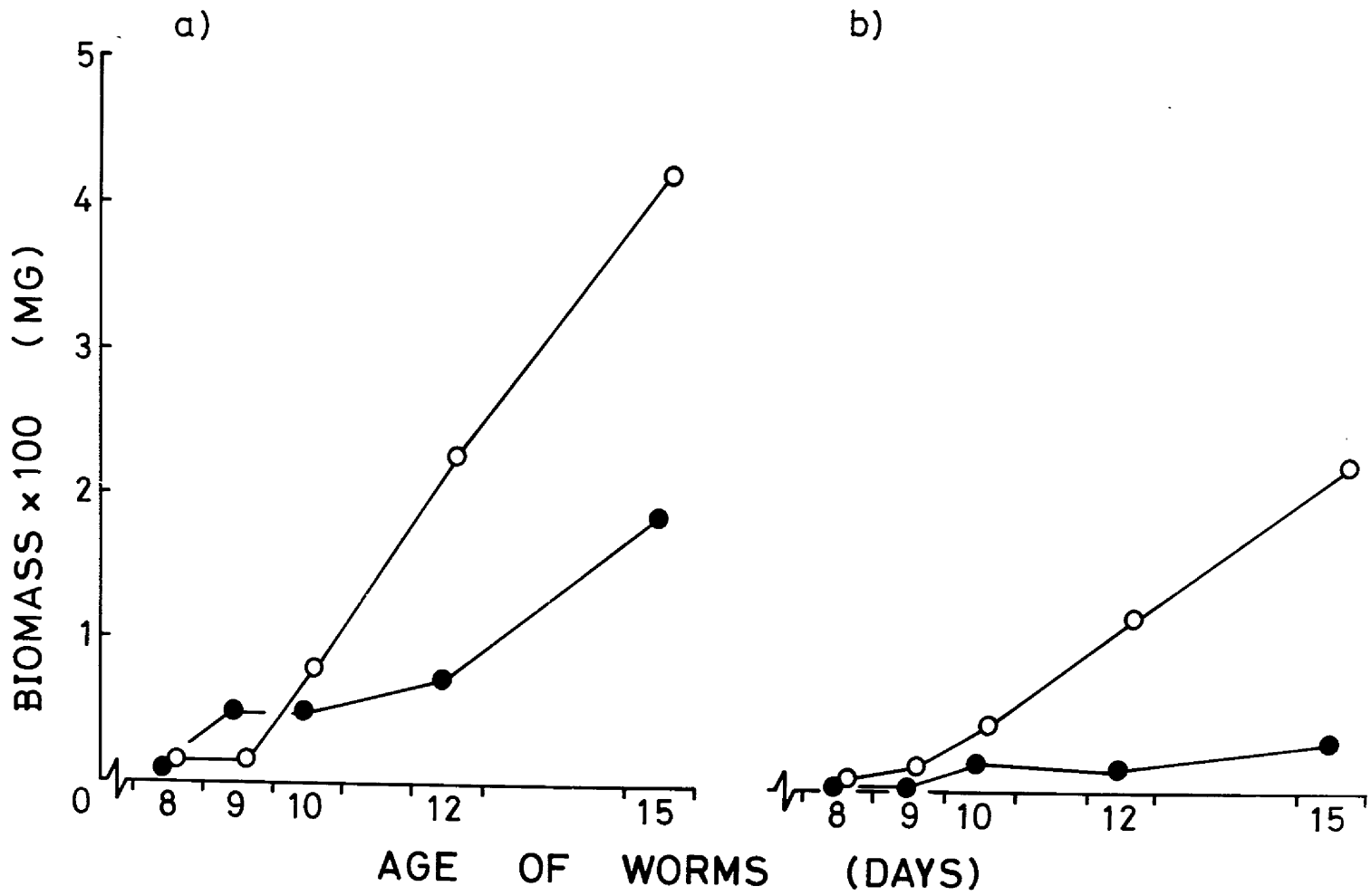
Figure 4 - 2

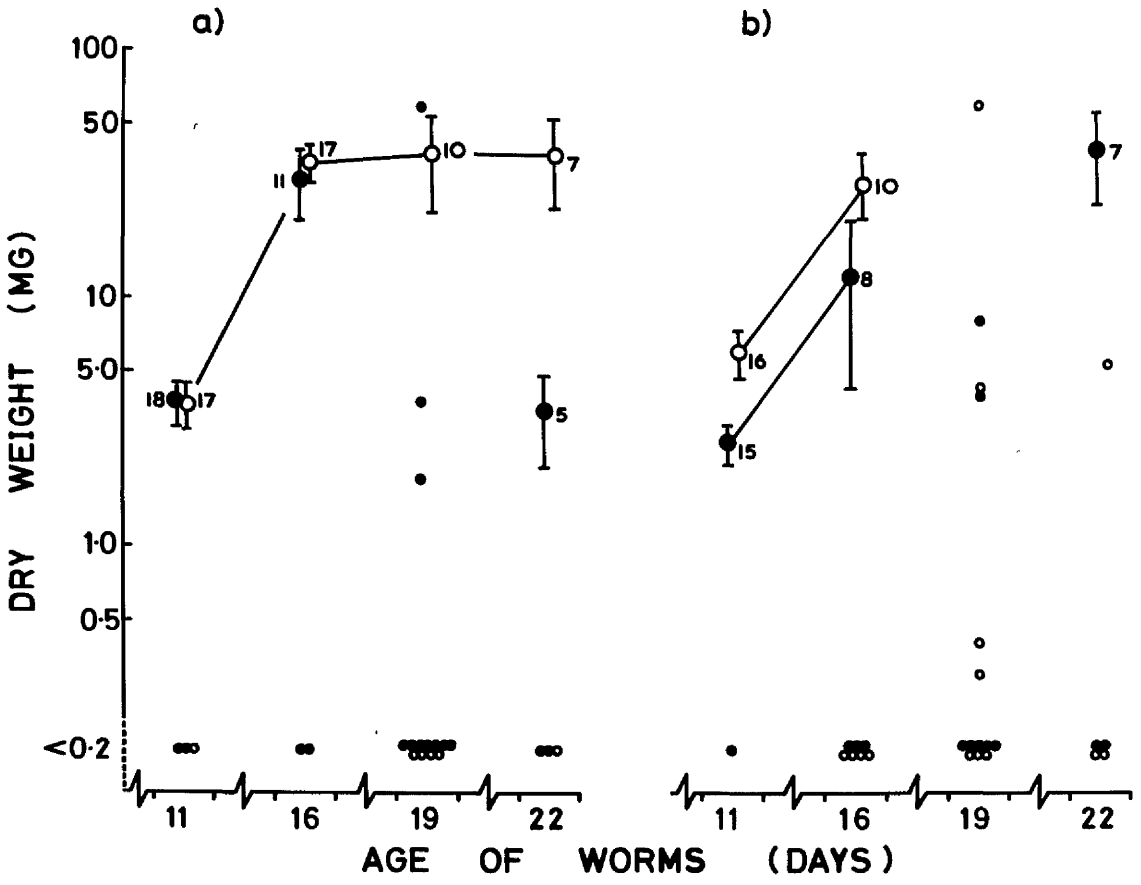
Biomass of Hymenolepis diminuta from single worm challenge infections of mice immunized for a) 3 or b) 12 days (Exp. 2); ● immunization for stated period with six H. diminuta; 0 sham immunization; n = 10-12 mice/group/day.


a)
b)
BIOMASS x 100 (MG)
0
1
2
3
4
5
8
9
10
12
15
AGE OF WORMS (DAYS)

Facing page 81

Figure 4 - 3

The growth and survival of single Hymenolepis diminuta in challenge infections of mice which received an immunizing infections for a) 3 or b) 12 days (Exp. 20). The large points with bars and adjacent numbers show the mean dry weights, ± standard errors and the number of undestrobilated worms; the small points without bars represent individual worms; ⊕ worms from mice which were immunized, for stated period, with six H. diminuta, ○ worms from mice given sham immunization; n = 20 mice/group/day days 11, 16 and 19; 12-21 days 22 and 23.


a)
b)
100
50
10
5·0
1·0
0·5
<0·2
DRY WEIGHT (MG)
18
17
11
17
10
7
5
16
15
10
8
7
11
16
19
22
AGE OF WORMS (DAYS)

In both ZD3 and ZD12 mice biomass increased until day 16 (Fig. 4 - 4a & b) as worm loss was compensated for by the growth of the surviving worms, but thereafter biomass declined as rejection proceeded. The relatively large biomass on day 23 in the experimental ZD12 mice may be due to chance although it may have significance (see below).

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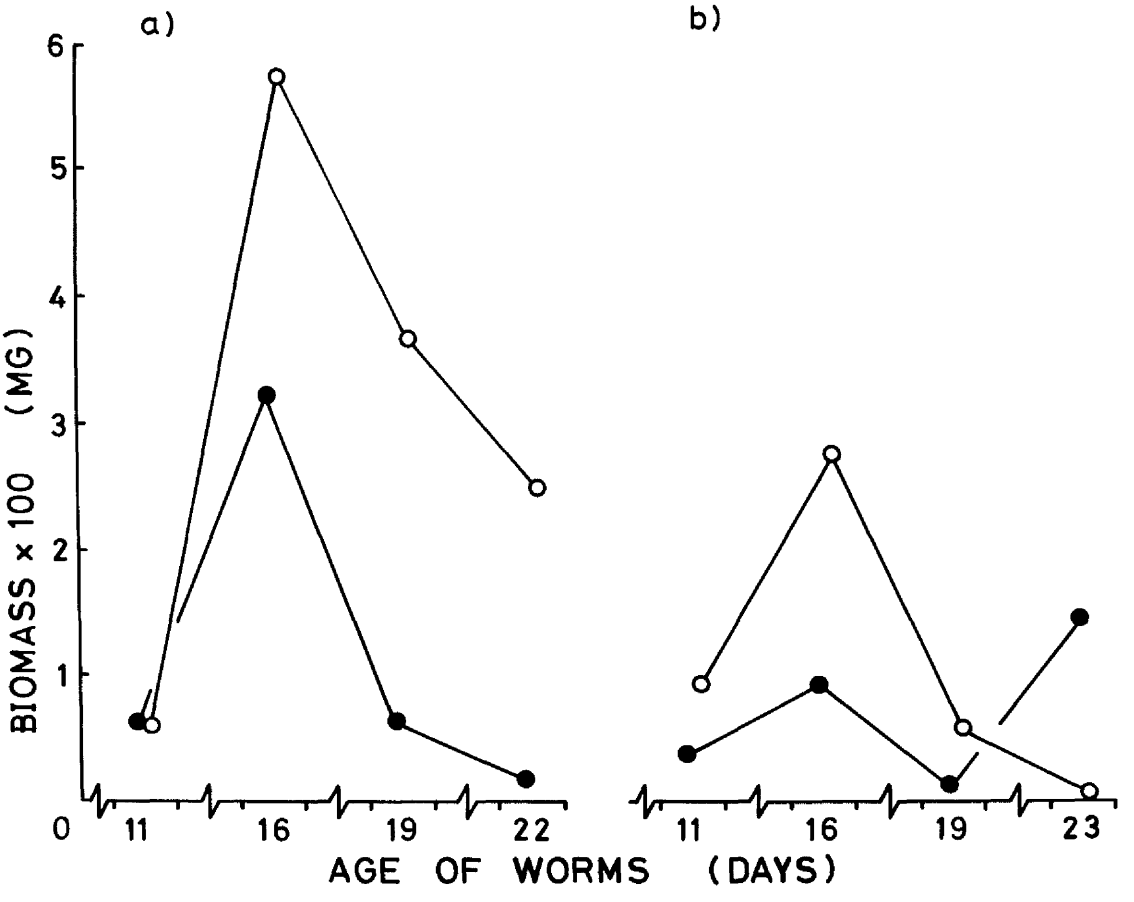
Figure 4 - 4

Biomass of Hymenolepis diminuta from single worm challenge infections of mice immunized for a) 3 or b) 12 days (Exp. 20); ◍ immunization for stated period with six H. diminuta, O sham immunization; n = 20 mice/group/day days 11, 16 and 19; 12-21 days 22 and 23.


a)
b)
BIOMASS x 100 (MG)
0
1
2
3
4
5
6
11
16
19
22
11
16
19
23
AGE OF WORMS (DAYS)

DISCUSSION

Immunizing infections of 6c of H. diminuta for 12 days result in the production of unequivocal memory in mice to homologous challenge with 1c 14 days later, which is expressed as a significant reduction in the growth of the challenge worms (Figs. 4 - 1b; 4 - 3b). Furthermore in Exp. 2, in which mice were killed on a number of days during the period of rejection, the mean survival time in immunized mice was less than in sham mice. In Exp. 20 where kills were widely spaced during the period of rejection differences between experimental and sham mice in the mean survival time of worms were not detected. Apart from day 23, Exp. 20 (see below), biomass was consistently less in the immunized than in the sham mice in both experiments (Figs. 4 - 2b; 4 - 4b). The rejection of worms from mice in Exp. 20 was atypically slow (see SECTION 1) as shown by the recoveries on days 22 and 23; why this occurred is unknown.

Results from the ZD3 mice, however, are less conclusive than those from the ZD12 mice. The consistent lack of a significant difference in the mean dry weight of worms from experimental and sham ZD3 mice agrees with the preconception that infection with six H. diminuta for 3 days is not immunogenic. On the other hand the shorter mean survival time of the challenge worms in the mice immunized for 3 days, Exp. 2 (Fig. 4 - 1a), and the consistently lower biomass in these

mice than in the sham mice in both experiments (Figs. 4 - 2a; 4 - 4a) imply that a 3 day immunization does sensitize mice to H. diminuta. This suggestion is strongly supported by the following: Campbell, Hartman & Cuckler (1963) showed that immunological memory occurred in mice given four 1 day infections with Trichinella spiralis; Rose (1974) demonstrated enhanced resistance to homologous challenge 3 days after immunization with Eimeria maxima; and Zinkernagel, Blanden & Langman (1974) established that by day 3 of a Listeria monocytogenes infection, lymphocytes are sensitized. Cunningham & Sercarz (1971) using a wide range of doses of red blood cells suggested that T cell memory develops rapidly over 2-4 days after immunization and is optimal by days 4-6. In confirmation, Black & Inchley (1974) showed that memory T helper cell populations appear by day 3, and that progenitors of secondary IgM producing cells are sensitized almost immediately after antigen injection, although sensitization of progenitors of secondary IgG producing cells requires at least 4 days. The development of this B cell memory is cumulative over 8 weeks (Cunningham & Sercarz, 1971).

It is important to realize, however, that from the experiments with H. diminuta, immunogenic stimulation probably did not cease on either day 3 or 12 when the anthelmintic was administered, but continued for some unknown length of time due to the efficient antigen retention mechanisms of medullary macrophages and follicles in lymphoid tissue (Nossal & Ada, 1971, p.141). Therefore, the different effects on challenge worms

administered 14 days after anthelmintic termination of the immunizing infection reflect an interaction of the facts that: 1) the mice whose infection was terminated after 12 days undoubtedly received more immunogen(s) than the mice infected for only 3 days, and 2) the immunogen(s) was/were retained in the lymphoid tissues of the mice for unknown periods of time which may or may not have been positively correlated with the dosage of immunogen(s) received.

The results suggest that the effect of the secondary immune response on the challenge worms increases progressively with the duration of the primary infection as demonstrated by Wakelin (1969) for Trichuris muris infections. If this be true then immunizing infections of between 3 and 12 days duration should produce an intermediate stunting of the challenge worms, and infections terminated naturally should produce stunting more severe than in mice infected for only 12 days. The latter is confirmed by the results shown in SECTION 3, namely that stunting of challenge worms is most severe when the immunizing infection is terminated naturally (compare Fig. 5, SECTION 3 with Figs. 4 - 1b; 4 - 3b). Exp. 7 and Exp. 16 included groups in which the immunizing infection was terminated after 5 days and although, as stated above, the recoveries in these experiments were unacceptably low, the results acquired suggest that the stunting was intermediate between that of 3 and 12 day immunizations. Fig. 4 - 5 shows a diagrammatic representation of this concept of increasingly severe stunting in secondary infections related to the duration of the immunizing infection; the

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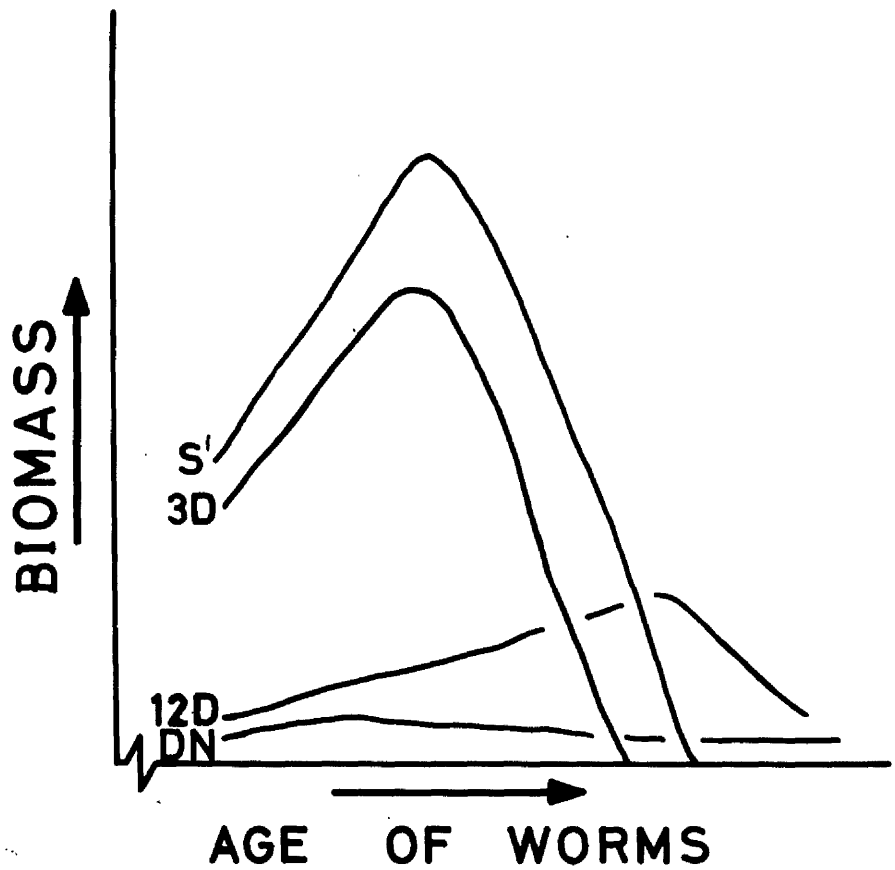
Figure 4 - 5

Diagrammatic representation of biomass of challenge single Hymenolepis diminuta following immunizing infections of various durations with six worms; S° - sham, 3D - 3 day, 12D - 12 day and DN - duration natural, immunizations.


BIOMASS
S'
3D
12D
DN
AGE OF WORMS

lines on the figure represent the response in all mice except those for which have unexpectedly large worms late in infections (e.g. Fig. 5, SECTION 3; Fig. 4 - 3b). The upper line (S') shows a representative result of a challenge infection in mice which had received a sham immunizing infection. The biomass initially increases as the worms grow but subsequently decreases as they are rejected. Following an immunizing infection of 3 days (3D line) the pattern is similar but the worms grow more slowly and are rejected more quickly. With an immunizing infection which is terminated naturally (DN), the challenge worms show little growth, whereas with a 12 day immunizing infection (12D) the challenge worms are stunted but do grow. The late peak in biomass is due to the fact that most worms are stunted and rejected but some survive longer than expected and continue to grow, albeit slowly. This may represent some form of adaptation by the worms as occurs in H. microstoma. With this latter worm, stunting occurs initially but subsequently the worms adapt and grow until most are virtually indistinguishable from worms in primary infections (R. Howard, personal communication).

At first sight the statement that the mean survival time of challenge worms is less in 3 and 12 day immunized mice than in the sham mice seems to contradict the conclusion in SECTION 3 that following natural termination of the primary infection expulsion of the challenge worms is not accelerated in comparison with in sham mice. In the determination of the mean survival time worms which have destrobilated are not considered to be surviving, whereas the conclusion in SECTION 3

includes all worms, because the stunting described therein was so severe that destrobilated worms, if they existed, could not be distinguished. The apparent contradiction is not in the data but in the difference, discussed further in SECTION 3, between 'rejection', used to determine mean survival time, and 'expulsion'. If expulsion rather than rejection was used to evaluate the results of Exp. 2 differences between experimental and sham mice would be minimized, especially considering that it was conducted early in this study when I was less efficient at finding very small worms than when I conducted the experiments in SECTION 3.

In conclusion, H. diminuta probably produces immunogen(s) from the moment it establishes in the host and the increasing effectiveness of memory on challenge worms is due to stimulation of the population of immunocompetent cells by greater amounts of immunogen(s) and for longer periods of time. This progressive development of the effectiveness of the secondary response may apply widely in helminth infections.

SUMMARY

1. An immunizing infection of 12 days with six *H. diminuta* produces unequivocal memory in mice to homologous challenge with lc 14 days later; it is expressed as a significant reduction in the growth of challenge worms and in one experiment hastened rejection.

2. Immunization for 3 days produced a detectable, but not significant, reduction in growth, and hastened rejection in one experiment.

3. It is concluded that the effectiveness of memory in suppressing growth of challenge worms increased with the duration of the immunizing infection. This suggests that the immunogen(s) is/are released from the beginning of the infection and that there is not a totally nonimmunogenic phase of worm development followed by an immunogenic phase.

SECTION 5

CHARACTERISTICS OF INTESTINAL IMMUNE RESPONSES WITH OBSERVATIONS ON INFECTIONS OF MICE WITH *HYMENOLEPIS DIMINUTA* AND *H. MICROSTOMA*

INTRODUCTION

All adult cestodes and, with the notable exception of *Schistosoma* spp., most other adult helminths which have been subject to immunological studies inhabit the gastrointestinal tract. The complexity of this environment has been reviewed recently by Mettrick & Podesta (1974) who showed that the approaches of parasitologists, studying physiological relations between the host and parasite, have not kept pace with recent advances in knowledge of intestinal physiology. One aspect not discussed in depth by Mettrick & Podesta (1974) but to which their criticism that parasitologists are slow to follow recent developments applies equally well, is the immunological capabilities of the intestine.

Mucosal surfaces of which the intestinal tract is probably the largest in the body, are the sites of natural entrance of most infectious organisms into the body. Hence it is not surprising that immune mechanisms at mucosal surfaces have received considerable attention by immunologists. Recently two large international

symposia have been held and the proceedings published subsequently (Dayton, Small, Chanock, Kaufman & Tomasi, 1971; Mestecky & Lawton, 1974). In addition, reviews of mucosal immune mechanisms have been published in journals representing diverse disciplines in science and below is a far from exhaustive list of reviews. Each review has its own emphasis and provides interesting practical and theoretical implications for consideration in studies of immunity to gastrointestinal helminths: Tomasi & Bienenstock (1968), Brandtzaeg, Fjellanger & Gjeruldsen (1970), Ginsberg (1971), Hobbs (1971), South (1971 a & b), Berg & Savage (1972), Doe (1972), Ferguson (1972), Fubara & Freter (1972 a), Jones (1972), Murray (1972 a), Shearman, Parkin & McClelland (1972), Tomasi & Grey (1972), Bazin, André & Heremans (1973), Brandtzaeg (1973, 1974 c), Porter (1973), Walker & Hong (1973 a & b), Bienenstock (1974) and Wardlaw & Watt (1974). Below I intend to summarize some of the characteristics of mucosal lymphoid tissue, immunoglobulins (Igs) and immune responses, and to discuss briefly current concepts of their biological significance so that subsequently some of these characteristics and concepts can be considered with regard to gastrointestinal helminth infections, particularly with Hymenolepis spp.

1. Précis of intestinal immunity

a) intestinal lymphoid tissue

The intestinal mucosa is continually exposed to vast amounts of antigenic material in or as food and it is well established that potentially immunogenic quantities

of these antigens are absorbed intact (Rothberg, Kraft & Michalek, 1974; Walker & Isselbacher, 1974). It is hardly surprising that a large portion of the gastrointestinal tract is lymphoid tissue. Within the epithelium 1:6 to 1:10 of the cells are thelio-lymphocytes; such an abundance that if aggregated they would represent an organ at least as large as the pancreas (Ferguson, 1972). Fichtelius (1968) introduced the terminology thelio-lymphocytes and Bienenstock (1974) reviewed some of the characteristics of these cells. Ultrastructurally they resemble lymphocytes but contain granular inclusions reminiscent of mast cells and can degranulate releasing histamine. It has been suggested that they are derived from mast cells (see Bienenstock, 1974), although recently Guy-Grand, Griscelli & Vassalli (1974) provided evidence that they are of T cell origin. Whatever their derivation, they may act as sentinels at the mucosal surface perhaps sensitized by IgE (Bienenstock, 1974).

Below the epithelium there is an abundance of cells including lymphocytes, plasma cells, macrophages, eosinophils and mast cells. Plasma cells are particularly numerous in the lamina propria of each villus and surrounding the crypts of Lieberkühn. The vast majority of these contain IgA, but plasma cells containing other Igs can be detected also; Igs in the gut will be discussed more fully below.

In the 1960's the elucidation of the roles of the thymus and avian bursa of Fabricius as central lymphoid organs, responsible respectively for cellular and humoral aspects of immune responses, created much excitement. As both these organs are derived from the

embryonic gut it follows logically that in the search for the mammalian equivalent of the bursa, the gut associated lymphoid tissue (GALT), specifically the Peyer's patches and to a lesser extent the appendix, has received considerable scrutiny (reviewed by Cooper & Lawton, 1972). The Peyer's patches are covered by a specialized epithelium which has pinocytotic capabilities not shared by the epithelium of adjacent villi (Bockman & Cooper, 1973) and this apparently functions in sampling the antigenic make-up of the lumen. Below this specialized epithelium there are three structural elements: 1) the domes and immediately below them 2) the follicles; between the follicles are the 3) thymus-dependent areas (see Waksman, 1973; Waksman, Ozer & Blythman, 1973). The early appearance of domes prior to significant antigenic stimulation (Waksman, 1973; Chapman, Johnson & Cooper, 1974) is consistent with the possibility of their being a bursa equivalent and it has been suggested that B cells arising from the domes proliferate in the underlying follicles following antigenic stimulation (Waksman, 1973; Waksman et al. 1973).

Peyer's patches are also areas where antigenic materials carried from the mucosal surface in the lymph accumulate while draining to the regional lymph nodes (Carter & Collins, 1974). However, primary antibody responses do not occur in vivo in Peyer's patches although secondary responses can be induced (Veldkamp, van der Gaag & Willers, 1973). Cells from Peyer's patches have considerable migratory capabilities and repopulate, predominately with IgA producing cells, the lamina propria of the guts, and the spleens of irradiated

animals (Bienenstock, 1974; Cooper, Kincade, Bockman & Lawton, 1974; Cebra, Craig & Jones, 1974; Guy-Grand et al. 1974).

Given these characteristics the current concept of the functioning of Peyer's patches and the appendix is that they are areas of early, antigen-independent development of B lymphocytes. Subsequently, after sensitization by intestinal antigens transported through the specialized epithelium overlying the domes, these cells proliferate and migrate via lymphatics and the bloodstream to predominantly the lamina propria of the intestine where they become IgA producing cells (Bienenstock, 1974; Cooper et al. 1974).

b) immunoglobulins in the gut

As stated above, plasma cells are abundant in the lamina propria of the villi and surrounding the crypts of the gastrointestinal tract. In all species studied the vast majority of these cells produce IgA but plasma cells producing IgD, IgE, IgG and IgM occur also (e.g. Jones, 1972). Normally the relative concentration of the various Igs in the gut reflects the abundance of the different types of plasma cells in the underlying lamina propria and does not correlate with the concentrations of the Igs in the serum. The situation is a complex one, however, and differences between the various external secretions of one species, between different species and between normal and pathological states lead to confusion (see Tomasi & Grey, 1972). Some of these differences will be enumerated below but unless stated otherwise descriptions will be of the human gastrointestinal tract, as this has been extensively

investigated.

i) IgA

An excellent review on IgA by Tomasi & Grey (1972) is available and the most recent advances and concepts can be found in Brandtzaeg (1974 c) and Mestecky & Lawton (1974). Herein only a brief summary is provided.

In human serum, IgA at 13% of the total is the second most abundant Ig following IgG (80%) (Roitt, 1974). However, it is commonly stated that IgA is the predominant Ig in external secretions, but this is not always true when absolute concentrations are studied (see Table 1 in Brandtzaeg, 1973). What is true, however, is that the ratio of secretion/serum IgA is greater than for other Igs. This suggests that there is a selective mechanism for the appearance of IgA in the secretions.

Mammalian IgA occurs in a variety of forms. Monomeric IgA has a sedimentation coefficient of about 7S (Svedberg units) with a molecular weight of approximately 160,000 and in other respects is fundamentally similar to IgG. As well as monomeric IgA there is polymeric IgA which occurs in a variety of states with sedimentation coefficients ranging from 10 to 18S corresponding to increasing numbers of monomers joined together. All polymeric IgA molecules apparently contain J (joining) chain, a component with a molecular weight of 15,000-26,000, which is generally considered essential for polymerization. Recent work by Eskeland & Brandtzaeg (1974), however, has suggested that J chain is not required for polymerization but is for the combination of

dimeric IgA with secretory component (SC) producing secretory IgA (s-IgA). SC has a molecular weight of 70,000-85,000 and seems to function in the transfer of s-IgA into the intestinal lumen, although as discussed below this has been disputed. It has also been suggested, although not proven, that SC confers resistance to proteolysis on s-IgA in the enzymatically hostile intestinal environment (Tomasi & Grey, 1972). In man there are two subclasses of IgA, namely IgA_1 and IgA_2. IgA_1 predominates in the serum whereas in the secretions IgA_1 and IgA_2 are in approximately equal amounts (Tomasi & Grey, 1972). At the time of writing this thesis the most recent model proposed for the structure of s-IgA is that of Heremans (1974).

About 85% of serum IgA in man is monomeric; the remaining, polymeric IgA, is largely dimeric and lacks SC. Only about 1% of serum IgA in man is s-IgA (Heremans, 1974). In contrast about 60% of IgA in secretions at mucosal surfaces is s-IgA and the remaining 40% is comprised of larger polymers with little monomeric IgA (Brandtzaeg, 1974 c). Animals such as cats, dogs, guinea pigs, mice and rats differ considerably from man as they have little 7S monomeric IgA in either the secretions or, more interestingly, the serum (Vaerman, André, Bazin & Heremans, 1973; Heremans, 1974). Also as distinct from man, about 80% of mouse serum IgA is dimeric, rather than monomeric (Tomasi & Grey, 1972). This has important implications in this thesis with reference to the specificity of the antisera employed in some experiments.

In man, monomeric and at least some polymeric IgA in the serum is presumed to originate in plasma cells

in the bone marrow and spleen, and in lymph nodes other than those associated with mucosal surfaces (Heremans, 1974; Radl, Schuit, Mestecky & Hijmans, 1974). Such IgA-containing plasma cells in extramucosal sites are rare in the cat (Heremans, 1974) and this has been used to explain the scant amount of 7S IgA in the serum of this animal. Presumably the same is true of the mouse and other animals with little 7S IgA in the serum. Until recently dimeric IgA was thought to be formed after the monomers were released from plasma cells situated near mucosal surfaces (Ginsberg, 1971; Hobbs, 1971). Presently the consensus is that a molecule of dimeric IgA plus J chain is produced and assembled in a single plasma cell and released into intercellular spaces (Tomasi & Grey, 1972). This dimeric IgA may enter the circulation via the lymphatics (Vaerman *et al.* 1973) or, cross the basal lamina of the mucosal epithelium, enter the epithelial cells and be released into the lumen. The basal lamina is permeable to large molecules (Threadgold, 1967) and IgA probably diffuses through it freely, although Allen, Smith & Porter (1973) hypothesized that pseudopodia from plasma cells pass through pores in the basal lamina and release vesicles containing IgA into the intercellular spaces of the epithelium. Regardless of how IgA gets into these intercellular spaces it must enter the epithelial cells for secretion into the lumen as it is agreed that access into the lumen is not readily available through the well-known tight junctions or *zona occludens* uniting the apices of adjacent epithelial cells (Tidball, 1971).

The site where dimeric IgA with J chain combines

with SC has been a matter of disagreement, some authors (e.g. Brandtzaeg, 1974 b; Poger & Lamm, 1974) claim that SC is produced by epithelial cells and s-IgA is formed on the membrane or within the cytoplasm of these cells prior to secretion. Other authors (Allen et al. 1973; Comoglio & Guglielmone, 1973) have suggested that SC is produced in goblet cells, not epithelial cells, and that complexing with IgA to form s-IgA occurs intralumenally. Brandtzaeg (1974 a) argued that antisera which have localized SC in goblet cells by immunofluorescent techniques were of poor specificity and he expressed confidently that epithelial cells, not goblet cells, are the general source of SC. In support of this argument of the nonspecificity of reactions with goblet cells, Bienenstock, Gauldie & Perey (1973) showed in chickens that a relatively crude anti-IgA serum reacted with goblet cells but a pure anti-IgA serum did not. Based upon present evidence intercellular dimeric IgA with J chain combines with SC, produced by the epithelial cells, on the cell membrane or within the cytoplasm to become s-IgA. The molecule enters the cytoplasm supranuclearly or infranuclearly (Allen et al. 1973) and is released to the lumen by an unknown mechanism (Brandtzaeg, 1974 a & b). The specificity of this transfer system probably involves the interaction of information in J chain and SC (Eskeland & Brandtzaeg, 1974; Brandtzaeg, 1974 c). Figure 5 - 1 provides a synthesis of the current concepts on IgA production and secretion in man.

ii) IgM

IgM occurs in the serum as a 19S (molecular weight of about 900,000) pentamer with an associated J

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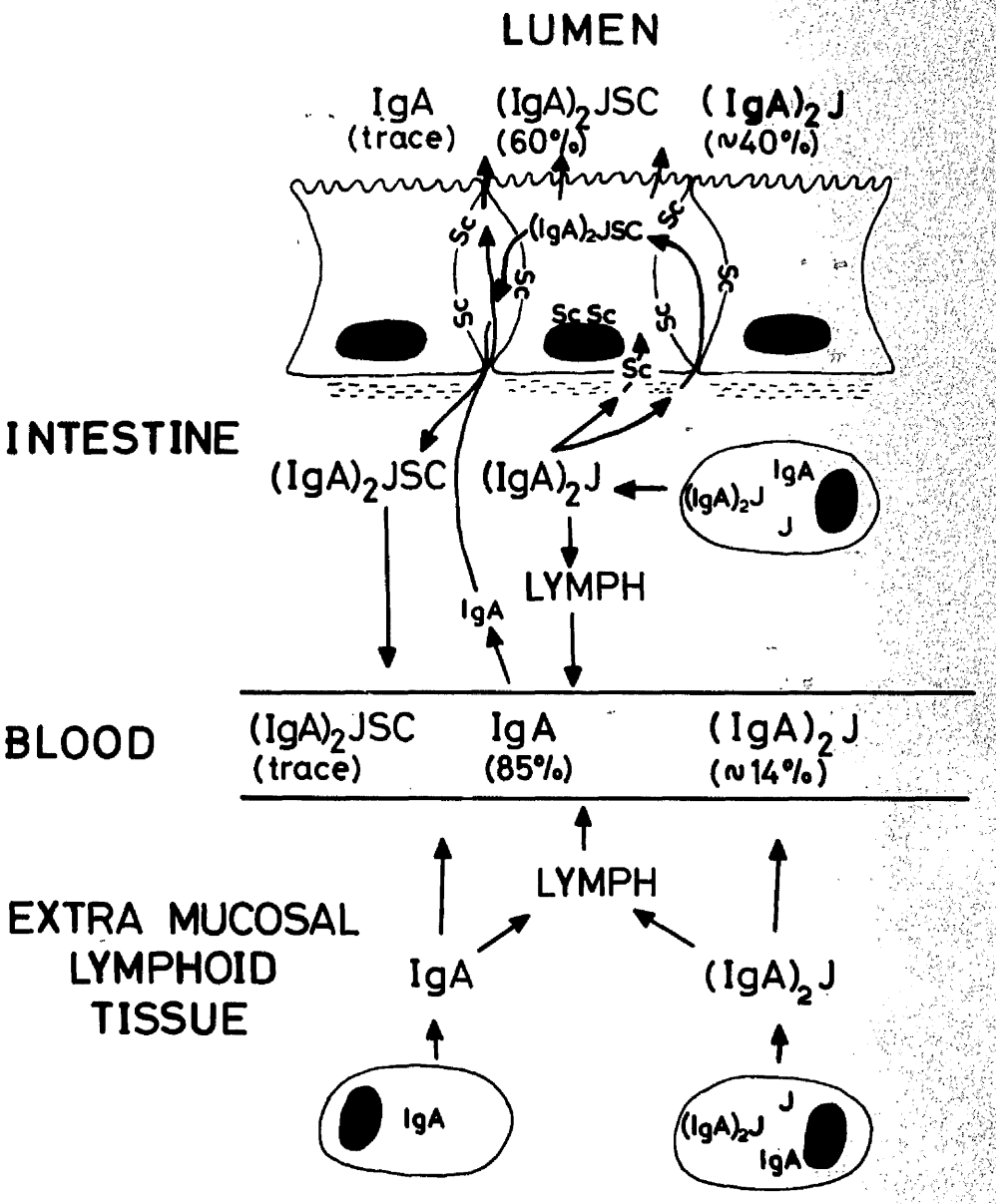
Figure 5 - 1

Current concepts on the sites of production of monomeric and dimeric IgA and secretory component (SC), and the distribution and relative abundances of the various types of IgA in man. Notice the differences between the intestinal lumen and the blood.


LUMEN
IgA (trace)
(IgA)2JSC (60%)
(IgA)2J (≈40%)
(IgA)2JSC
Sc
Sc.Sc
INTESTINE
(IgA)2JSC
(IgA)2J
(IgA)2J
IgA
J
LYMPH
IgA
BLOOD
(IgA)2JSC (trace)
IgA (85%)
(IgA)2J (≈14%)
LYMPH
EXTRA MUCOSAL LYMPHOID TISSUE
IgA
(IgA)2J
IgA
(IgA)2J
J
IgA

chain and in nonpathological states represents about 6% of the total serum Ig in man (Roitt, 1974). At a given time about 25% of serum IgM is extravascular (Tomasi & Grey, 1972). In intestinal secretions IgM normally occurs in concentrations lower than IgA but in excess of IgG (Tomasi & Grey, 1972; Brandtzaeg, 1973). It has been shown (quoted by Hobbs, 1971) that, like IgA, IgM enters the gut 30 fold faster than serum albumin and hence as for IgA there must be a mechanism for local production and/or selective secretion of IgM.

Whereas in the human parotid gland about 97% of the plasma cells produce IgA with the remaining 3% comprised equally of IgM and IgG producing plasma cells, in the intestine average values are 81.5% for IgA, 16% for IgM and 2.7% for IgG producing plasma cells (Brandtzaeg, 1973). Further evidence for the importance of local production of IgM in intestinal immune responses was provided by Brandtzaeg et al. (1968 a) when they demonstrated significant increases in IgM producing plasma cells in IgA deficient patients. Brandtzaeg (unpublished, quoted in Eskeland & Brandtzaeg, 1974) also has shown that IgM has a weak affinity for SC and that 60-70% of secreted IgM contains SC. Hence, there is abundant evidence of significant local production of IgM in the intestine and of its secretion by the same J chain - SC specific mechanism as hypothesized for s-IgA.

iii) IgG

IgG represents 80% of the total serum Ig and occurs as four major subclasses, namely IgG_1 - IgG_4, with molecular weights of about 150,000 (7S) (Roitt, 1974).

About 50% of serum IgG is extravascular (Tomasi & Grey, 1972) and hence IgG is abundant in intercellular ground substance and at basal laminae of epithelial surfaces (Brandtzaeg, 1973). As shown above, however, the number of IgG producing plasma cells near mucosal surfaces is, in normal states, insignificant although in cases of chronic inflammation this can be altered (see below). Despite the abundance of extravascular, but systemically produced, IgG near mucosal surfaces, IgG is not abundant in the intestinal lumen nor is it considered significant in many intestinal responses (Tomasi & Grey, 1972). The IgG in the intestinal lumen is not structurally different from serum IgG and this, along with its paucity in the lumen, suggests that there is no selective secretion, only epithelial leakage, of IgG into the intestine. Unlike s-IgA and probably to a limited extent secreted IgM with weakly bound SC, IgG is highly susceptible to proteolysis in the intestine and, therefore, the IgG entering the intestinal lumen has a short half-life (Doe, 1972). In conclusion, unlike IgA and IgM, IgG does not appear to have specific adaptations to facilitate its action in intestinal resistance.

iv) IgD

Unlike IgA, IgM and IgG, IgD has not been detected in intestinal secretions although plasma cells containing this poorly known Ig occur in low numbers in the lamina propria of the intestine (Shearman et al. 1972; Doe, 1972; Jones, 1972). In the serum, IgD represents about 1% of the total Ig and is a 7S molecule with a molecular weight of about 185,000 (Roitt, 1974).

v) IgE

IgE occurs in human serum at the very low concentration of about 0.002% of the total serum Ig. It is an 8S molecule with a molecular weight of about 200,000 (Roitt, 1974). Plasma cells producing IgE are rare in the spleen and peripheral lymph nodes but large numbers occur in the gastrointestinal lamina propria and other mucosal associated lymphoid tissue (Ishizaka, Ishizaka, Tada & Newcomb, 1971; Tomasi & Grey, 1972). Despite this local production of IgE it has not been detected in the intestinal secretions, although it has been detected in sputum, nasal washings and saliva (Ishizaka et al. 1971; Waldman, Virchow & Rowe, 1973). Presumably IgE occurs in the gastrointestinal secretions but is rapidly degraded by proteolytic activity in the lumen and hence with existing techniques cannot be detected.

c) gastrointestinal immune responses and their biological significance

As in so many fields today and especially those with direct medical relevance there is a tremendous volume of literature on immune responses to infections localized at, or gaining entrance to the body through mucosal surfaces. An extensive review occurs in Tomasi & Grey (1972) and recent but more restricted reviews include Bienenstock (1974), Wardlaw & Watt (1974) and the various papers presented in sessions D (Function) and F (The IgA antibody response, implications for oral health) of the International Symposium on the Immunoglobulin A System (Mestecky & Lawton, 1974). A summary of responses of the gastrointestinal tract is given below.

An approach commonly used to provide evidence of local immune responses is to study the levels of various intestinal Igs in normal and diseased states. For example, increased levels of IgA and IgM occur in the jejunum of patients with dermatitis herpetiformis (McClelland, Parkin, Heading, Barnetson, Warwick & Shearman, 1972). The stools of patients with Shigella sp. dysentery have increased levels of IgA but not IgG or IgM (Read & Williams, 1971) and those of convalescent cholera patients have elevated IgA levels (Waldman, Benčić, Sakazaki, Sinha, Ganguly, Deb & Mukerjee, 1971). An increased concentration of IgA does not necessarily mean that there is increased synthesis, although a technique for measuring IgA synthesis in vitro has shown increased production in biopsies from convalescent, nonbacterial gastroenteritis patients (Agus, Falchuk, Sessoms, Wyatt & Dolin, 1974), and increases in the numbers of lamina proprial plasma cells occur in patients with coeliac disease, Crohn's disease and ulcerative colitis (Baklien, quoted by Brandtzaeg, 1974 c; Savilahti, 1972; Söltoft, Binder & Gudmand-Höyer, 1973).

Although there are increases in the synthesis and concentrations of Igs in the gastrointestinal tract in response to many local infections and diseases, are these Igs specific antibodies or merely due to nonspecific stimulation of Ig synthesis? There is copious evidence with respiratory viral infections that abundant specific antibody, largely but not exclusively s-IgA, occurs in the nasopharyngeal and bronchial secretions (see Tomasi & Grey, 1972; Kilbourne, Butler and Rossen, 1973). Undoubtedly the most widely quoted studies of viral

infection at the human gastrointestinal mucosa are by Ogra and associates on poliovirus (reviewed by Tomasi & Grey, 1972; Ogra, Wallace, Umana, Ogra, Grant & Morag, 1974). They showed that similar serum antibody titres arose after either live oral or killed parenteral vaccination but that only after oral immunization did significant antibody titres occur in the nasal and duodenal secretions. Following oral vaccination, antibody in the secretions was entirely IgA. In contrast, however, serum antibody was predominantly IgG with some IgM and IgA. The s-IgA is considered protective against poliovirus by preventing the virus from crossing the gastrointestinal and nasopharyngeal mucosa into the circulation. In summary, their results show that locally produced IgA is the secretory antibody response to poliovirus and the induction of this secretory antibody is dependent upon the route of immunization and the type of viral antigen used (live versus attenuated or inactivated).

The work of Ogra et al. (1974) clearly demonstrates the dissociation between secretory and serum antibody responses which has been realized for many years but understood poorly (Tomasi & Bienenstock, 1968; Tomasi, 1971). In mice differences between oral and parenteral immunization have been elucidated using ferritin as antigen (Crabbé, Nash, Bazin, Eyssen & Heremans, 1969). Parenteral immunization stimulated production of IgM and IgG antibodies in the serum and IgA antibody producing cells in the intestine, whereas oral immunization produced exclusively IgA in the serum and IgA producing cells in the intestine. Differences between the results of Ogra et al. (1974) and Crabbé et al. (1969) in characteristics

such as the occurrence of serum antibody following oral immunization may be misleading; Tomasi & Grey (1972) emphasized that caution is necessary when comparing studies on mucosal versus systemic routes of immunization in which variations occur in the species employed and the nature and dosage of antigen used.

Bacterial infections of the gastrointestinal tract have received much study notably because of the important humon pathogens, Shigella spp. and Vibrio cholerae, which respectively cause shigellosis (a type of dysentery) and cholera. Escherichia coli has been studied also, partly because it occurs in the human large intestine throughout the world, causes infantile diarrhoea (South, 1971 b) and is suspected in the etiology of ulcerative colitis (Walker & Hong, 1973 b), but also because it is an important pathogen of piglets causing severe diarrhoea and loss of production (Porter, 1973; Porter, Kenworthy & Allen, 1974). Immunity, often considered largely due to secretory responses (Tomasi & Grey, 1972), occurs against these gut bacterial infections but what is perhaps most interesting is the immunological paradox of the indigenous bacterial flora surviving despite eliciting antibody production (Berg & Savage, 1972).

Of those bacterial infections which produce immunity, cholera is widely studied because of its unique biology. Unlike most other intestinal infections V. cholerae is completely restricted to the mucosal surface, it is never found within the gut wall or systemically and there is no obvious morphological pathology in the intestine. The pathophysiology of the disease, namely excessive intestinal secretion, is caused by cholera toxin

binding to receptors on the brush border membrane of epithelial cells resulting in their malfunction (quoted in Pierce & Reynolds, 1974). Immunity to cholera therefore is two fold: a) antitoxic, preventing the binding of toxin to the receptors and hence the intestinal malfunction and b) antibacterial, suppressing or depressing colonization of the gut by the vibrios. To be functional both antitoxic and antibacterial immunity must occur in the lumen of the intestine. This seems the ideal situation in which s-IgA should be important and suggests that Igs without specific adaptations for intestinal existence would be of little significance. Antibodies to V. cholerae are well known in intestinal secretions and are predominantly IgA although IgM and IgG occur (Northrup & Hossain, 1970; Fubara & Freter, 1972 a & b; Shimamura, 1972; Waldman, Benčić, Sinha, Deb, Sakazaki, Tamura, Mukerjee & Ganguly, 1972). Curlin & Carpenter (1970), however, concluded from experiments on isolated, perfused canine ileum that antitoxic immunity resides primarily in the circulation. Tomasi & Grey (1972) summarized their review of cholera immunity by concluding that whether locally formed antibody, serum antibody or a combination of both mediates immunity at the mucosal surface remains to be clarified. Subsequently Fubara & Freter (1972 a, 1973) have demonstrated protective immunity to cholera with s-IgA antibodies and Pierce & Reynolds (1974) have demonstrated protection using passive immunization with humoral IgG antitoxin. The conclusion of Tomasi & Grey (1972) remains justified although it seems that both systemically and locally produced antibody can function

in protective immunity to cholera. A qualification is necessary. Tomasi & Grey (1972) elsewhere in their extensive review stated "experiments designed to test the efficacy of passively-transferred serum antibody in providing gastrointestinal protection are confusing and provide no clear answers as to the significance of this route (serum to intestine antibody leakage) in the normal animal" (p.131) (explanation in parenthesis is mine). Tomasi & Bienenstock (1968) discussed this more fully and significantly noted that although passively administered antiserum quickly appears in the faeces, within a few days it is no longer detectable despite continued high serum levels of antiserum. The kinetics of antiserum appearing in the faeces are not consistent with simple diffusion from the serum.

With disease states characterized by chronic inflammation significant increases in the numbers of IgG producing plasma cells can occur locally (Tomasi & Grey, 1972; Brandtzaeg, 1973). This local production plus the abundant extravascular IgG led Brandtzaeg (1973) to suggest that IgG could act as a second line of mucosal defence by leakage into the lumen. The problem remains of the short survival of IgG in the intestine but this could be partially overcome if large quantities were entering the lumen in a short period of time. Perhaps this is a function of locally produced IgE, viz. to potentiate short term leakage of IgG into the lumen (Ishizaka et al. 1971).

In summary, there is abundant evidence of local intestinal responses to disease, immunization or infection with increased synthesis of Ig. s-IgA antibodies are

predominant in the secretions and in most cases are considered protective. Serum antibody is produced in response to intestinal stimulation and is generally IgM and IgG. This circulating antibody can enter the intestinal lumen but its significance in nature remains to be clarified. How then does antibody, regardless of its origin, provide protection in the intestinal environment?

Introductory textbooks on immunology generally have one or more sections dealing with the specific actions of serum antibody. Properties of antibodies frequently discussed include: precipitation, agglutination, opsonization, complement fixation and neutralization (e.g. Nossal & Ada, 1971). Expression of such properties results in the localization and subsequent destruction of antigen. It is important to realize that many functions of antibody do not destroy antigen directly but simply identify it so that other components such as the phagocytic cells or complement system can deal specifically with it - antibody molecules provide recognition. Another important albeit inadequately understood function of serum antibody is regulation of immune responses. The potential complexity of this function is discussed in Jerne (1973, 1974). The question of relevance in this thesis is: what are the specific actions of s-IgA or serum antibody in the intestinal lumen?

If intestinal antibody serves to label antigen, as is a common function of serum antibody, what is the effector mechanism which recognizes the resulting antigen-antibody complex? A priori it seems unlikely to be

phagocytic cells which have migrated into the lumen engulfing the complexes, although this suggestion appears in the literature and requires further investigation (Mestecky & Lawton, 1974, p.311). s-IgA agglutinates efficiently and may opsonize antigen for phagocytosis (Bienenstock, 1974) but is phagocytosis necessary in the gut as it is in the serum? There is considerable agreement that although complement may occur in the secretions the intestine is anticomplementary and accordingly complement fixation does not occur there (Mestecky & Lawton, 1974, see index re complement). Despite some conflicting reports, the majority of evidence shows that neither serum IgA nor s-IgA can fix complement (Tomasi & Grey, 1972; Bienenstock, 1974; Boackle, Pruitt & Mestecky, 1974; Colten & Bienenstock, 1974). However, aggregates of serum IgA or s-IgA produced by lyophilization or evaporation do interact with complement (Boackle et al. 1974) apparently by the alternate rather than classical pathway (Mayer, 1973). Whether this has significance in vivo remains to be determined. What may be important is that Adinolfi, Glynn, Lindsay & Milne (1966) demonstrated bacteriolytic action of s-IgA, but not serum IgA, in the presence of complement and lysozyme. In a paper which is difficult to follow, Burdon (1973) demonstrated bactericidal action of both serum IgA and s-IgA when various components of complement and lysozyme were in the medium. Recently Hill and Porter (1974) have confirmed that s-IgA, but not serum IgA, is bactericidal in the presence of complement and lysozyme. The role of complement, particularly in collaboration with lysozyme, in the intestine may yet provide a fruitful area of research.

The best understood role for s-IgA is the inhibition of adherence of bacteria to the mucosal surface (Williams & Gibbons, 1972; Freter, 1972; Fubara & Freter, 1973; Gibbons, 1974; Genco, Evans & Taubman, 1974). As adherence is essential for successful colonization (Gibbons, 1974), its prevention is protective. With Streptococcus mutans, adherence is a property of polymers of sucrose produced by glucosyltransferases. Genco et al. (1974) have shown that s-IgA antibodies inhibit these enzymes and hence probably have elucidated the specific immunological action which inhibits adherence in this species.

Walker and his associates have conducted a series of studies on the absorption of soluble antigens from the intestine (reviewed by Walker & Hong, 1973 a; Walker et al. 1974). In orally immunized animals antigen uptake is reduced; and similar reduction in uptake can occur after parenteral immunization using large doses of antigen. Walker et al. (1974) showed that this reduction was due to enhanced degradation of the antigen within the lumen. The hypothesis which these authors are investigating is that s-IgA combines with soluble antigen in the lumen preventing its uptake and leading to enzymatic degradation. If true, this phenomenon has some important consequences. For example, whether or not the IgA system has immunological memory. This matter has received much discussion (Dayton et al. 1971, p.417); Hobbs (1971) quoted studies showing long lasting memory and Gerbrandy & van Dura (1972) showed a clear secondary s-IgA response, whereas Heremans (1974) and Porter, Kenworthy, Noakes & Allen (1974) showed a lack of secondary responses. The lack

of a clear secondary s-IgA response could be due to depressed antigen uptake in the immunized animal, a situation which Heremans (1974) called 'negative memory', the existence of antibody prevents a detectable secondary response and hence can be considered regulatory. This 'negative memory' phenomenon could explain the partial tolerance or refractory phase identified by some authors following oral immunization (Thomas & Parrott, 1974; André et al. quoted by Heremans, 1974). Closely associated with this 'negative memory' function of s-IgA is the proposal that the normal flora and food antigens are blanketed with s-IgA. Thus it is suggested that the flora and food antigens would be protected from other immune responses, such as complement fixing antibodies, which would be detrimental to the flora or to the host (Beale, Douglas, Parish & Hobbs, 1971; South, 1971 b). Other possible examples of s-IgA 'blocking antibodies' are given in Tomasi & Grey (1972) and Bienenstock (1974).

Unlike antibody action in the serum which is largely a recognition function, it seems that antibody in the intestinal lumen can, or perhaps even must, act without the subsequent intervention of complement or phagocytes. Antigenic material neutralized, agglutinated or prevented from adhering to the mucosa would not 'clutter' the gut as it would the serum; it would be quickly disposed of by proteolysis or the peristaltic flow of the intestinal contents. Thus phagocytosis is probably not an essential function of the immune system of the intestine.

It is obvious from the above summary of intestinal immune responses that since the initial

demonstration in the early 1960's of the predominance of IgA in secretions there has been rapid progress in characterizing the immunological potentials of mucosal-associated tissues. There is, however, an embarrassing lack of information on the actions of cell-mediated responses. Waldman & Ganguly (1974) reviewed the information available which largely has originated from studies on respiratory infections. There are local cell-mediated responses which differ from systemic responses, just as local antibody responses differ from systemic ones. Dr. Robert Good concluded at the International Symposium on the IgA System that "cell-mediated immunity is a very important component of the local immunity system" (Mestecky & Lawton, 1974, p.311). Recently described techniques for the isolation of intestinal proprial cells will make studies to elucidate the role of this component a less formidable task (Castro, Roy & Stockstill, 1974; Rudzik & Bienenstock, 1974).

2. Immunity to intestinal helminth parasites

Considerable effort has been spent recently in attempting to elucidate the mechanisms of immunological rejection of helminth parasites. Within vertebrate hosts some helminths lead a solely parenteral existence, some a solely enteral existence, many spend time in both environments and still others have intimate contact with the lamina propria or deeper tissues of the intestinal wall while part of their body is also in the lumen. It is self-evident that the protective immune response must act directly on the parasite *in situ* or indirectly by making the environment unsuitable for continued survival.

Thus the simplest hypothesis regarding protective immunity is that, regardless of what environment parasites inhabit, the protective mechanisms are essentially similar. The alternative is that protective immune responses to intestinal parasites are different from those effective against parenteral parasites. This difference could be due to the stimulation of different lymphoid tissues with specific adaptations to produce effector units capable of acting in the intestinal lumen rather than in the tissues or circulation. Given our current concepts on intestinal immunity (see above) it seems extremely likely that some dissociation must occur between protective responses to parenteral and enteral helminth parasites, and that s-IgA has some function in the relationship between intestinal helminths and their hosts.

Herein, immune responses to enteral and parenteral helminths will not be extensively reviewed as recent reviews are plentiful (Valdivieso & Tamsitt, 1970; Ogilvie & Jones, 1971, 1973; Murray, 1972 a; Soulsby, 1972; Kelly, 1973; Larsh & Weatherly, 1974; Ogilvie, 1974 a; Ogilvie & Love, 1974; Soulsby, 1974). Nippostrongylus brasiliensis is the most extensively studied model of helminth immunity and within the rat definitive host the larvae which have entered by penetrating the skin migrate to the lungs and ultimately are swallowed and become adults in close association with the jejunal villi. Presently it is accepted that rejection of N. brasiliensis from the rat intestine involves antibody-mediated damage and lymphoid and myeloid cell components (see ADDENDUM, SECTION 2). From passive transfer experiments it has been concluded that IgG_1

antibody is of major importance, but IgG_2, IgM and circulating IgA may be involved; IgE apparently is not essential for protection (Jones, Edwards & Ogilvie, 1970). These authors qualified their work by saying that although serum IgG_1 may be important in passive protection, active immunity may involve other antibody.

IgA antibody has not been demonstrated in N. brasiliensis infections, however, and currently it is not thought to be important in the protective responses to this nematode. Anti-worm antibodies have been demonstrated in intestinal extracts in other nematode infections (Douvres, 1962; Dobson, 1967) but their Ig class is not known. Weinmann (1966) showed that intestinal extracts from immune mice were more active in vitro against Hymenolepis nana than control intestinal extracts but did not identify any specific antibody in the extracts. Rose (1972) reported some preliminary work on Eimeria spp. infections of chickens in which caecal Ig preparations may be protective but like previous workers she did not demonstrate specific antibodies in the preparations. Zinnerman & Kaplan (1972) showed, however, a correlation between infection with another protozoan, Giardia lamblia, and reduced intestinal s-IgA levels and argued that patients with depressed s-IgA levels were predisposed to such intestinal infections. Crandall, Cebra & Crandall (1967) showed both IgA and IgG in gut extracts of rabbits infected with Trichinella spiralis but specific antibody was detected only as IgG; specific IgG and IgM antibodies were detected in the serum also. With T. spiralis infections of mice Crandall & Crandall (1972) showed approximately similar levels of IgA, IgG_1

and IgM antibodies in the serum, but in the gut predominantly IgA antibody with some IgG_1 antibody occurred. In mice infected with Heligmosomoides polygyrus (= Nematospiroides dubius) IgA, IgG_1, IgG_{2a} and IgM antibodies occurred in the serum but only IgG_1 antibodies were detected in the intestine (Crandall, Crandall & Franco, 1974). IgA and IgG_1 antibodies against Trichostrongylus colubriformis occur in the serum of infected sheep (Rothwell & Merritt, 1974). Therefore, although IgA antibodies have been demonstrated in some helminth infections, there is no evidence that these antibodies are of any significance in passive or active immunity. But, it has not been demonstrated that intestinal antibodies and specifically s-IgA are not of significance, and hence because of their known importance in procaryotic infections further research is needed on this aspect of helminth infections.

However, rather than continue attempts to passively transfer immunity against intestinal helminths with serum antibody, the characteristics of the immune response within the intestinal lumen and wall must be investigated more fully. Studies localized upon the intestine have included those on mast cells and antibody leakage (Murray, 1972 a & b) and localized eosinophilia (Ismail & Tanner, 1972); the demonstration of successful adoptive transfers only with mesenteric lymph node cells (MLNC) and not spleen cells (Selby & Wakelin, 1973); studies on lymphoid cell proliferation in the Peyer's patches, mesenteric lymph nodes and the lamina propria of the intestine (Dobson & Soulsby, 1974); studies on the migration of lymphoid cells to the intestine (Dineen,

Wagland & Ronai, 1968; Dineen, Ronai & Wagland, 1968; Parrott & Ferguson, 1974); and studies on worm expulsion from intestines infused with histamine and 5-hydroxytryptamine (Rothwell, Prichard & Love, 1974). With the approach of concentrating on the intestine in mind the present study was begun.

3. The objective, and approaches used in the study

Despite the convincing arguments that local immune responses, as distinct from systemic, and particularly s-IgA cannot be excluded from consideration of protective immunity to intestinal helminths, there is very little published work on this aspect of the host responses. Therefore, the major objective of this portion of the study was to acquire information which would support or refute the involvement of local immune responses. *H. diminuta* appeared to be an excellent model because of its lack of obvious mucosal damage (Turton, 1968) and its totally enteric existence, eliminating the complication of a parenteral migration which would probably influence the subsequent immune responses to the intestinal worm.

At the time, the logical starting point was to determine the extent to which the intestinal and serological responses were dissociated throughout the course of an infection. Using the single radial immunodiffusion (SRID) technique of Mancini, Carbonara & Heremans (1965) levels of IgA, IgG_1, IgG_2 and IgM in the serum were studied as were levels of IgA in the intestinal contents (other Igs were not detected by immunodiffusion, see below).

To provide further information about possible local immune responses the distribution and relative abundance of Igs in the intestines of mice was studied. Direct anti-globulin immunofluorescence was used to study IgA, IgG_1, IgG_2, and IgM and later the third component of complement (C_3). For comparative purposes, infections of H. diminuta in mice immunodepressed with cortisone and in untreated mice, and infections of H. microstoma in mice were studied. Unlike H. diminuta which often destrobilates and is expelled quickly from mice, H. microstoma seldom destrobilates (Howard, personal communication) and survives well in mice despite causing a chronic inflammatory response in the bile duct where its scolex attaches. It was of interest to determine if Igs could be detected associated with the worms in vivo as was reported briefly by Coleman (1961), Coleman & Fotorny (1962) and Coleman, McMorrow & Fimian (1963) for antibodies to H. nana or whether differences occurred between the various Hymenolepis spp. in this respect.

MATERIALS AND METHODS

1. Immunodiffusion

a) collection of serum

For collection of serum from mice or rats the animal was heavily anaesthetized with ether, the pleuropericardial cavity was exposed by removing the sternum and the major vessels entering and leaving the heart were severed. Blood collected in the cavity and was transferred to centrifuge tubes using a Pasteur pipette. In the tubes the blood coagulated and after standing at room temperature for 1-4 h it was centrifuged (1100 g, 30 min, 4°C), the serum collected and stored in bijou bottles at -20°C. Occasionally coagulated blood was stored in the refrigerator overnight prior to separation and storage of the serum. In all cases the sera from mice of a group were pooled for study.

b) collection of intestinal contents

The satisfactory collection of intestinal contents from small animals such as mice presents considerable difficulty and many methods have been used. Herein, the method is a combination of other people's with some original components. In man, wide and unpredictable variation in the concentration of Igs in intestinal aspirates occurs following a meal or the administration of the gastrointestinal hormones, secretin and pancreozymin, which are normally produced following a meal (McClelland, Warwick & Shearman, 1973; Samson,

McClelland & Shearman, 1973). Hence, in the present studies mice were fasted 18-24 h (water provided ad libitum) prior to collection of their intestinal contents. To minimize coprophagy during fasting the mice were maintained in cages with wire screen bottoms through which the majority of faeces would pass.

Mice were weighed, anaesthetized and killed by exsanguination. Immediately thereafter the entire small intestine (pylorus to ileo-caecal junction) was removed and the cut ends closed with 3.5 cm Blalock clamps to prevent loss of contents. The intestine thus sealed was washed in cold 0.85% NaCl to remove peritoneal fluid on the serosa, which otherwise could contaminate the samples with extraintestinal Igs. The cleaned, chilled gut was cut into two approximately equal lengths. The first was opened longitudinally, placed on a clean working surface with the mucosa uppermost and then gradually drawn under a vertical glass slide (7.5 x 5 cm) which scraped off the mucosa and intestinal contents and collected them on its posterior surface. From here they were rapidly transferred with forceps to a graduated centrifuge tube maintained in iced water. To secure the glass slide and ensure that a relatively constant pressure was applied for scraping the mucosa and contents from each gut, the slide was supported from above within a groove of an inverted metal staining tray. The tray was secured to a retort stand by two clamps and a bosshead. Fig. 5 - 2 shows the apparatus. The second section of each gut was scraped immediately after the first.

The samples from all mice in a group were pooled and mixed well by shaking, after the volume of the pool

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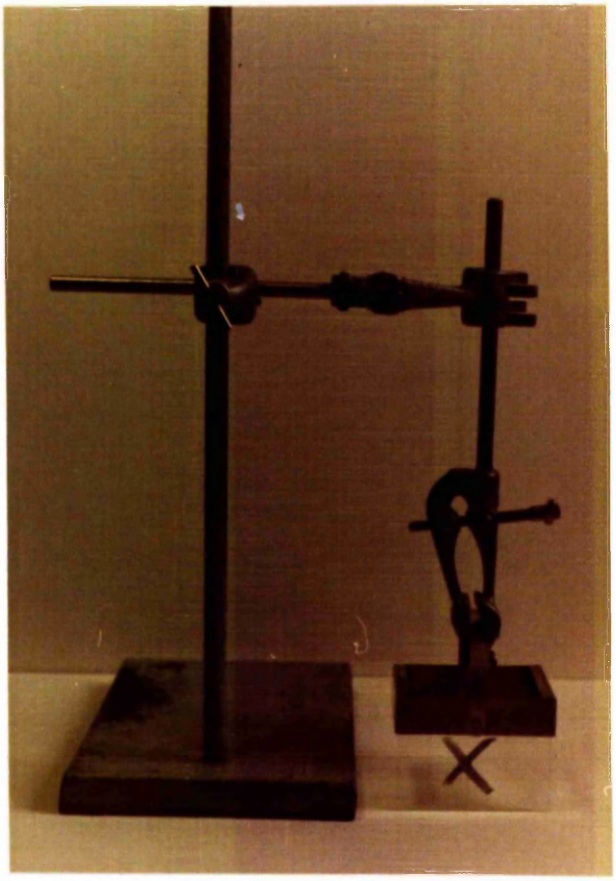
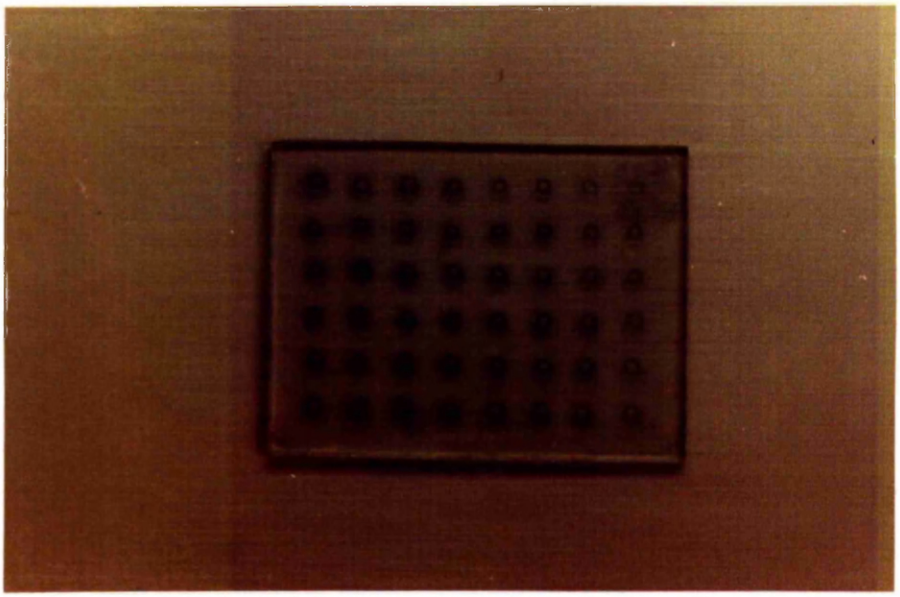
Figure 5 - 2

Apparatus used for scraping the mucosa and contents from the small intestine of mice; the glass slide suspended from the inverted staining tray is marked (X).

Figure 5 - 3

A dried and stained SRID plate showing the pattern of wells and precipitation rings surrounding each well.





had been increased to 1.0 ml/mouse with cold barbitone buffer (pH 7.0; Dawson, Elliott, Elliott & Jones, 1969, p.492). To inhibit proteolytic action of the intestinal contents and hence the possibility of enzymic degradation of Ig, the samples were incubated at 56°C for 30 min in a water bath (Plaut & Keonil, 1969). The samples were then centrifuged at 1100 g, 4°C, for 30 min and the supernatant filtered through a No. 2 Gooch crucible and stored at -20°C in bijou bottles.

c) Hymenolepis diminuta antigen

This antigen preparation was used for double immunodiffusion (DID) studies only. Ten H. diminuta were recovered from two female rats (each weighing approximately 240 g) given six cysticercoids (6c) 35 days previously. The worms were rinsed in cold 0.85% NaCl, blotted and then mixed with three volumes of cold 0.85% NaCl. In this solution they were homogenized initially in a blender and subsequently in a ground glass tissue homogenizer. The resultant milky solution was ultracentrifuged at 100,000 g for 1 h and the supernatant divided into aliquots in 1 ml polythene specimen containers and stored at -20°C. Once thawed this antigen was not refrozen but stored in a refrigerator and used within a week. The protein concentration of the antigen was 16.8 mg/ml, measured by quantitative ultraviolet absorption (280 nm) and compared with a standard of bovine serum albumin.

d) antisera

In all cases sera tested for anti-H. diminuta antibodies by DID were from mice or rats infected by

administering cysticercoids. For studies employing SRID, specific anti-mouse IgA, IgG_1, IgG_2 and IgM antisera produced in goats (Meloy Laboratories, Inc., U.S.A.) were purchased from the British distributor, Flow Laboratories, Irvine, Scotland. An outline of the procedure for the production of these antisera is given in a booklet provided by Meloy. The mouse serum IgA used for producing the antiserum in goats was the 11S fraction from a Sephadex G 200 column (information courtesy of the Manager of Quality Assurance, Meloy).

On one occasion anti-mouse IgM serum produced in rabbits by Nordic Immunological Laboratories, The Netherlands was purchased from Sera Service Ltd., Berkshire and tested for SRID, as it was less expensive per ml than Meloy antiserum. However, it required about 10 fold more of this cheaper antiserum to get a reaction equivalent to that of the Meloy antiserum. Hence, in the long term the Meloy antiserum was the more economical. All commercially prepared antisera used for immunodiffusion were stored in the refrigerator until used.

e) stock agar

Agar for immunodiffusion plates was prepared by boiling 3% (wt/vol) Ionagar No. 2 (Oxoid, London) with 0.02% (wt/vol) of the preservative sodium azide (NaN_3) in barbitone buffer (pH 8.2; Godfrey & Coulombe, 1970). Agar which was still molten was used for the preparation of plates or alternately the molten agar was placed in 28 ml universal containers and stored in the refrigerator until needed. Normally this agar was melted only once as repeated melting and solidification affects the

diffusion properties of an agar solution (Oudin & Williams, 1971).

f) double immunodiffusion (DID)

Using a warmed pipette, glass plates (7.5 x 2.5 to 7.5 x 5.0 cm) were covered with a layer of molten agar (2.5-5.0 ml) which had been diluted from the stock agar to 1.5% agar and 0.01% NaN_3 using the barbitone buffer (pH 8.2). Once the agar had solidified, wells were cut and the plugs removed by suction. The distribution and size of the wells varied from one preparation to the next depending upon the material under study. The wells were filled with appropriate antigen or antisera and the plates allowed to develop for 24-96 h in a humid chamber at room temperature.

g) single radial immunodiffusion (SRID)

Mancini et al. (1965) described in detail the technique of SRID and showed that it could be used to quantify the antigen present in a sample. The method was later simplified by Hobbs (1970). The technique, as described below, incorporates aspects from both these descriptions.

i) preparation of antiserum-agar plates

To facilitate the preparation of the antiserum-agar mixture and its subsequent transfer to the glass plate molten agar, buffer, test tubes and pipettes were warmed in a water bath maintained at 56°C. To 4.5 ml of the molten stock agar 4.32 ml of barbitone buffer (pH 8.2) and 0.18 ml of the appropriate antiserum were added, producing 9.0 ml of 1.5% agar with 0.01% NaN_3 and 2% antiserum. The mixture was shaken well and with a 10 ml

pipette about 8.5 ml was poured evenly over a clean 9 x 7 cm glass plate. The plate was on a level surface so the agar covered it to a near uniform thickness.

ii) cutting the wells

Once the agar had solidified, wells were cut using a fine aluminium tube (o.d. 2.1 mm) with an inside bevel, and the agar plugs were removed by suction. On all plates the well size and distribution was identical. Borders of 1 cm were left on the four edges and wells were cut 1 cm apart using graph paper as a template. When completed each plate had 48 equally spaced wells comprised of six rows and eight columns (Fig. 5 - 3).

iii) filling the wells

Mancini et al. (1965) placed 2 µl of antigen solution in each well but Hobbs (1970) filled each well to exactly level and argued that "where there are minor flaws ... in the thickness of the agar, filling to the brim compensates for test and antiserum alike whereas a constant volume in the well cannot allow for varying depths of well" (p.5). Hence, all wells were filled to the brim, with care not to overfill or under fill which would produce inaccuracies. A new 5 µl disposable microcapillary tube (Drummond Scientific Co., U.S.A.) was used with a bulb dispenser for each sample. Appropriate dilutions of samples in barbitone buffer (pH 7.0) were prepared using 0.5-1.0 ml pipettes and 10 and 20 µl disposable micropipettes (Becton, Dickinson & Co., U.S.A.) with suction tubes. Care was taken not to contaminate samples with used pipettes. Each sample was applied to three (first experiment) or four (second experiment) adjacent wells on a plate and in both experiments each

plate was duplicated 1-12 weeks after the first was completed. For the two experiments a total of 50 plates were prepared. The samples were refrozen between setting up the two plates, and therefore, as the DID and SRID plates were prepared at the same time, the samples were thawed only twice and each time for less than 4 h. Once the wells were filled the plates were developed for 72 $\pm$ 12 h at room temperature in a humid chamber.

iv) reference serum

When the study commenced no commercial source of mouse Igs of known concentration existed, so a reference serum was prepared with which all samples could be compared. This reference serum was collected from 100 CFLP male mice, 2 star category, 42 days old. After overnight refrigeration, the serum was placed in 0.5 ml aliquots in polythene specimen containers and stored at -20^{o}C until needed. Once an aliquot of serum was thawed it was completely used the same day, any excess was discarded. On each SRID plate 4-10 wells were filled with appropriate dilutions of reference serum.

During the study a commercial source of known concentrations of mouse Igs became available (Meloy). The reference serum previously prepared was compared with this commercial standard and the concentrations of IgA, IgG_1, IgG_2 and IgM were determined according to Hobbs (1970), and the values for all samples in the experiments were calculated. The commercial standard contained (mg/dl): IgA 447, IgG_1 133, IgG_2 597 and IgM 111. The IgA in the standard was a 7S molecule (information courtesy of Quality Assurance Manager, Meloy). The reference serum contained (mg/dl): IgA 24.5, IgG_1 175.0,

IgG_2 227.5 and IgM 25.5.

v) reading plates

Once the precipitation rings were stained (described below; see Fig. 5 - 3) their diameter was determined using an ocular micrometer on a dissecting microscope at X 6 magnification.

vi) analysis of data

From the seven wells on the two plates which had been filled with a given sample the mean precipitation ring diameter was determined together with the coefficient of variation (Colquhoun, 1971). This mean diameter was compared with the mean diameter of the reference serum precipitates from the two plates and expressed as a % of the reference serum. Ultimately when the concentrations of the Igs in the reference serum were determined from comparisons with the commercial standard, the mg/dl of the Igs in each sample were determined. Figures were prepared showing the changes in Ig concentrations with time in serum and gut samples from infected and uninfected mice.

h) washing and staining immunodiffusion plates

When the specified development time for the immunodiffusion plates was complete they were gently rinsed with tap water, covered with moistened filter paper and allowed to dry overnight. Then they were washed in changes of 0.85% NaCl for about 24 h and subsequently stained with 0.1% amido black in 1% acetic acid for 10-30 min (Damian, 1970). Excess stain was removed by gentle rinsing in running tap water for up to 30 min and then the plates were air dried.

2. Immunofluorescence

a) preparation of tissues

Prior to staining with immunofluorescent reagents the tissue was prepared either by: a) immediate fixation with subsequent freezing and cryostat sectioning or b) immediate freezing and cryostat sectioning followed by fixation. When the tissue was to be fixed first the method of Eidelman & Berschauer (1969) was used. A mouse was killed by cervical dislocation and, following closure of the cut ends with 3.5 cm Blalock clamps, the small intestine was removed quickly and placed in cold 10% formalin buffered to pH 7.0 (Godfrey & Coulombe, 1970). A total of 1 ml of the cold fixative was injected into the lumen in small quantities at intervals along the intestine to hasten fixation. After about 5 min of initial fixation the intestine was laid on a measuring board and 1-2 cm long sections were removed from desired areas of the gut to bijou bottles containing the cold neutral buffered formalin and fixed for a further 4 (3.5-10) h in the refrigerator. If the mouse to be studied was infected with H. diminuta the gut sections were normally from 10-20 cm from the pylorus and were selected so that pieces of worm were present in the lumen. If, however, the mouse was infected with H. microstoma, the hypertrophied bile duct containing the worms, as well as jejunal sections with worms, were fixed for further study. For mice which were uninfected, bile duct or intestinal sections were selected as appropriate for comparison with infected mice. Rats infected with H. diminuta were treated similarly to mice for the fixation of pieces of intestine with worms in situ.

At the end of the fixation period the formalin was replaced with cold 30% sucrose in deionized water and the specimens were refrigerated overnight (7-18 h). Eidelman & Berschauer (1969) claimed that the tissue was ready to be frozen now, but it was found that a wash of about 1 h in 0.85% NaCl made sectioning easier and improved the subsequent fluorescence. Following this wash the tissue was frozen onto a metal chuck for sectioning in the cryostat following in part Johnson & Holborow (1973). To facilitate adherence of the tissue to the chuck a small piece of filter paper was frozen to the prechilled chuck with a drop of water. A drop of OCT embedding medium (Ames Co., U.S.A.) was placed on the filter paper and the tissue supported appropriately in the medium to allow transverse sections to be cut. Two blocks of solid CO_2 were held against the chuck to hasten the freezing of the tissue and additional OCT medium was gradually added to support the tissue for subsequent sectioning. The chuck with tissue was placed in the freezing cabinet (-25°C) of the cryostat (Slee of London) and allowed to freeze thoroughly for 30 min prior to sectioning. Sections were cut at 6 μm thickness and attached to 76 x 25 mm glass slides previously coated with a thin layer of glycerin-albumen (Gurr Ltd., London) (only slides 1.0-1.2 mm thick were used as the high power Leitz darkfield condenser (D 1.20 A) which was used for some of the microscopy has a short focal distance and thicker slides made proper focusing of the condenser impossible). Prior to staining the sections, they were air dried for at least 30 min.

If the tissue was to be fixed after sectioning

rather than before, the mouse was killed, its intestine removed and 1-2 cm sections were immediately frozen in OCT medium on cryostat chucks as described above. Every effort was made to freeze the tissue as quickly as possible once the mouse was killed to minimize alterations in the in vivo distribution of Ig and tissue morphology. After freezing for at least 30 min sections were cut as above, attached to slides (glycerin-albumen not required), air dried and fixed in 100% methanol (AnalaR) for 10-15 min. They were air dried again prior to staining.

b) staining procedures

i) antisera

The sources, characteristics and working dilutions, in phosphate buffered saline (PBS) pH 7.2 (Godfrey & Coulombe, 1970), of the antisera conjugated with fluorescein isothiocyanate (FITC) used in this study are given in Table 5 - 1. For one of the controls used in direct anti-globulin immunofluorescence (see below) analogous antisera from the same producers but not conjugated with FITC were used. Rabbit anti-rat (RAR) IgG and RAR C_3 (β_1C globulin) produced and supplied by Mercia Diagnostics Ltd., Watford were the antisera (working dilutions 1:10) besides FITC swine anti-rabbit (SAR) IgG used for indirect anti-globulin immunofluorescence.

ii) general procedure and controls

For direct anti-globulin immunofluorescence, sections on slides were covered with an appropriate dilution of FITC conjugated antiserum and incubated at room temperature in a humid chamber prepared by placing

Table 5 - 1

Fluorescein isothiocyanate (FITC) conjugated antisera used for immunofluorescence

Antisera	Producer	British Distributor	Protein Concentration mg/ml	FITC/Protein	Dilution
Direct anti-globulin immunofluorescence					
GAM* IgA	Meloy Lab. Inc. U.S.A.	Flow Lab. Irvine	17.0	4.0	1:20
GAM IgG_1	"	"	10.0	3.1	1:10
GAM IgG_2	"	"	10.0	3.2	1:10
GAM IgM	"	"	17.8	4.9	1:10
RAM C_3	Nordic Immunological Lab., Netherlands	Sera Service Ltd., Maidenhead	18.5	1.2	1:5
RAM C_3	Cappel Lab. Inc. U.S.A.	Dynatech Lab. Ltd., Billingshurst	10.0	2.0-4.0	1:5
Indirect anti-globulin immunofluorescence					
SAR IgG	Dako-immunoglobulins Denmark	Mercia Diagnostics Ltd., Watford	NA	2.3	1:50

*Abbreviations: GAM-goat anti-mouse, RAM-rabbit anti-mouse, SAR-swine anti-rabbit, NA - information not available.

moistened filter paper in a petri dish (14 cm diameter) with cover. After 30 min the antiserum was washed off the sections with PBS using a Pasteur pipette followed by a prolonged wash (30-150 min) with the PBS in a Coplin staining jar. Following this wash the sections were covered with 90% glycerol in PBS, and a coverslip, and were ready for viewing. To determine if the fluorescence observed was specific, control sections were used as follows: a) treatment with conjugated antiserum was eliminated from the above procedure; b) initial 30 min treatment with neat analogous nonconjugated antiserum (excess washed off) prior to treatment described above, a 'blocking control'; and c) treatment of comparable sections with conjugated antiserum of differing specificity. When the specificity of the staining procedure with each antiserum was established, controls were used only irregularly as the examination of sections from infected and uninfected mice with the variety of antisera required considerable time. The 'blocking control', (b) above, never entirely obliterates the specific staining but markedly reduces its intensity (cf. Rubin, Fauci, Sleisenger & Jeffries, 1965; Johnson & Holborow, 1973) although this is seldom stated in publications.

Indirect anti-globulin staining of sections of rat intestine with H. diminuta in situ involved an incubation for 30 min with either RAR IgG or RAR C_3 nonconjugated antiserum followed by a 30 min PBS wash, and then incubation with conjugated SAR IgG antiserum and wash and mount as above.

c) microscopy and photography

For most of the study a transmitted-light illuminating system was used but near the completion an incident-light Ploem illuminating system was available (see Koch, 1972). The results obtained were independent of the illuminating system used.

A Leitz Orthoplan microscope fitted with a 75 W xenon high pressure lamp in a 100Z Leitz lamp housing, mounted from the side or behind the microscope, was used in the transmitted-light fluorescent microscopy. With the movement of a mirror the specimens could be exposed to white light for normal observation, or blue light for excitation of the FITC to produce fluorescence. Two filter combinations were used for transmitted-light fluorescence: a) BG 12, 3 or 5 mm excitation filter and K 510 suppression filter or b) BG 38 red suppression filter, GG 475 edge filter, FITC KP 490 selective excitation filter and K 530 suppression filter. A heat filter was in the lamp housing with both combinations. Filter combination (a) produces broad-band excitation whereas combination (b) gives selective excitation (Koch, 1972). The latter gives superior fluorescence and once the FITC KP 490 filter was purchased, selective excitation, (b), was used routinely. A darkfield condenser of either low power or high power, as appropriate, was used.

The Ploem incident-light fluorescent system (Koch, 1972) was fitted on a Leitz Ortholux I microscope with a 50 W ultra high pressure mercury lamp in a Leitz 100Z lamp housing. Apart from the BG 38 red suppression filter, the filters are built into the Ploem system and

were set for FITC fluorescence as instructed by Leitz. A K 530 suppression filter was used also.

A Leitz Orthomat photographic system was used with the transmitted-light fluorescent system. With the Ploem system, however, a Wild Photoautomat was used. The films used were: for black and white, Ilford HP 4 or Kodak Tri X both ASA 400; for colour, GAF Anscochrome ASA 500. The colour prints were produced by dying black and white prints prepared from the above black and white and colour films.

d) collection of data

Immunofluorescence is an useful method and presents little difficulty when differences are qualitative. If, however, quantitative differences are suspected, caution is required to assess these objectively. To standardize the recording of data a data sheet was prepared with space for a description of fluorescence on the worm surface, in the gut lumen, in the lamina propria of the villi and between the crypts of Lieberkühn, and associated with the muscle layers of the gut. The sheet included a diagrammatic sketch of a transverse section of intestine with a worm in the lumen on which the distribution and relative intensities of the fluorescence could be recorded in coloured pencil.

To determine the abundance of various types of plasma cells in the lamina propria, cells with specific cytoplasmic fluorescence and lying between the crypts and the villus immediately above (a villus-crypt unit; Jarrett, Jarrett, Miller & Urquhart, 1968) were counted. Five units were counted per slide; oblique sections were not counted.

RESULTS

1. Immunodiffusion

Experimental design:

For the first experiment 6 week old CFLP male mice graded 2 star were purchased from Anglia Laboratory Animals. The experiment, designed as shown below, commenced 2 days later:

day 0,

- -kill six mice collect serum and gut samples
- -infect 108 mice with 6c of H. diminuta (1)
- -sham infect 78 mice (2)

days 5, 8, 10, 12, 15, 20,

- -kill six mice from each of groups (1) and (2) and collect serum and gut samples

day 21,

- -give Zanil to 36 mice of group (1)

day 26,

- -infect the mice given Zanil day 21 with 6c of H. diminuta (3)
- -kill six mice from groups (1) and (2) and collect samples

days 31, 34*, 36, 41, 46, 51

- -kill six mice from groups (1), (2) and (3) and collect samples (*group (1) mice excluded).

The second experiment commenced 3 days after the 6 week old 4 star CFLP male mice arrived from Anglia Laboratory Animals. The experimental design was:

day 0,

-infect 68 mice with 6c of H. diminuta (1)

-sham infect 60 mice (2)

day 1,

-kill 10 mice not treated on day 0

days 5, 8, 11, 13, 18, 25, 30, 35, 40, 45,

-kill six mice from groups (1) and (2) and collect serum and gut samples

day 8,

-kill eight mice (1) and recover worms to establish if infection was successful; recovery was 81% of administered worms, hence the infection established well.

Serum and gut samples from both experiments were studied by DID and SRID.

a) DID (double immunodiffusion)

Not unexpectedly when commercially available goat antisera to specific mouse Igs were diffused against serum samples, IgA, IgG_1, IgG_2 and IgM were detected in all cases. When the same antisera were tested against gut contents from infected and uninfected mice, however, only a single line representing IgA was present in all cases; the other Igs were never detected. All the serum and gut samples from both experiments were tested for precipitating anti-H. diminuta antibodies using the homogenized antigen preparation but none was found.

In an exploratory experiment using homebred CFHB male rats about 8 week old infected with 6c of H. diminuta, apparently single, broad but faint precipitation lines occurred on the unstained plates between wells containing the H. diminuta antigen and intestinal contents

collected from infected rats days 10, 15, 23 and 29 post infection. Such lines did not occur with serum samples or uninfected intestinal contents. The significance of these lines is uncertain as when the plates were routinely stained with amido black (two separate preparations) the lines apparently disappeared. Unfortunately this experiment was not repeated.

b) SRID (single radial immunodiffusion)

As with DID, only IgA was detected in the intestinal samples from infected and uninfected mice using SRID, and its concentration ranged in the two experiments from about 24-37 mg/dl (Figs. 5 - 4 a & b). In the serum samples the ranges of concentrations of Igs (mg/dl) were approximately: IgA 21.5-34.5, IgM 23-32 (Figs. 5 - 5 a-d), IgG_1 110-250 and IgG_2 150-410 (Figs. 5 - 6 a-d). On the 50 SRID plates from the two experiments the mean coefficient of variation (a measure of the reproducibility of the technique in my hands) was 2.5% (range 0-11.7%), a value similar to that of Hobbs (1970).

In one experiment the levels of IgA in the intestine of infected mice (O) were greater (or equal) than in uninfected mice (●) on days 5-20 post infection and on a total of 10 of the 12 days studied up to day 51 (Fig. 5 - 4 a). The levels of intestinal IgA in mice given a secondary infection (X) showed no consistent pattern in relation to the levels in the other two groups. In the other experiment (Fig. 5 - 4 b) secondary infections were not studied and in contrast to the first experiment no consistent differences occurred in the levels of intestinal IgA in infected and uninfected mice.

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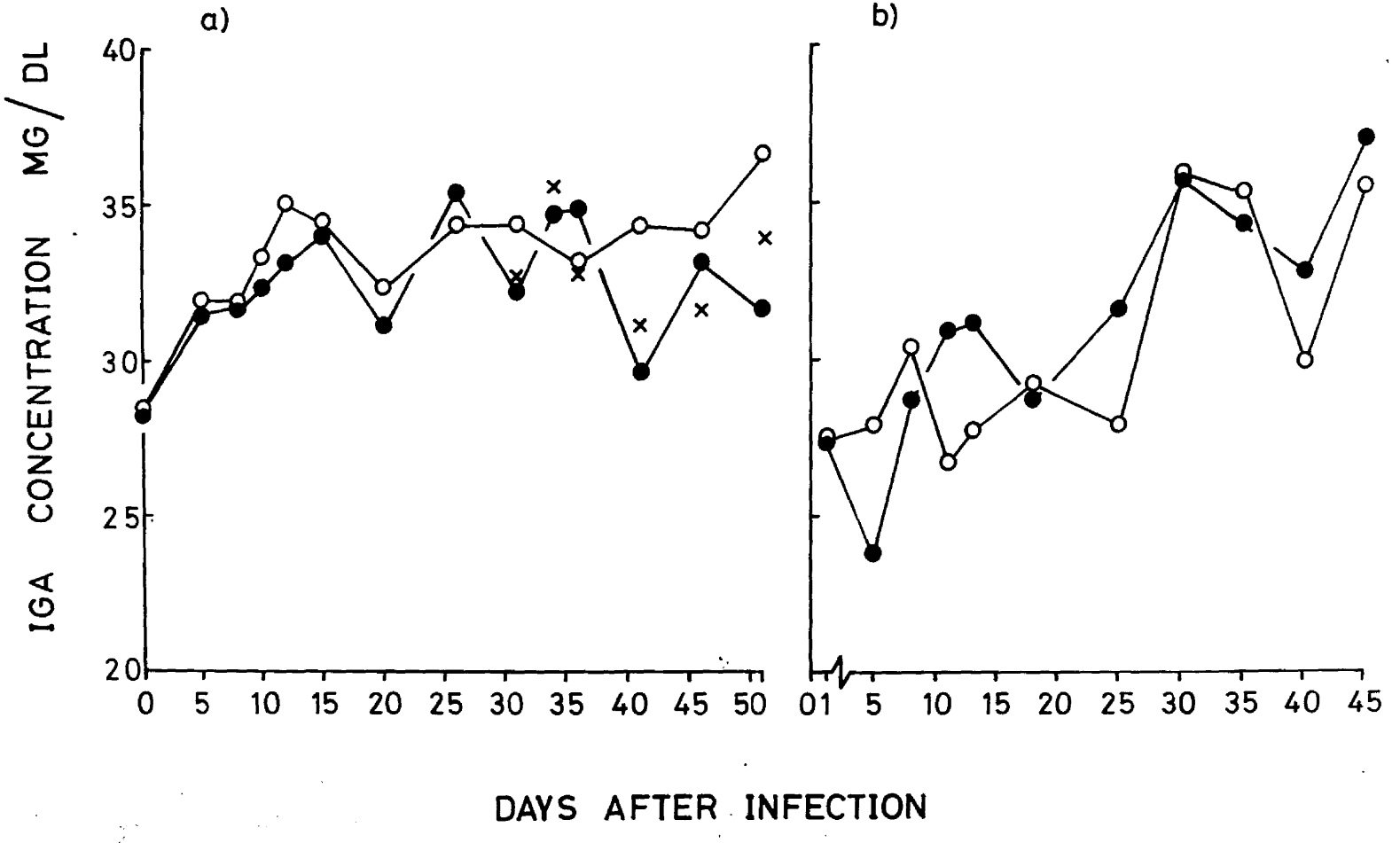
Figure 5 - 4

Changes in the concentrations of IgA in the intestinal contents of mice given a 6c (0) or sham (●) primary infection, or a 6c secondary after a 6c primary infection (X); (a) and (b) are different experiments. Each point is from the pooled contents of six mice, except on day 1 (b), where n = 10 mice.


a)
b)
IGA CONCENTRATION MG / DL
40
35
30
25
20
0 5 10 15 20 25 30 35 40 45 50
01 5 10 15 20 25 30 35 40 45
DAYS AFTER INFECTION

Facing page 139

Figure 5 - 5

Changes in the serum concentrations of IgA (a & b) and IgM (c & d) in two experiments; 0, mice given a 6c or ●, sham primary infection, or X, mice given a 6c primary followed by a 6c secondary infection. Each point is from the pooled serum of six mice, except on day 1 in (b) and (d), where n = 10 mice.

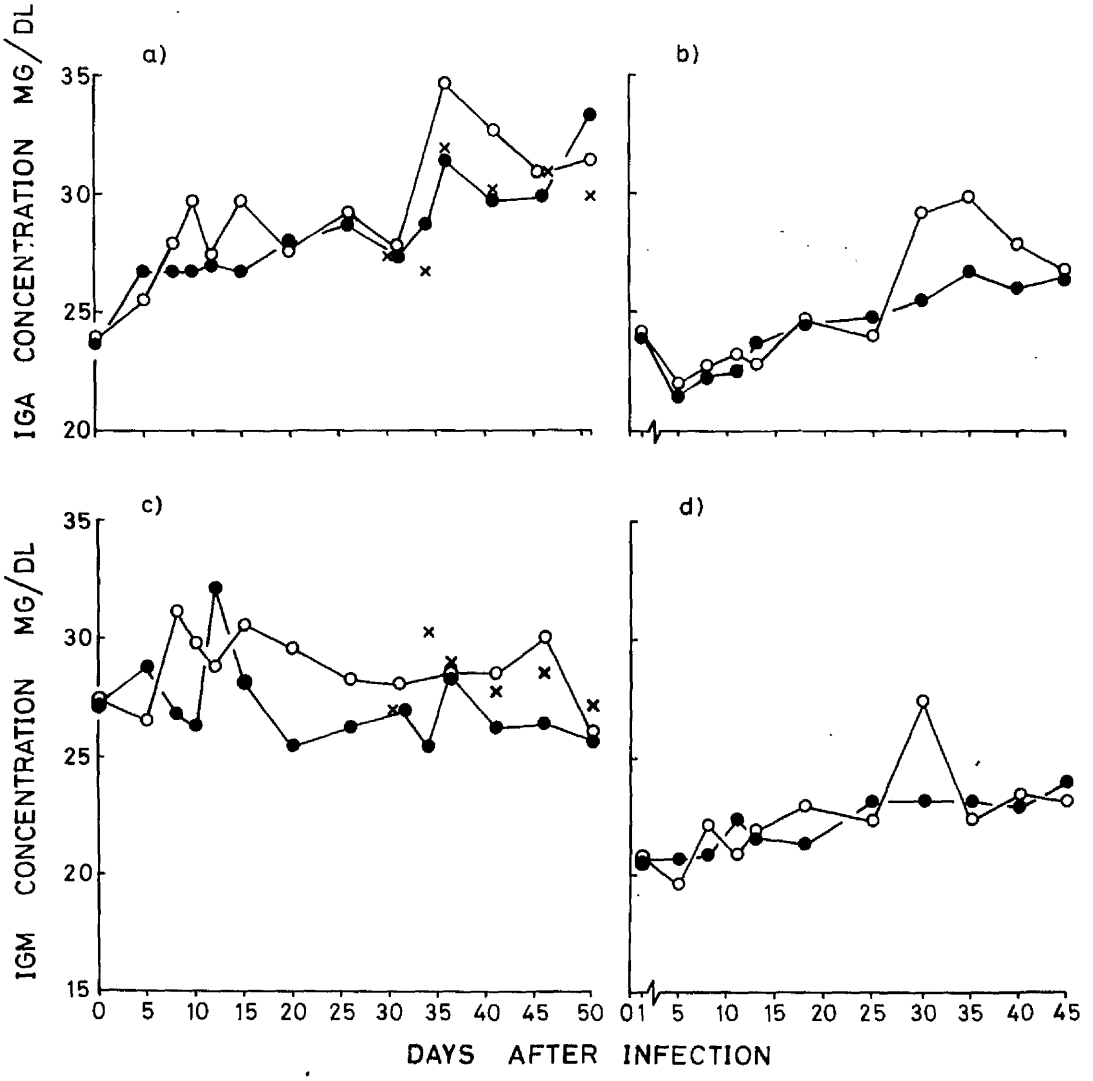

a)
b)
c)
d)
IGA CONCENTRATION MG/DL
IGM CONCENTRATION MG/DL
35
30
25
20
15
0 5 10 15 20 25 30 35 40 45 50
0 1 5 10 15 20 25 30 35 40 45
DAYS AFTER INFECTION

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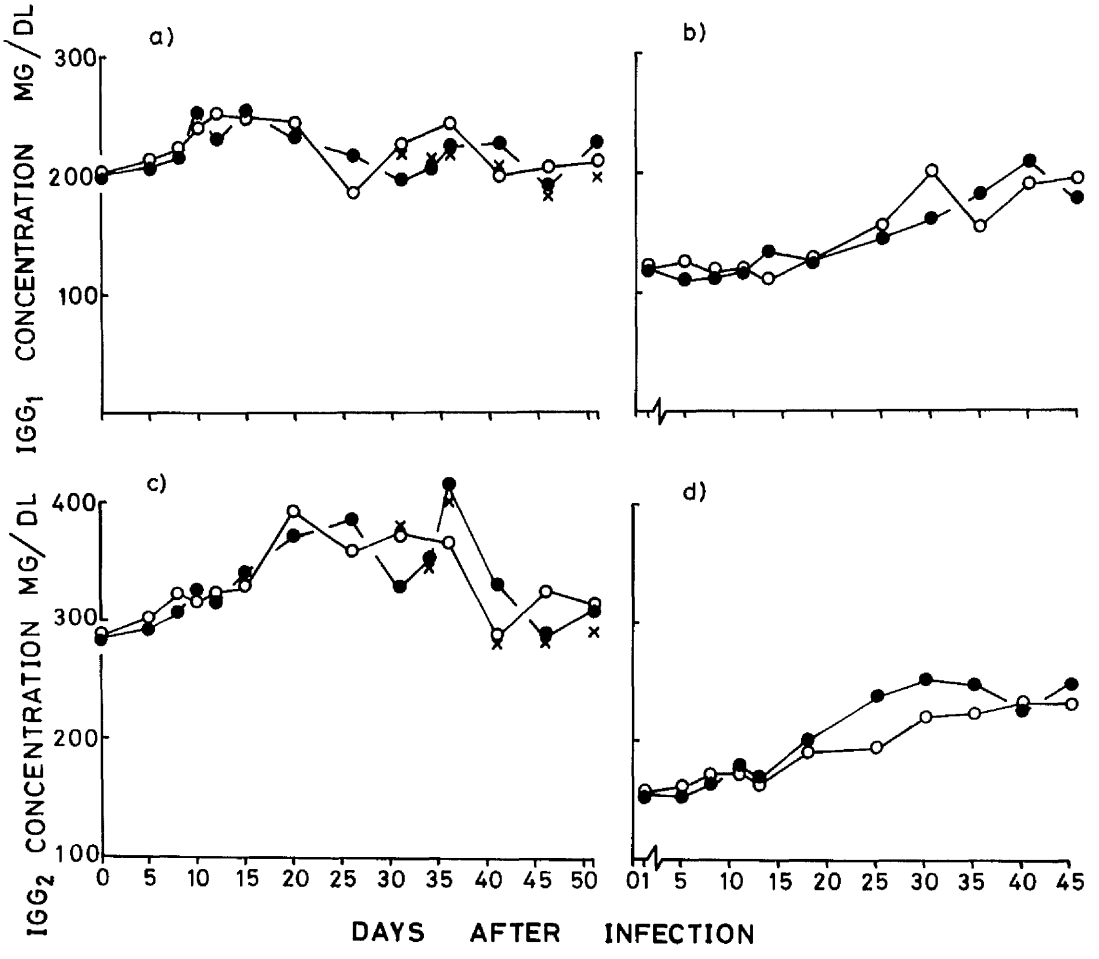
Figure 5 - 6

Changes in the serum concentrations of IgG_1 (a & b) and IgG_2 (c & d) in two experiments; 0, mice given a 6c or ●, sham primary infection, or X, mice given a 6c primary followed by a 6c secondary infection. Each point is from the pooled serum of six mice, except on day 1 in (b) and (d), where n = 10 mice.


a)
b)
c)
d)
300
200
100
400
300
200
100
0 5 10 15 20 25 30 35 40 45 50
01 5 10 15 20 25 30 35 40 45
IGG1 CONCENTRATION MG/DL
IGG2 CONCENTRATION MG/DL
DAYS AFTER INFECTION

In both experiments, however, the levels of IgA in infected and uninfected mice increased after the experiments commenced and at the end the levels were greater than at the beginning. It was concluded that differences, if they exist, in the levels of intestinal IgA in infected and uninfected mice could not be detected with the methods employed.

Although in one experiment the levels of IgA (Fig. 5 - 5 a) and IgM (Fig. 5 - 5 c) in the serum seemed to be greater in infected than in uninfected mice, these differences were not large and could not be confirmed in the second experiment (Figs. 5 - 5 b & d). As for intestinal IgA, levels of serum IgA and IgM were not elevated in secondary infections with H. diminuta. Levels of IgA in the serum increased as the experiments progressed but levels of IgM remained relatively constant. There was no consistent increase in IgG_1 or IgG_2 concentration during the experiments and no differences occurred between mice given primary or secondary infections, or uninfected mice. A consistent difference noted, however, was that when the second experiment started the concentrations of the various Igs in all the samples were lower than in the first experiment (Figs. 5 - 4 a & b; 5 - 5 a-d; 5 - 6 a-d). In summary it was concluded that infection of mice with H. diminuta did not affect the concentrations of serum Igs when compared with uninfected mice, although differences in concentrations apparently unrelated to infection were noted.

2. Immunofluorescence

Direct anti-globulin immunofluorescence was used to study the distribution of IgA, IgG_1, IgG_2, IgM and C_3 in the intestines of uninfected mice and mice infected with H. diminuta or H. microstoma. Preliminary observations were made of H. diminuta infections in rats also. Initially a single mouse was studied each day, but later to facilitate assessing responses to infection, experiments were conducted so that infected and uninfected mice were studied concurrently. The characteristic distribution and abundance of specific fluorescence in uninfected mice will be described first and then the characteristics in mice infected with H. diminuta or H. microstoma will be described.

For most of the studies and unless stated otherwise, the tissue was prepared by formalin fixation prior to freezing and sectioning, but rapid freezing with methanol fixation post sectioning was used in some studies (see MATERIALS AND METHODS). Differences in the characteristics of the specific fluorescence produced by these two methods of fixation will be described. Many of the general characteristics of the specific fluorescence were similar in uninfected and infected mice. Hence, as superior photographs often are available from infected mice, in some cases they are used for figures in the initial description of uninfected mice, but only where they are representative of these mice also. A diagrammatic representation of intestinal morphology with the terminology used below is provided (Fig. 5 - 7). Except where stated descriptions are based upon CFLP or C_3H male mice from commercial suppliers.

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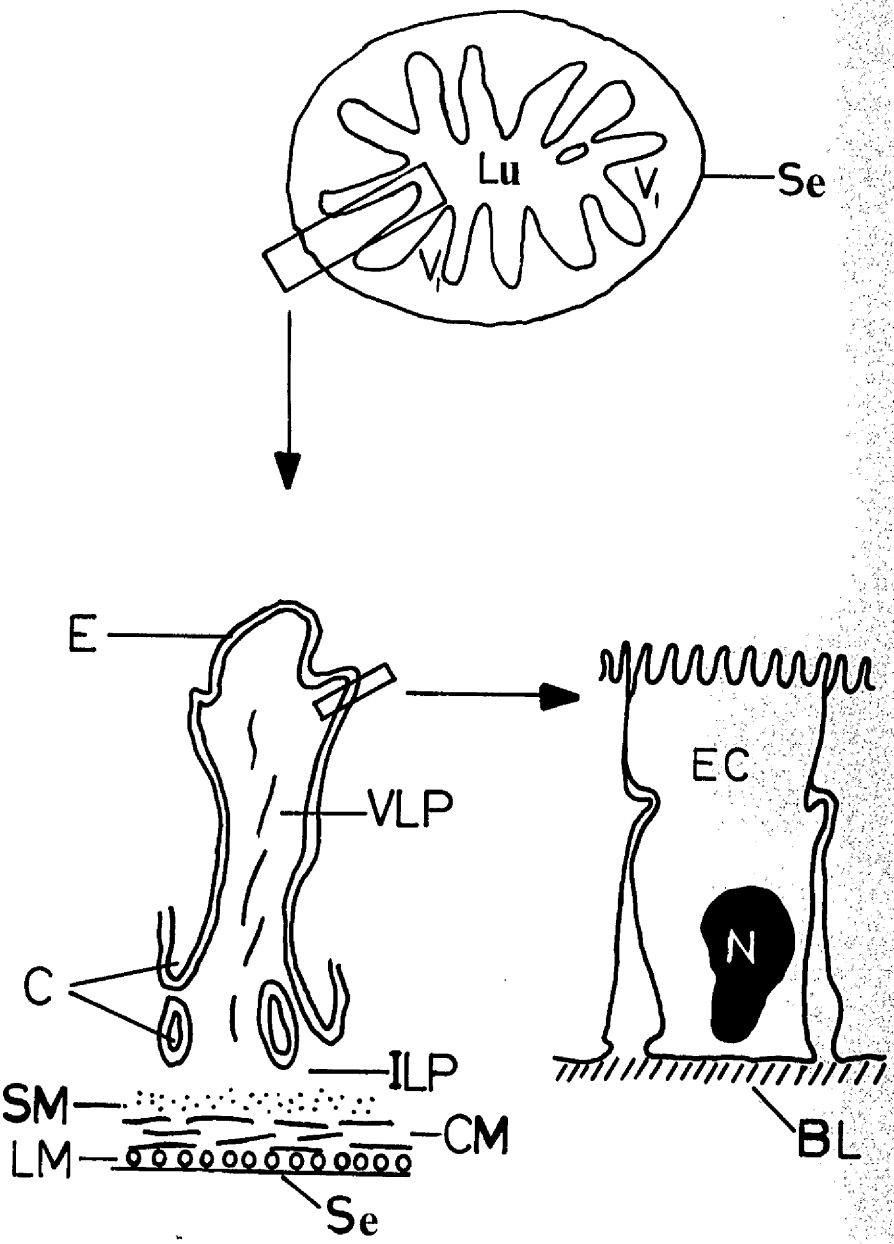
Figure 5 - 7

Diagrammatic representation of a transverse section of the small intestine of a mouse with enlargements of a villus and an epithelial cell to show their basic morphology and the terminology used.

Key to abbreviations used in this SECTION:

BL	Basal lamina	Lu	Lumen
C	Crypt of Lieberkühn	Me	Medulla of Peyer's patch
CM	Circular muscle	N	Nucleus
E	Epithelium	Se	Serosa
EC	Epithelial cell	SM	Submucosa
H. dim	H. diminuta	Su	Subepithelium
H. mic	H. microstoma	V_1	Villus
ILP	Intercryptal lamina propria	VLP	Villous lamina propria
LM	Longitudinal muscle		


Lu
Se
E
VLP
C
ILP
SM
CM
LM
Se
EC
N
BL

a) uninfected mice

i) intestine

IgA

Using tissue fixed before sectioning, undoubtedly the most striking feature of immunofluorescence produced by FITC conjugated anti-IgA serum was the abundance of brilliantly fluorescing cells in the intestinal lamina propria, from near the tips of the villi to the bases of the crypts (Fig. 5 - 8). These oval mononuclear cells had eccentric nuclei and presumably were plasma cells (Fig. 5 - 9). The numbers of these IgA containing cells per villus-crypt unit (see MATERIALS AND METHODS) varied considerably; of 30 units studied from six uninfected mice (Exp. 22, 23 and 24 see below) the mean (range) number was 45.3 (24-62). About 80-90% of these cells were in the lamina propria of the villi with the remainder in the lamina propria surrounding the crypts. The fluorescence of these IgA containing cells was the characteristic apple-green colour produced by specific FITC staining but other cells in the lamina propria contained a granular yellowish fluorescence shown by controls to be nonspecific (Fig. 5 - 10). Such fluorescence is commonly reported (e.g. Allen & Porter, 1973; Johnston & Bienenstock, 1974) and is nonspecific fluorescence of eosinophils. Generally in the uninfected intestine the lamina propria contained no other fluorescence, although occasionally a longitudinal area of fluorescence occurred in the centre of a villus. This was considered to be due to either circulating IgA in a section of the central non-branching arteriole of the villus (see Svanvik, 1973) or IgA in a section of the central lacteal of the villus, or both.

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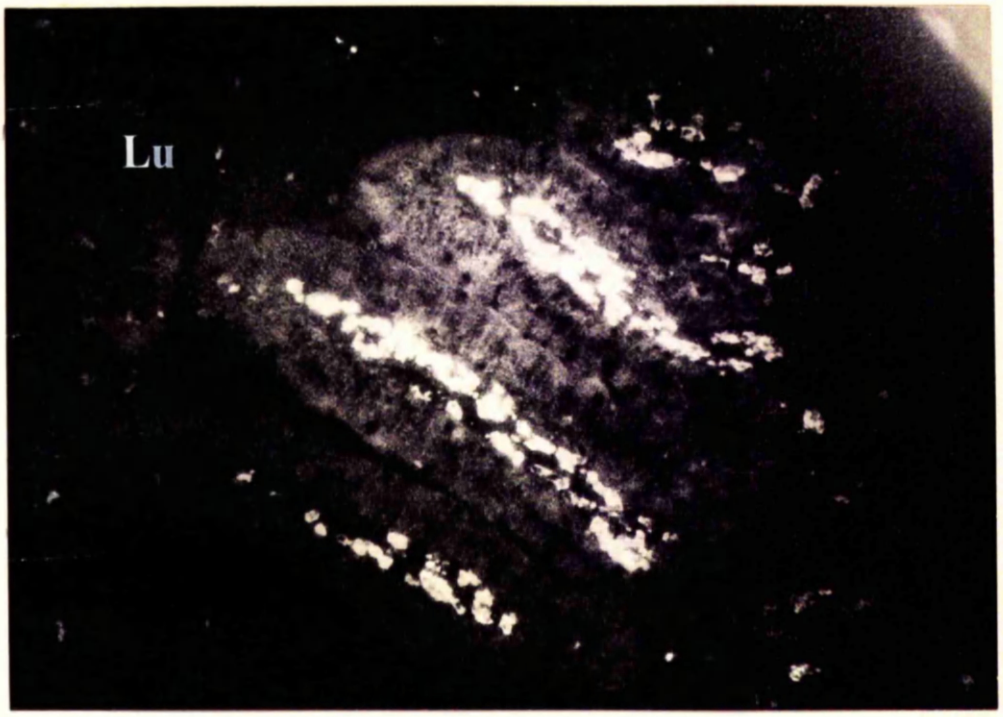
Figure 5 - 8

Section of the small intestine showing specific cytoplasmic fluorescence in IgA containing plasma cells of the lamina propria of villi; tissue fixed in formalin prior to freezing and sectioning. (approx. X 250.)

Figure 5 - 9

Section of a villus showing specific cytoplasmic fluorescence in IgA containing plasma cells of the lamina propria; tissue fixed in formalin prior to freezing and sectioning. (approx. X 600.)


Lu

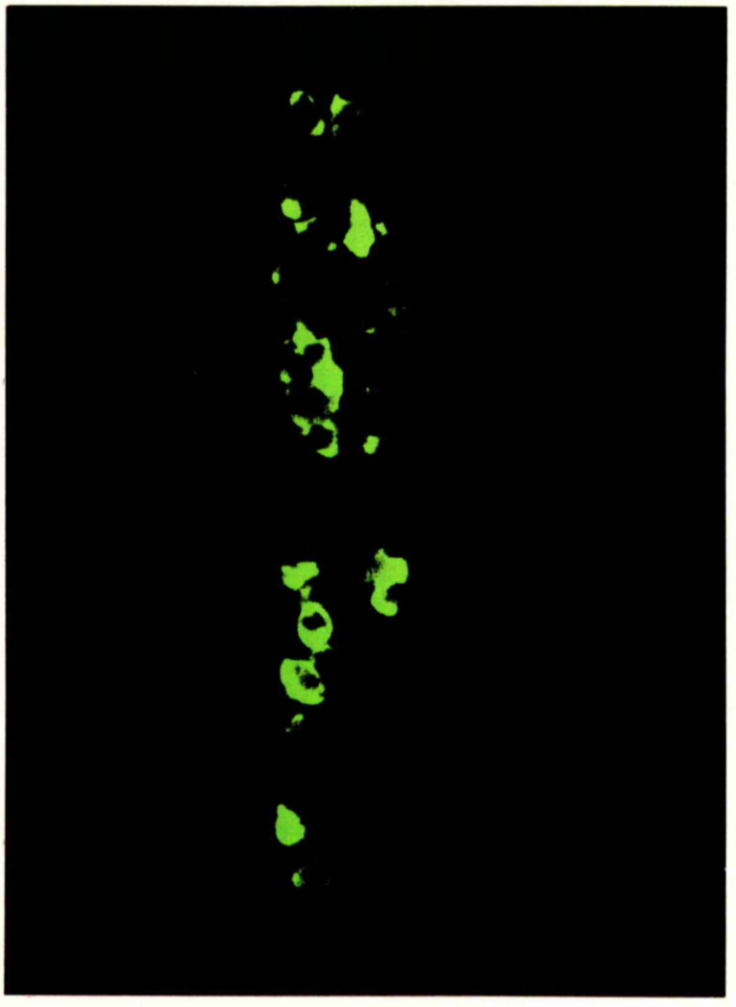


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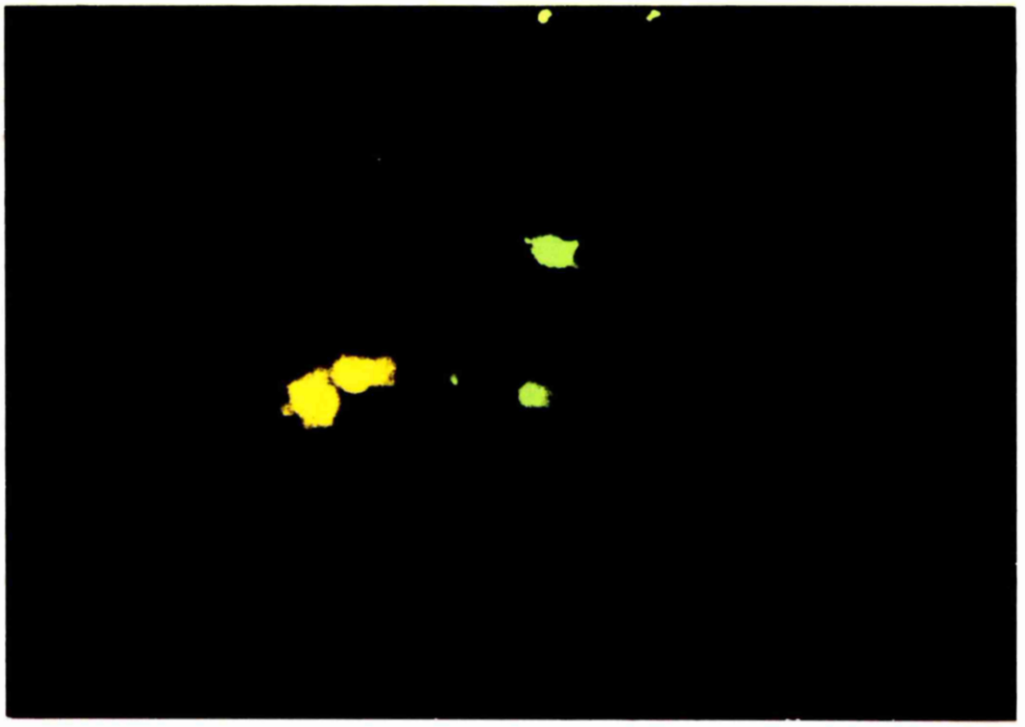
Figure 5 - 10

Section of a villus of a mouse stained with antiserum against mouse IgA showing nonspecific yellow fluorescence of eosinophils; tissue fixed in formalin prior to freezing and sectioning. (approx. X 575.)

Figure 5 - 11

Section of intestine of a mouse infected with five Hymenolepis microstoma for 328 days showing specific IgA fluorescence on the serosa and in the muscle layers. The fluorescence is in considerable excess of that normally seen in uninfected mice; tissue fixed in formalin prior to freezing and sectioning. (approx. X 600.)




CM
Se

IgA also occurred on the serosal surface of the intestine closely associated with the outer, longitudinal muscle layer, and on the inner surface of the circular muscle layer adjacent to the area of the submucosa (Fig. 5 - 11). The submucosa never was clearly seen as it is not extensive in the mouse intestine. A region normally visible between the circular muscle and the lamina propria surrounding the crypts was thought to represent the submucosa. The fluorescence on the serosal and mucosal surfaces of the muscle layer were fine discontinuous lines whose extent varied from one preparation to another. The lumen of the intestine also contained particles which fluoresced (Fig. 5 - 12). Much of this fluorescence was specific IgA staining, but some was nonspecific and could be predicted accurately using white light illumination as these particles, which often could be identified as plant material from the diet, showed birefringence. Sections of Peyer's patches showed little fluorescence, particularly within the medullary region, although IgA containing cells occurred in the periphery especially near the overlying villi of the mucosa (Fig. 5 - 13). No fluorescence occurred on the epithelium, other than that on intralumenal particles, and none occurred within or between epithelial cells of the villi or crypts. The basal lamina of the epithelium was negative also.

Except where stated above for nonspecific staining of eosinophils and birefringent particles in the intestinal lumen, controls with the tissue fixed before sectioning were successful. The nonconjugated 'blocking control' suppressed the specific fluorescence on particles

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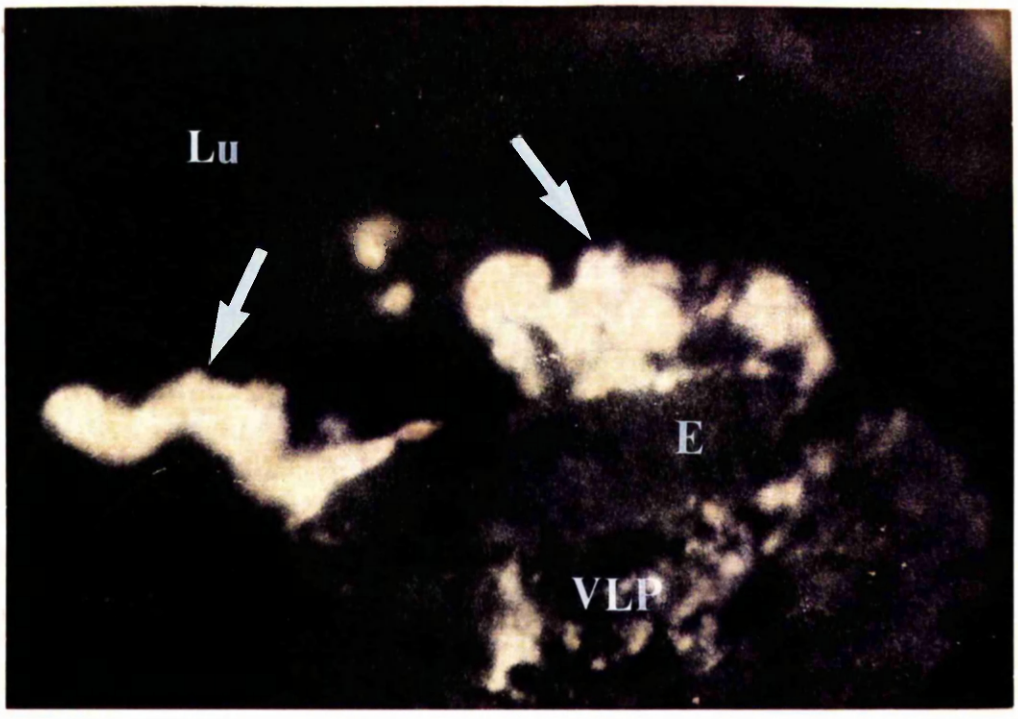
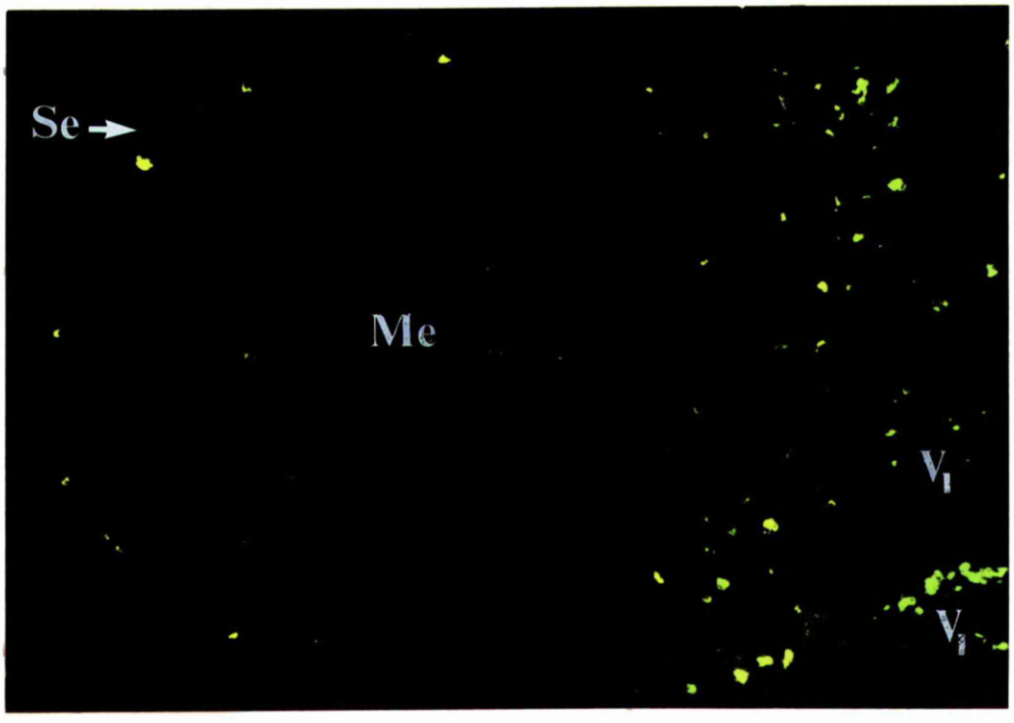
Figure 5 - 12

Section of the intestine of a mouse infected with five Hymenolepis microstoma for 328 days to show specific IgA fluorescence on particles in the intestinal lumen (arrows). Similar fluorescence occurs in the intestine of uninfected mice; tissue fixed in formalin prior to freezing and sectioning. (approx. X 700.)

Figure 5 - 13

Section of a Peyer's patch showing IgA containing plasma cells near the periphery, especially at base of villi, but little fluorescence in the medulla; tissue fixed in formalin prior to freezing and sectioning. (approx. X 100.)


Lu
E
VLP


Se
Me
V1
V1

in the lumen and on the muscle layers and, to varying degrees, depressed the fluorescence of IgA containing cells in the lamina propria. It seems probable that the range from complete suppression to slight depression of fluorescence reflects increasing abundance of IgA, with the greatest concentration in the IgA producing plasma cells.

In tissue fixed with methanol following freezing and sectioning in the cryostat, diffuse specific IgA fluorescence of relatively weak intensity occurred throughout much of the lamina propria and IgA containing cells were less easily distinguished than in tissue fixed before sectioning (compare Fig. 5 - 14 with 5 - 8 & 5 - 9). More intensely staining regions, presumably representing IgA containing cells, could be distinguished in some cases on a background of diffuse fluorescence but often the intensity throughout the lamina propria was uniform and cells could not be distinguished. Where the latter was true, cell counts were impossible and even when some stained cells were visible the reliability of counts seemed questionable; hence no counts of IgA containing cells were conducted on tissue fixed after sectioning. As in tissue fixed before sectioning, nonspecific fluorescence of eosinophils occurred with tissue fixed after sectioning.

The distribution of specific IgA fluorescence on the muscle layers of tissue fixed after sectioning was more extensive than on preparations fixed before sectioning: more of the mucosal surface of the muscle layer was positive and generally the entire serosal surface fluoresced. Specific fluorescence in the lumen

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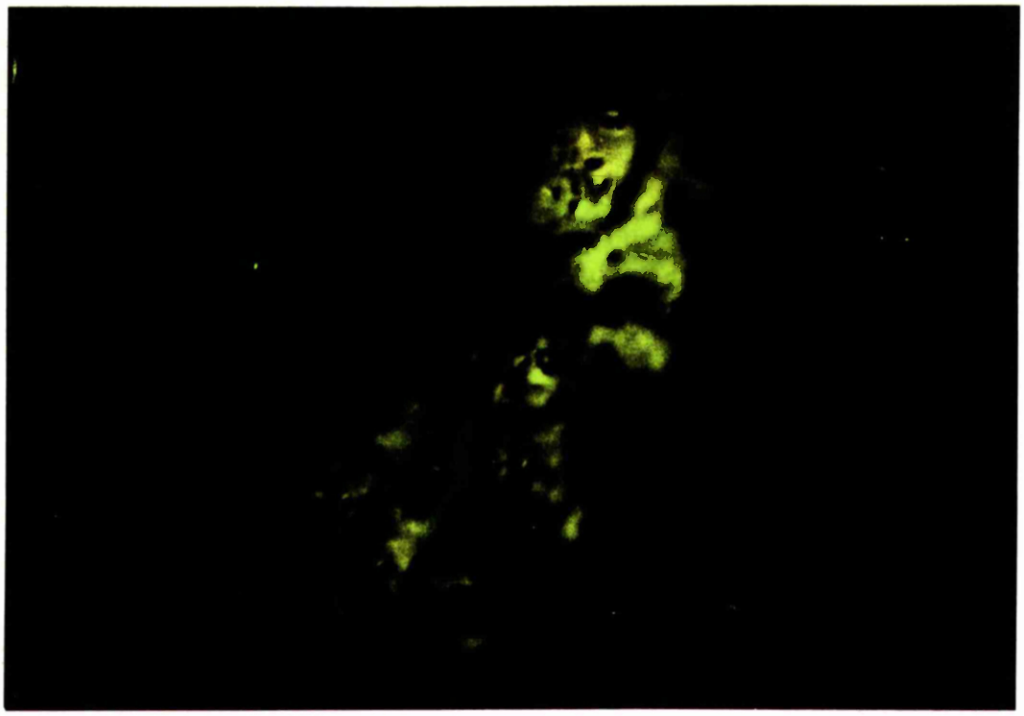
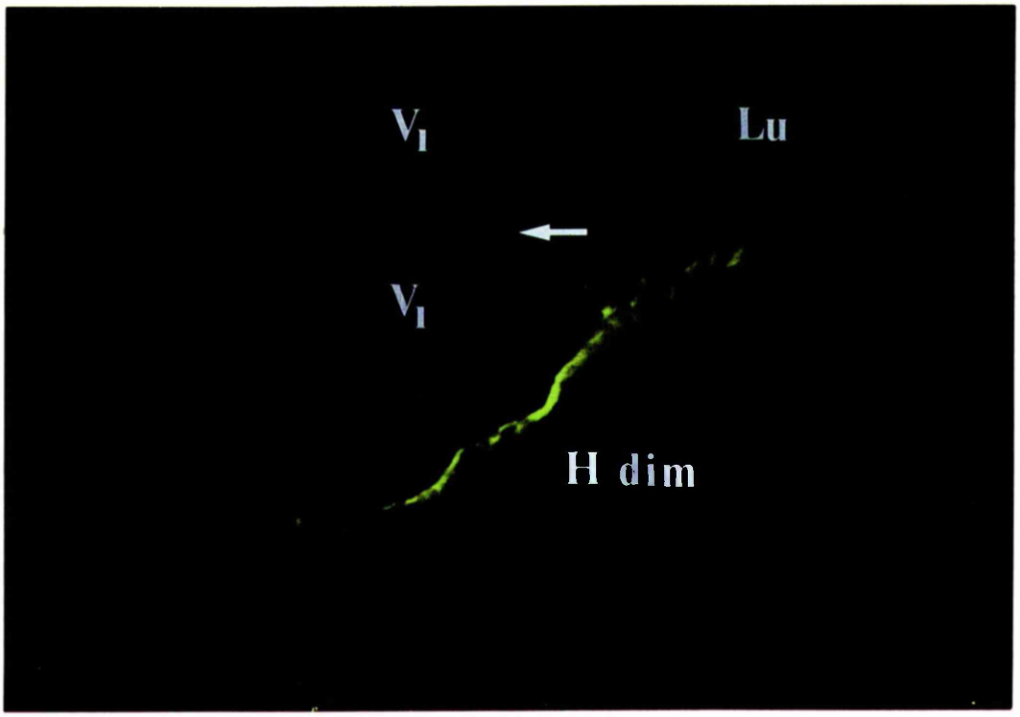
Figure 5 - 14

Section of a villus showing specific, but diffuse, IgA fluorescence throughout the lamina propria; tissue fixed in methanol after freezing and sectioning. (approx. X 800.)

Figure 5 - 15

Section of intestine showing the tips of two villi with diffuse IgA fluorescence on epithelial surface (arrow), and with Hymenolepis diminuta with a more intense, broad layer of fluorescence on its surface; tissue fixed in methanol after freezing and sectioning. (approx. X 400.)




V_1
Lu
V_1
H dim

was seldom intense but, in contrast to fixation prior to sectioning which yielded no fluorescence associated with the epithelium, with fixation after sectioning the epithelium was covered with a coating of fluorescence (Fig. 5 - 15) of variable intensity and thickness. The intensity was greatest nearest the epithelium and decreased towards the centre of the lumen. Another qualitative difference between the two fixation methods was that, whereas no fluorescence occurred in the epithelium or crypt lumen with prior fixation, with fixation after sectioning the apical portions and surfaces of many crypt epithelial cells showed specific fluorescence (Fig. 5 - 16) which occasionally extended up the neck of the crypt. Overall the distribution of fluorescence was greater but its intensity less in post rather than pre-sectioning fixed tissue. Controls with the former, as with the latter, suppressed specific fluorescence.

The above description of the distribution of IgA in uninfected mice is a synthesis of results collected over about 1 year. The following, similarly derived, descriptions for other Igs and for infected mice often will be compared with the above. Where similarities are considerable photographs will not be included but where notable differences occur photographs are provided. Unless otherwise stated, controls were successful as for IgA.

IgM

In tissue fixed before sectioning IgM containing cells occurred in the lamina propria of the villi and surrounding the crypts as did IgA containing

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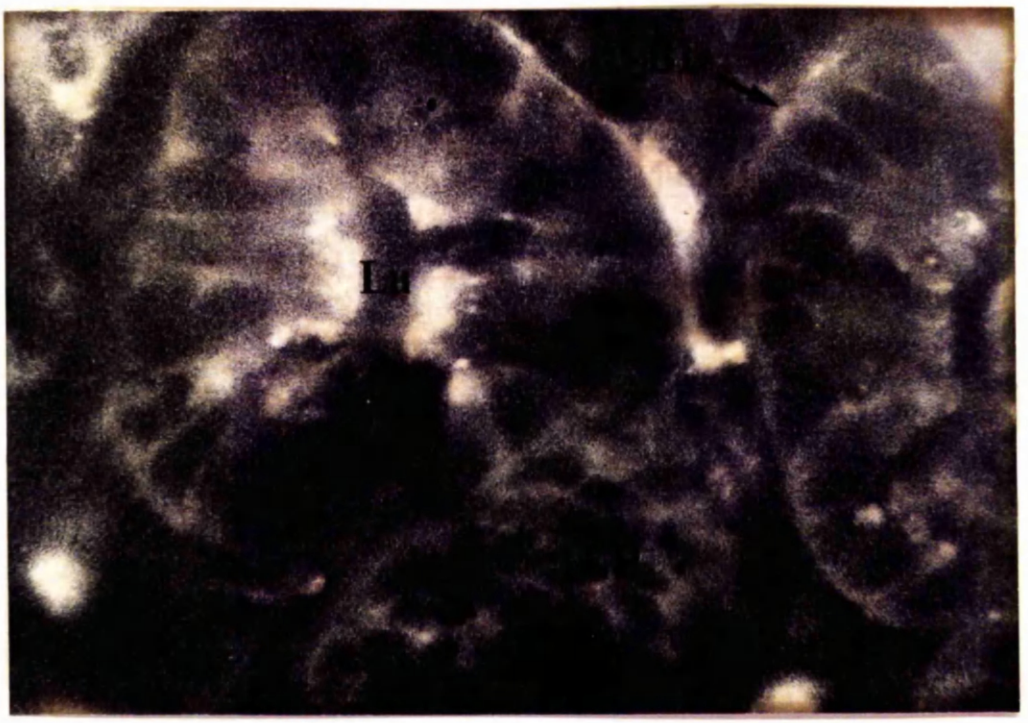
Figure 5 - 16

Section of the cryptal region of the intestine of a mouse infected with five Hymenolepis microstoma for 328 days showing specific IgA fluorescence in the intercryptal lamina propria, in and on the apical portions of cryptal epithelial cells, on the basal lamina of the epithelium and in some intercellular regions of the epithelium. The fluorescence is in considerable excess of that normally seen ir uninfected mice; tissue fixed in formalin prior to freezing and sectioning. (approx. X 1350.)

Figure 5 - 17

Section of the cryptal and muscle regions of the intestine of a mouse infected with five Hymenolepis microstoma for 266 days showing specific IgG_1 fluorescence on the serosa, muscle layer and basal lamina of the cryptal epithelium and in the intercryptal lamina propria. The fluorescence is in excess of that normally seen in uninfected mice but less so than above (Fig. 5 - 16); tissue fixed in formalin prior to freezing and sectioning. (approx. X 600.)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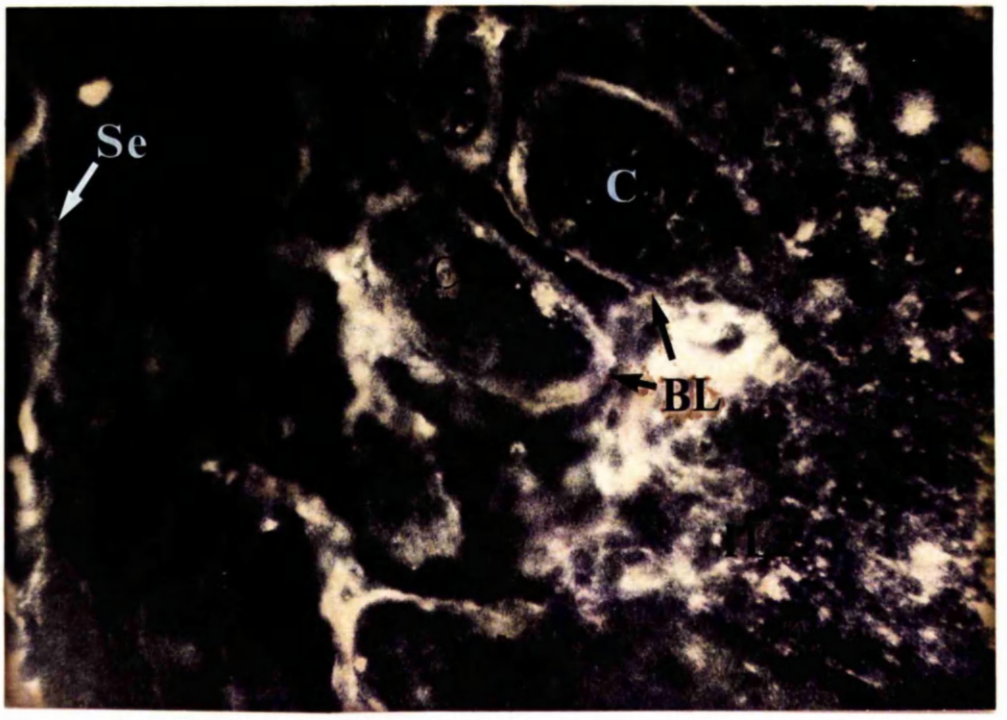
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cells. The mean (range) number of IgM containing cells per villus-crypt unit, from 30 such units in six mice was 18.6 (6-40). The ratio of mean number of IgA:IgM containing cells was 2.4:1 and in the different mice ranged from 1.8:1 to 4.4:1. Nonspecific fluorescence of eosinophils occurred as in IgA stained tissue. Specific fluorescence occurred in the central cores of some villi and was considered to be due to IgM in sections of the central arterioles or lacteals of the villi. Once fluorescence occurred closely associated with the basement lamina of the epithelium of some villi, but in uninfected mice this was exceptional.

As with IgA fluorescence, IgM was present on the serosal and mucosal surfaces of the muscle layer but the distribution of IgM fluorescence was greater, as the serosal surface normally was positive throughout and the mucosal surface had more extensive fluorescence. The intestinal lumen contained some specific fluorescence but generally much less than for IgA; nonspecific fluorescence occurred also. Further, in common with IgA, IgM containing cells occurred around the periphery of Peyer's patches; the medullary region contained some intercellular fluorescence of weak intensity. In mice purchased from commercial suppliers of 2 star or 4 star grading, the epithelium of the villi and crypts did not stain for IgM in tissue fixed before sectioning, but in one homebred mouse, IgM occurred near the basement lamina of the cryptal epithelium.

Tissue fixed after sectioning had diffuse fluorescence throughout the lamina propria, had fluorescence in the apical portions and on the surface of

cryptal epithelial cells and had fluorescence on the entire serosal and mucosal surfaces of the muscle layer; characteristics similar to IgA fluorescence. The specific IgM staining in the lamina propria was in general less extensive than that of IgA but the IgM associated with the musculature was greater, particularly on the mucosal surface.

IgG_1 and IgG_2

Unlike the abundance of IgA and IgM containing cells, there were few IgG_1 and IgG_2 containing cells in the lamina propria of the intestine. In fact many villus-crypt units contained none and when counts were attempted the maximum number of IgG_2 containing cells in one unit was four. There was IgG_1 and IgG_2 fluorescence in the central cores of some villi, which again was probably in the central arterioles or lacteals. Fluorescence also occurred, albeit with variable abundance in association with the basement laminal region of the cryptal and villous epithelium (Fig. 5 - 17). Whether this was truely on or in the basement lamina was not determined; if separate from the basement lamina the most probable explanation is that it was in the capillaries near the basement lamina. The serosal and mucosal surfaces of the muscle layer were often stained but the distribution of fluorescence was not extensive. There was nonspecific fluorescence of eosinophils in the lamina propria and of intralumenal particles; no specific fluorescence occurred in the lumen. Fluorescence associated with Peyer's patches was similar to that with IgA and IgM but was less abundant.

IgG_1 and IgG_2 were more abundant in homebred than in purchased mice as was found to be the case with IgM. Fluorescence was most obvious in the intercryptal lamina propria and appeared to be intercellular. Some may have been extravascular but its frequent dendritic appearance suggested that some was within small venules or the capillary network.

Tissue fixed after sectioning had fluorescence in the central cores of some villi as above, but in contrast to tissue fixed before sectioning, had extensive fluorescence on the mucosal and serosal surfaces of the muscle layer. Fluorescence occurred in the intercryptal lamina propria, especially where the adjacent fluorescence on the mucosal surface of the muscle layer was abundant. With both fixation methods the distribution and abundance of IgG_2 was generally greater than that of IgG_1.

C_3

Using tissue fixed before sectioning FITC conjugated RAM C_3 serum produced by Nordic Immunological Laboratories stained the central core of many villi, portions of both surfaces of the muscle layer and, particularly where adjacent areas fluoresced, some regions associated with the basement lamina of cryptal and villous epithelium. No intralumenal specific fluorescence occurred, although nonspecific fluorescence occurred in the lumen and in eosinophils in the lamina propria.

ii) bile duct

Although the bile duct in an uninfected mouse is small, it can be removed with little difficulty and

the epithelium and scanty subepithelial connective tissue can be studied. Generally no specific IgA fluorescence occurred in the lumen or wall of the bile duct. On a few occasions, however, IgA containing cells (a maximum of four) were detected in the subepithelium as was specific, but weak and diffuse background fluorescence; specifically stained particles occurred in the lumen also. IgM fluorescence was similar to IgA but the weak background fluorescence in the subepithelium was more common.

With IgG_1 and IgG_2 the subepithelium contained diffuse fluorescence of variable intensity. No IgG_1 and IgG_2 containing cells were detected, but it is not known if some occurred and were masked by the diffuse fluorescence. On one occasion fluorescence, not associated with birefringent particles occurred in the lumen. The distribution and abundance of specific fluorescence produced by C_3 antisera was indistinguishable from that produced by IgG_1 or IgG_2 antisera.

b) infections with H. diminuta in mice

For immunofluorescent studies a number of homebred mice of various ages and strains were infected with H. diminuta and killed days 8-16 post infection, and some information from these studies is provided below. Most of the following description, however, comes from three experiments with male 6 week old mice from commercial suppliers. The protocol of these experiments is shown in Table 5 - 2.

i) IgA

When intestinal tissue fixed before sectioning with H. diminuta in situ was incubated with FITC

Table 5 - 2

Protocol of experiments for immunofluorescent studies on Hymenolepis diminuta infections of mice

Exp.	Mice	Groups	Treatment	Days tissue studied: fixation before sectioning	Days tissue studied: fixation after sectioning
22	CFLP	sham		10, 12	
	4 star	2c H. diminuta		10, 12	14
		2c H. diminuta	cortisone	10, 12	14
23	C_3H	sham		10, 12, 14	11
		2c H. diminuta		10, 12, 14*	11
24	C_3H	sham	0.85% NaCl	9, 11, 13	
		6c H. diminuta	0.85% NaCl	9, 11, 13	
		6c H. diminuta	cortisone	9, 11, 13	

* worms absent (rejected)

conjugated GAM IgA serum, a fine line of fluorescence occurred on the worm tegument (Fig. 5 - 18). Specific fluorescence did not occur elsewhere on the worm. In one experiment (23) with 2c infections tegumental fluorescence occurred on day 10 but was of relatively weak intensity and incomplete. In the other experiment (22) no tegumental fluorescence occurred by day 10. By day 12 in both experiments the tegumental fluorescence had increased in distribution, completely covering the tegument in Exp. 22, but not completely in Exp. 23. In Exp. 24 with 6c infections the entire tegument had fluorescence of relatively strong intensity by day 9. On days 11 and 13 the intensity of the fluorescence had increased. The time of appearance and relative abundance of Igs on H. diminuta is summarized in Table 5 - 3. Using tissue fixed after sectioning, on days 11 and 14 (see Table 5 - 2) the tegumental surface was entirely covered with a diffuse broad layer of fluorescence (see Fig. 5 - 15).

With either method of fixation of tissue from homebred mice, worm fluorescence was similar to that described above, but in general these mice were more rapidly responsive than commercially raised mice and fluorescence of relatively high intensity covered the entire worm surface by days 8-10.

In Exp. 22 IgA was detected on H. diminuta in the cortisone treated mouse on day 10, 2 days earlier than in the untreated mouse. However, on neither day 10 nor 12 was the entire surface covered as was the case in the untreated mouse on day 12. With 6c infections in Exp. 24 worm fluorescence in cortisone treated mice was

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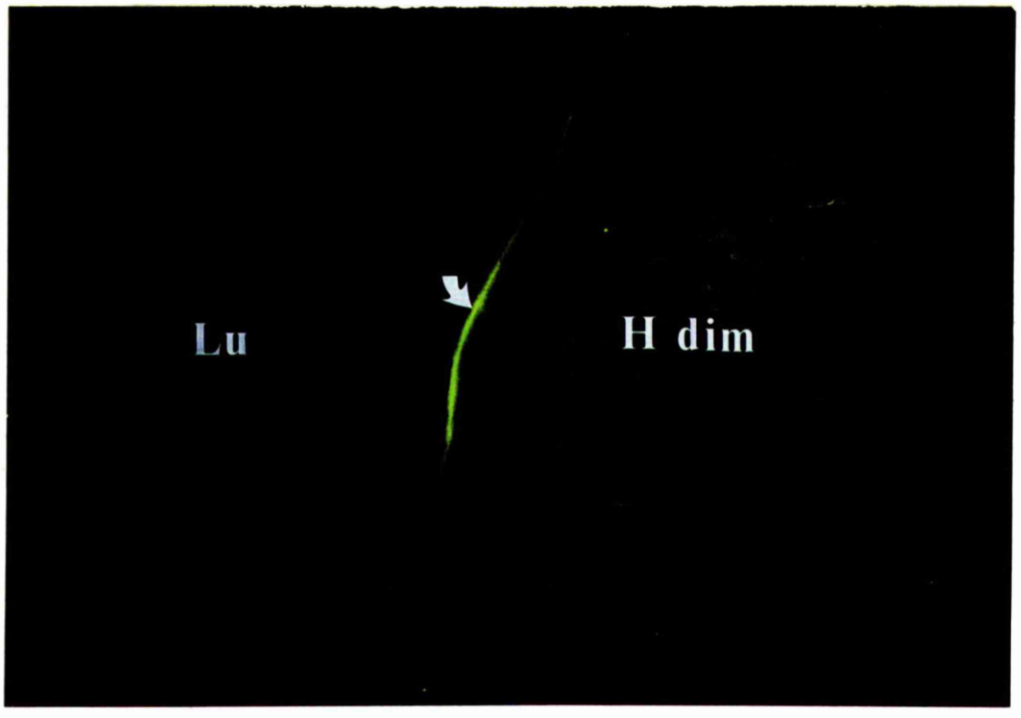
Figure 5 - 18

Section of a 13 day old Hymenolepis diminuta from a mouse with intense linear IgA fluorescence on the tegument (arrow); tissue fixed in formalin prior to freezing and sectioning. (approx. X 1000.)

Figure 5 - 19

Section of the intestine of a mouse infected with five Hymenolepis microstoma for 266 days showing apparent partial villous atrophy which also occurred in infections with H. diminuta (compare to the long slender, normal, villi in Fig. 5 - 8 & 5 - 24). Specific IgA fluorescence is shown in the villous, cryptal and muscle regions; tissue fixed in formalin prior to freezing and sectioning. (approx. X 275.)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H dim

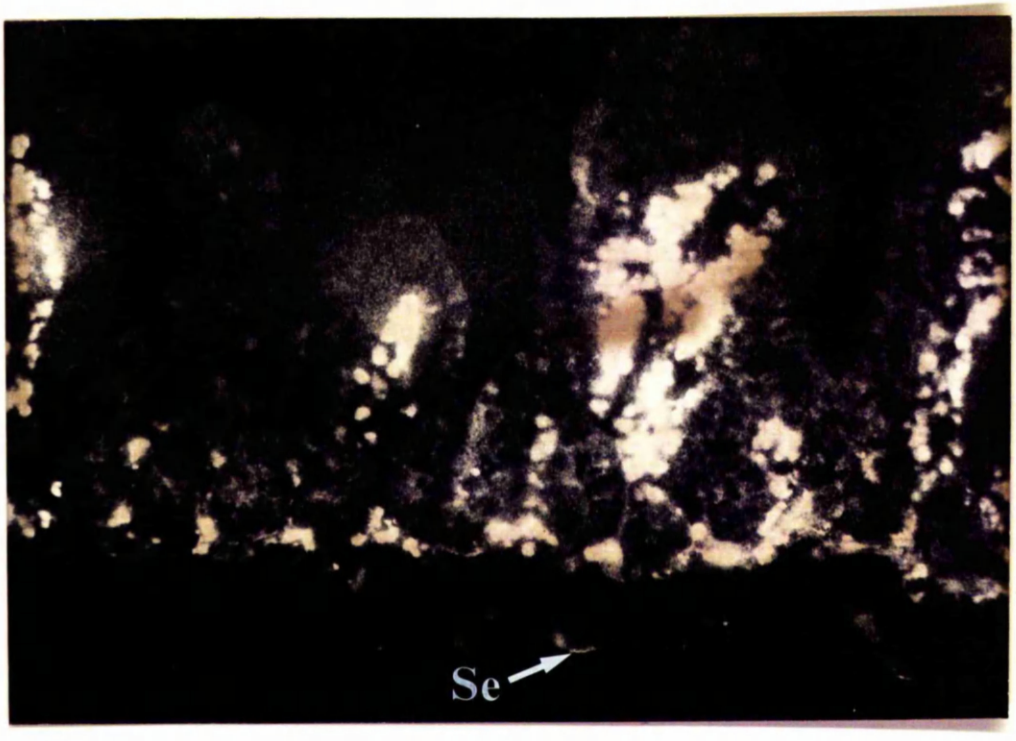
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Table 5 - 3

The time of appearance and relative abundance of immunoglobulins (Igs) on the tegument of Hymenolepis diminuta

Igs	Group	Age of worms (days) 9	10	11	12	13
IgA	2c H. diminuta		-, +2*		+2, +3	
	2c H. diminuta + cortisone		+2		+2	
	6c H. diminuta	+3		+4		+4
	6c H. diminuta + cortisone	+2		+2		+3
IgM	2c H. diminuta		-		+2, +3	
	2c H. diminuta + cortisone		+2		+2	
	6c H. diminuta	+3		+4		+2
	6c H. diminuta + cortisone	+2	-	+2		+3
IgG_1	2c H. diminuta		-		+, +2	
	2c H. diminuta + cortisone		-		+	
	6c H. diminuta	+2	+3			
	6c H. diminuta + cortisone	+2	+3			
IgG_2	2c H. diminuta		-		+2	
	2c H. diminuta + cortisone		-		+	
	6c H. diminuta	+2		+3		+3
	6c H. diminuta + cortisone	+2				+3

* Relative abundance of Igs estimated as: - negative, + 1-50% of worm surface with fluorescence, +2 51-99% with fluorescence, +3 100% with fluorescence, +4 100% with intensive fluorescence; a blank indicates that the records are incomplete.

both less extensive and intensive than in untreated mice on days 9 and 11 and less intensive on day 13.

The mean (range) number of IgA containing cells per villus-crypt unit (n = 29) in five infected mice killed days 9-14 post infection was 40.3 (20-62); a value not different from that of uninfected mice, 45.3 (24-62). Three infected mice treated with cortisone had 28.4 (22-48) IgA containing cells per villus-crypt unit (n = 15). These results suggested that cortisone treatment reduced the numbers of lamina proprial IgA containing cells, but unfortunately only three mice were studied. This was because cortisone treatment, particularly in 6c infections, resulted in partial villous atrophy (see Fig. 5 - 19) and there seemed little point in continuing to count cells in villi of reduced volume. If a reduction in IgA containing cells occurred in cortisone treated mice it would be difficult to determine if this was due to reduced villous volume or to some other cause (e.g. direct action of cortisone on lymphoid cells). Partial villous atrophy also occurred in untreated mice given six H. diminuta but appeared to be considerably less severe than in infected mice treated with cortisone. This slight atrophy makes comparisons of the numbers of IgA containing cells in infected and uninfected mice questionable also.

Other than an apparent increase in specific fluorescence in the intestinal lumens of infected mice, and on one occasion fluorescence associated with the basement lamina of the villous epithelium, no consistent differences occurred in the distribution and abundance of IgA in the intestines of uninfected, infected untreated

and infected cortisone treated mice fixed by either of the two methods.

ii) IgM

Using tissue fixed before sectioning, when fluorescence, induced by FITC conjugated GAM IgM serum, occurred on H. diminuta it was similar to IgA fluorescence. In both experiments using 2c infections (22 and 23) no IgM was detected on H. diminuta surface on day 10 but by day 12 in Exp. 22 the entire surface stained, whereas in 23 the staining was discontinuous but still covered >50% of the tegument (Table 5 - 3). However, on both days 9 and 11 in the 6c infections (Exp. 24) the entire surface of each worm fluoresced; unexpectedly, on day 13 parts of some worms were without fluorescence. Using fixation post sectioning no fluorescence occurred on worms in Exp. 22 and 24.

As with IgA, in Exp. 22 worms from the cortisone treated mouse had a discontinuous line of fluorescence covering 50% of their surface, and on day 12 the fluorescence remained discontinuous, whereas in the untreated mouse fluorescence had appeared and covered the entire worm surface. In Exp. 24 on days 9 and 11 fluorescence occurred on >50% of the surface of the worms from cortisone treated mice and by day 13 covered the entire tegument. It is clear that IgM occurs on the surface of worms from cortisone treated mice just as does IgA, whether the abundance is depressed requires confirmation.

Studies with homebred mice confirmed the findings with IgA, namely that in general these mice were more rapidly responsive than commercially raised mice,

because as early as day 10 specific IgM fluorescence covered the entire worm surface in 2c infections. As with commercially raised mice IgM was not detected on worms prepared by fixation post sectioning.

The mean (range) number of IgM containing cells per villus-crypt unit in five infected mice killed days 9-14 post infection was 15.1 (5-26) (n = 23), which is not different from the number, 18.6 (6-40), in uninfected mice. The ratio of IgA:IgM containing cells was 2.7:1 in infected mice and 2.4:1 in uninfected mice. Three infected mice treated with cortisone had 9.7 (4-22) IgM containing cells per villus-crypt unit (n = 15) and a ratio of IgA:IgM containing cells of 2.9:1. The comments above on partial villous atrophy and counts of IgA containing cells apply to IgM containing cells also.

Specific IgM fluorescence other than in cells was more extensive in the intestines of some infected mice than in uninfected mice. Cortisone treatment suppressed this extra fluorescence in some cases, but not in others, most commonly the basement lamina of cryptal and villous epithelium stained in infected mice but not in uninfected mice, and intercellular fluorescence not seen in uninfected mice occurred in the villi of some infected mice.

iii) IgG_1 and IgG_2

In infections with two H. diminuta IgG_1 and IgG_2, like IgM, did not appear on the worm surface until day 12 post infection; on this day from about 30 to 80% of the surface fluoresced (Table 5 - 3). With 6c infections, by day 9 50-80% of the tegument stained in the untreated mouse but, as with IgA and IgM, the

fluorescence was more extensive (80-90%) in the cortisone treated mouse early in the infection. On days 11 and 13 the entire tegument fluoresced in treated and untreated mice, and the fluorescence was more intensive in the latter. In both 2c and 6c infections specific IgG_2 fluorescence was normally more extensive and intensive than IgG_1 fluorescence on the worms. Unlike IgA and IgM the distribution and abundance of IgG_1 and IgG_2 on the worms did not depend on whether the mice were homebred or commercially raised. In contrast to material fixed before sectioning, just described, no IgG_1 or IgG_2 were detected on worms fixed after sectioning.

Cells containing IgG_1 and IgG_2 were not abundant in the lamina propria of uninfected mice and no increase in cell numbers was detected in infected mice. With 6c infections intercellular fluorescence in the lamina propria and fluorescence associated with the cryptal and villous basement lamina were more extensive on days 9 and 13 than in uninfected or infected, cortisone treated mice; on day 11, however, notable differences did not occur. Similar, inconsistent changes occurred in 2c infections.

iv) C_3

Using FITC conjugated RAM C_3 serum (Nordic) on material fixed before sectioning fluorescence occurred on the surface of H. diminuta on days 9, 11 and 13 in 6c infections of mice given cortisone or 0.85% NaCl (Exp. 24). The fluorescence, was granular (Fig. 5 - 20) rather than linear (Fig. 5 - 18). This granular fluorescence is characteristic of specific C_3 fluorescence in some types of glomerulonephritis and has

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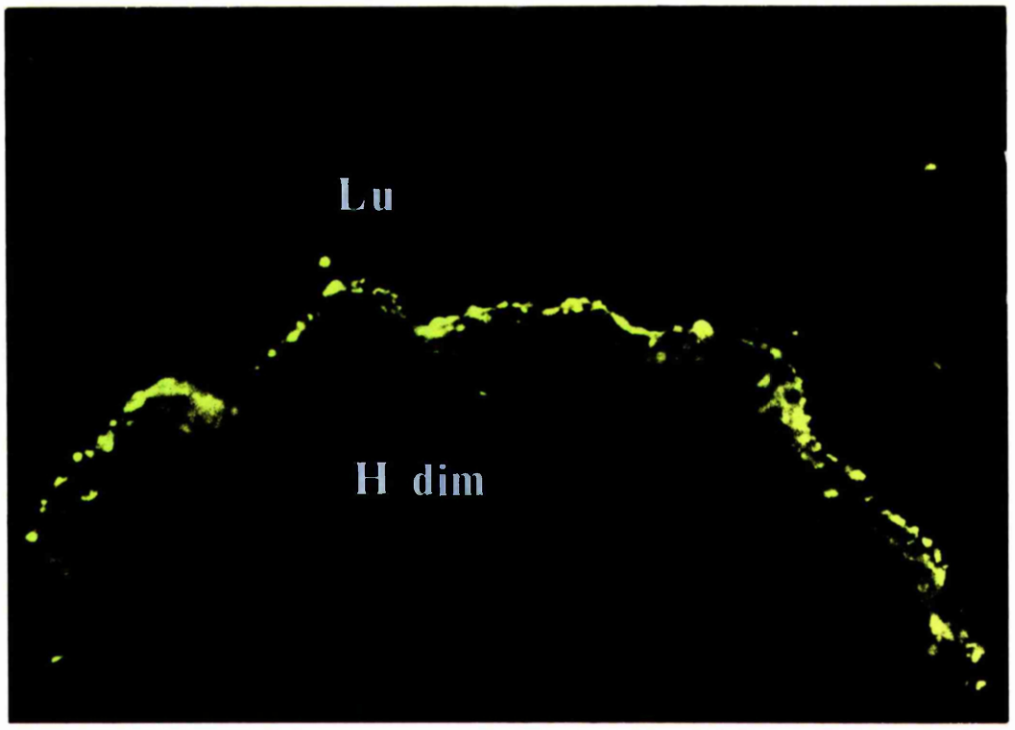
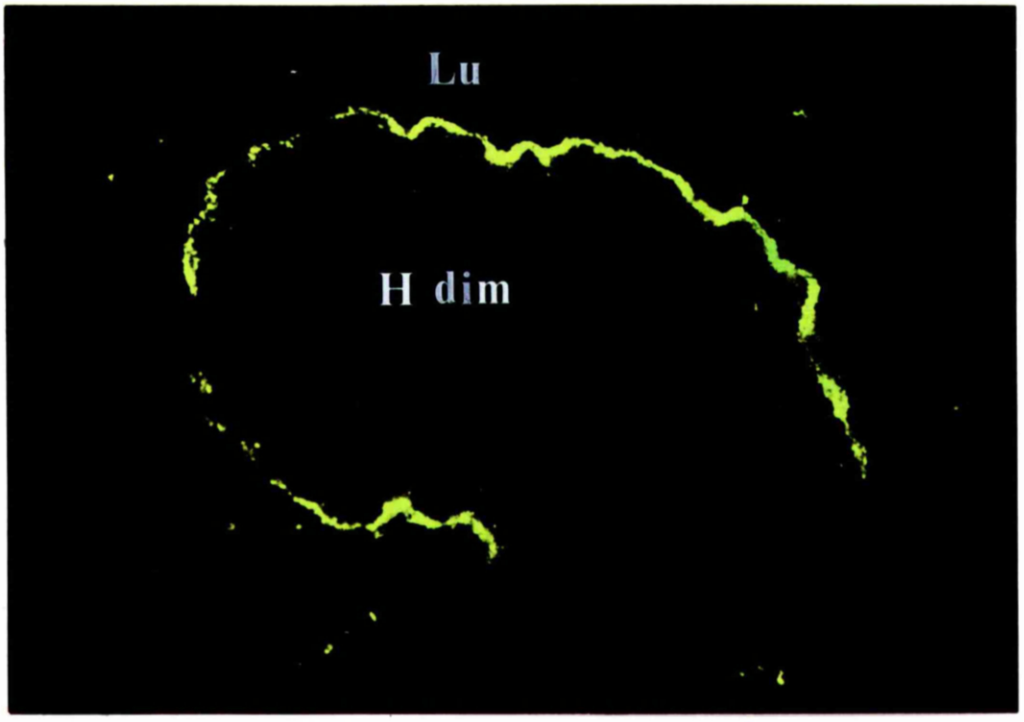
Figure 5 - 20

Section of an 11 day old Hymenolepis diminuta from a mouse showing lumpy bumpy C_3 fluorescence (Nordic antiserum) on tegument; tissue fixed in formalin prior to freezing and sectioning. (approx. X 400.)

Figure 5 - 21

An unsuccessful blocking control which attempted to depress the lumpy bumpy C_3 fluorescence (Nordic antisera) on the tegument of an 11 day old Hymenolepis diminuta; tissue fixed in formalin prior to freezing and sectioning. (approx. X 200.)


Lu
H dim


Lu
H dim

been termed "lumpy bumpy" in character (Turk, 1969). With tissue fixed after sectioning, fluorescence was present on the worm surface but was relatively thick and linear rather than lumpy bumpy.

In Exp. 24 blocking controls were not attempted for the lumpy bumpy fluorescence as nonconjugated antisera had not arrived from the supplier. In a subsequent study with purchased CD-1 mice and homebred hairless mice given 2c infections the occurrence of lumpy bumpy fluorescence on H. diminuta 9, 11 and 13 days old was confirmed. Nonconjugated RAM C_3 serum (Nordic) was available for blocking controls in these studies, but the controls were unsuccessful in all cases (Fig. 5 - 21), even when the incubation period with the nonconjugated antiserum was extended from the normal 30 min to about 24 h, and when successful blocking controls for IgA fluorescence were run concurrently. Blocking controls were unsuccessful on preparations fixed after sectioning also.

As the blocking control failed to depress the lumpy bumpy fluorescence, another commercial FITC conjugated RAM C_3 antiserum, namely that produced by Cappel (Table 5 - 1), was studied. The antiserum incubated on preparations fixed before sectioning produced lumpy bumpy fluorescence on the worms (Fig. 5 - 22), albeit of relatively weak intensity and with smaller lumps (compare with Fig. 5 - 20). The blocking control with RAM C_3 (Cappel) depressed the fluorescence and altered it from lumpy bumpy to a fine line (Fig. 5 - 23) suggesting that it was in part specific fluorescence.

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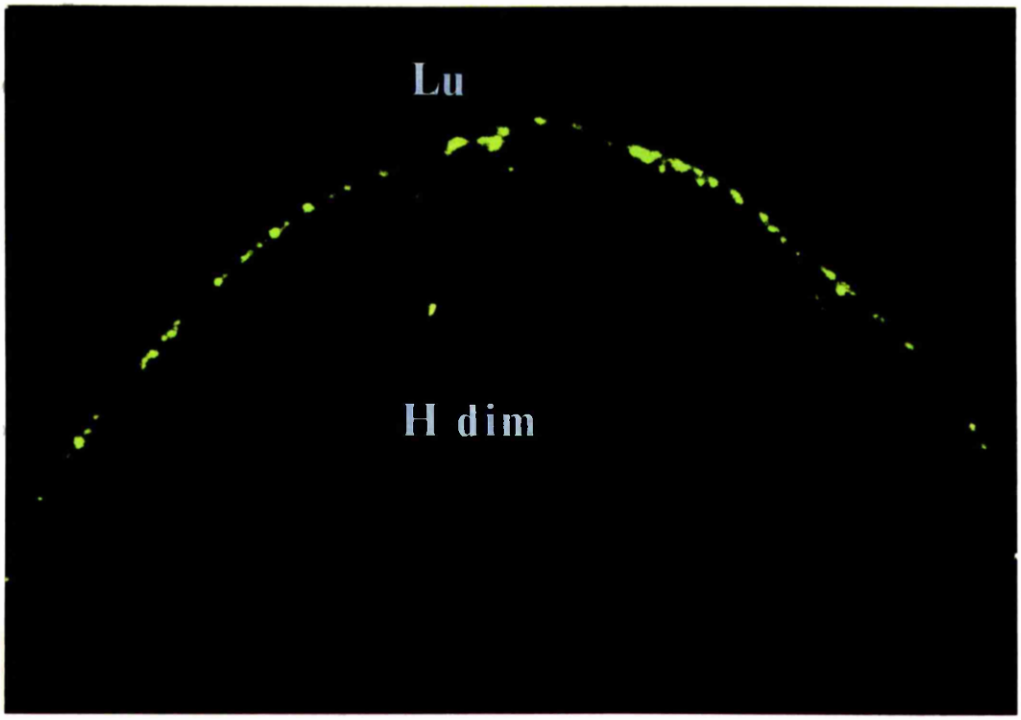
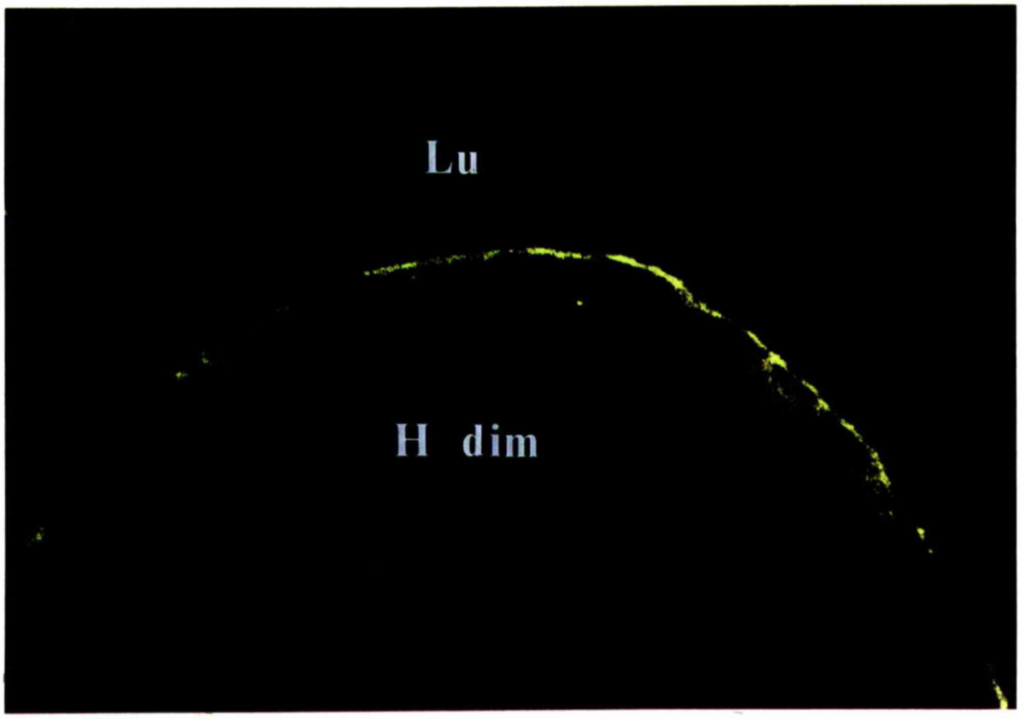
Figure 5 - 22

Section of a 13 day old Hymenolepis diminuta from a mouse showing lumpy bumpy C_3 fluorescence (Cappel antiserum) on tegument; tissue fixed in formalin prior to freezing and sectioning. (approx. X 750.)

Figure 5 - 23

A blocking control which altered C_3 fluorescence (Cappel antisera) on the tegument of a 13 day old Hymenolepis diminuta from lumpy bumpy to linear in nature; tissue fixed in formalin prior to freezing and sectioning. (approx. X 750.)


Lu
H dim


Lu
H dim

In the intestinal wall of infected mice the fluorescence produced by both Nordic (Fig. 5 - 24) and Cappel antiserum was similar and in general was more extensive than in uninfected mice. As with Igs, however, differences between infected and uninfected mice were inconsistent and require more study.

c) other, preliminary observations on H. diminuta infections

i) infection of a nude (athymic) mouse

Concurrently with the above studies Mr. P. Bland was characterizing the dynamics of H. diminuta infections in nude (athymic) mice and in their normal littermates. He found that whereas the littermates reject single H. diminuta in about 2 weeks as is typical of other mice, in nude mice H. diminuta survives well for at least 5 weeks (as long as studied). To determine if Igs and C_3 occur on the surface of worms from nude mice, Mr. Bland kindly provided a female nude (57 days old) given one H. diminuta 13 days previously for immunofluorescent studies. The mouse was homebred and from stock originally acquired from Dr. E.M. Pantelouris, University of Strathclyde, Glasgow; at autopsy no thymus was found in the mouse.

The intestine with the worm in situ was fixed before sectioning and using the appropriate antisera, IgA, IgM, IgG_1, IgG_2 and C_3 were detected on the worm. IgA covered the entire tegument with a fine linear fluorescence; similarly IgM covered about 50% and IgG_1 and IgG_2 25-50% of the tegument. The C_3 (Nordic antiserum) fluorescence was indistinguishable from the

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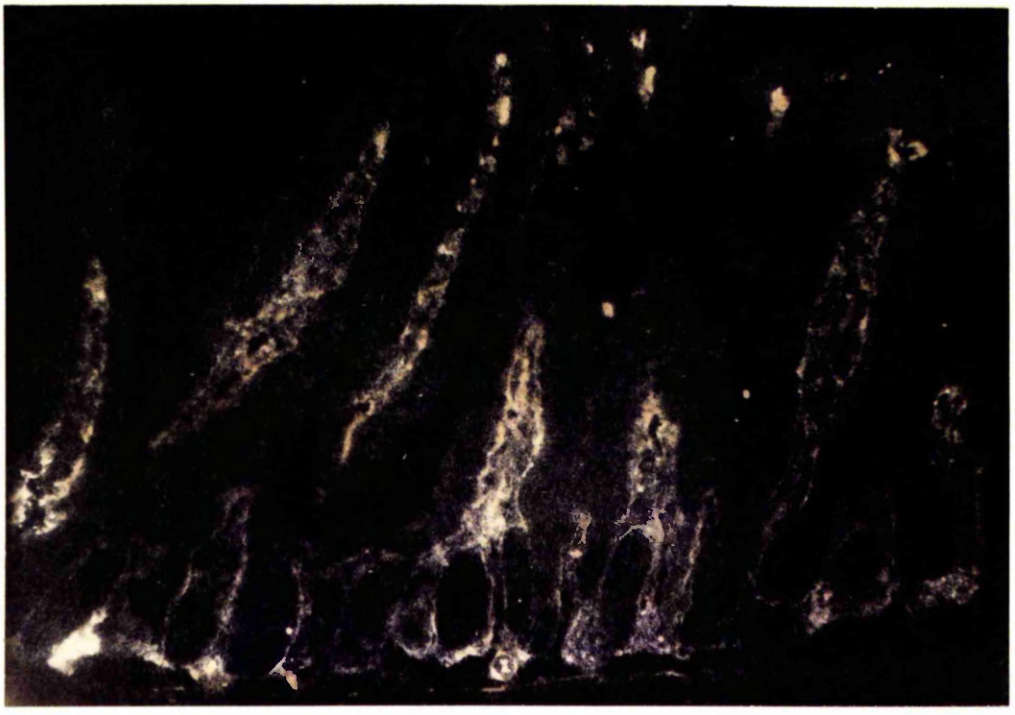
Figure 5 - 24

Section of the intestine of a mouse infected with two Hymenolepis diminuta for 11 days showing specific C_3 fluorescence on the basal lamina of the epithelium and intercellularly throughout the lamina propria; tissue fixed in formalin prior to freezing and sectioning. (approx. X 200.)



lumpy bumpy fluorescence described above. A blocking control for IgA worked well (no controls were conducted for the other Igs) but one for C_3 was unsuccessful. In the intestinal wall a few IgA containing cells occurred (maximum in a villus-crypt unit was five) and IgM was detected on about 10% of the mucosal and serosal surfaces of the muscle layer. No other fluorescence occurred in the intestine.

These are preliminary results but they suggest that the surface of H. diminuta in nude mice does have Ig and C_3 on it, albeit perhaps less than on worms from normal mice. Further studies on infected nude mice, and concurrently on infected normal littermates must be conducted to confirm these suggestions.

ii) infection of a rat

As H. diminuta survives well in rats at low intensities of infection (see DISCUSSION SECTION 3), it was of interest to determine if fluorescence induced by various FITC conjugated antisera occurred on the tegument of worms in rats. Therefore, tissue from one homebred male rat (age unknown) given six H. diminuta 67 days previously was fixed before sectioning for immunofluorescent studies. Few antisera against rat Igs are commercially available, hence FITC conjugated GAM IgA, IgM and IgG_1 and RAM C_3 (Nordic) were used in the studies as cross reactions between the various mouse and rat Igs are extremely likely (Tomasi & Grey, 1972). Indirect anti-globulin immunofluorescence was conducted with RAR IgG and RAR C_3, and FITC conjugated SAR IgG as described in the MATERIALS AND METHODS.

Using the GAM sera a fine linear fluorescence of IgA covered the entire tegument, IgM stained only about 25% and IgG_1 did not stain the worm surface at all. With the RAM C_3 serum lumpy bumpy fluorescence occurred as described above. Fluorescence in the intestinal wall appeared similar to that in mice with the exception of the notable abundance of nonspecific fluorescence of eosinophils in the rat. Whether this abundance is characteristic of rats or of the infection in rats is unknown. Studies with RAR IgG revealed fluorescence on about 25% of the worm surface and with RAR C_3 linear, rather than lumpy bumpy, fluorescence covered at least 75% of the worm surface. Unfortunately no controls were studied and, as suggested for the above observations on the infection of a nude mouse, confirmation is essential.

d) infections of H. microstoma in mice

Most of the information below is from two experiments conducted, as shown in Table 5 - 4, in 6 week old mice purchased from commercial suppliers. Occasionally results from studies on homebred mice of various ages and strains are presented. About 4-5 days post infection H. microstoma migrates into the bile duct and attaches there, subsequently causing extensive hypertrophy and chronic inflammation of the duct (Bogitsh, 1966; Lumsden & Karin, 1970). Accordingly, the distribution and abundance of Igs in both the bile duct and the intestine, immediately posterior to the opening of the bile duct, are described. When specific Ig fluorescence occurred on the tegument of H. microstoma (Fig. 5 - 25 & 5 - 26) it was indistinguishable from the fluorescence on H. diminuta. The time of appearance and

Table 5 - 4

Protocol of experiments for immunofluorescent studies on Hymenolepis microstoma infections in mice

Exp.	Mice	Groups	Days studied (all tissue fixed in formalin before sectioning) bile duct	intestine
26	C_3H	5c H. microstoma	8, 12, 20, 48, 79, 100	8, 12, 20, 48, 79, 100
		sham	8, 12, 20, 48, 79	8, 12, 20, 48, 79
29	CFLP 4 star	5c H. microstoma	8, 12, 25, 34, 40, 52	8, 12, 25, 34, 40, 52
		sham	8, 12, 25, 40, 52	8, 25, 40, 52

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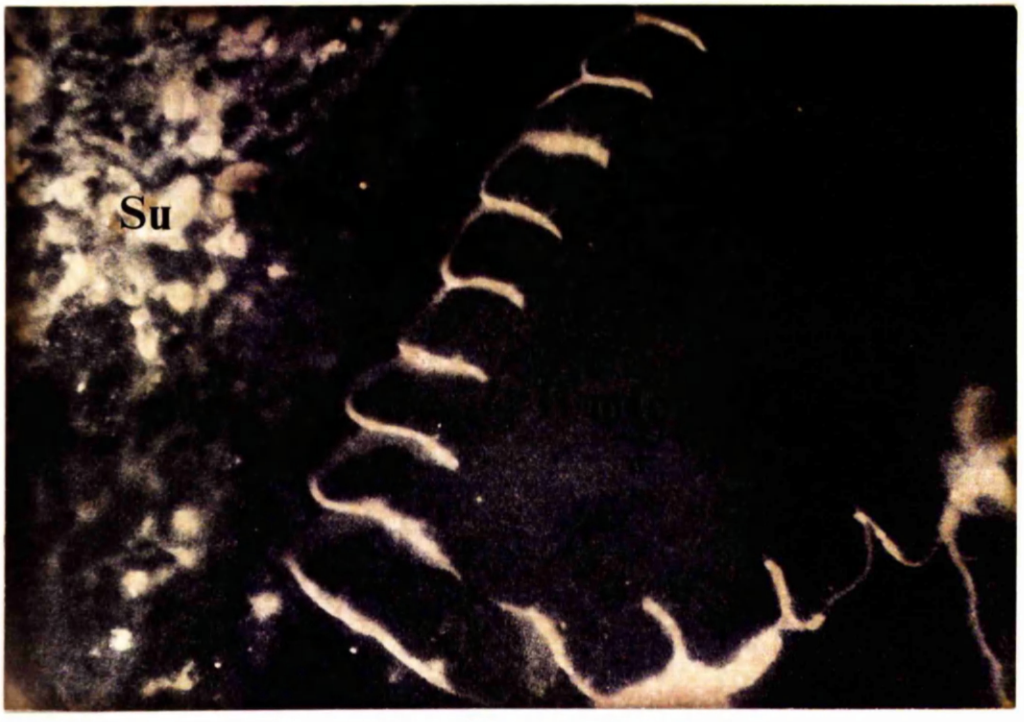
Figure 5 - 25

Section of the hypertrophic bile duct of a mouse infected with Hymenolepis microstoma for 266 days showing specific IgA fluorescence in the subepithelium of the bile duct and as a fine layer on the worm tegument; tissue fixed in formalin prior to freezing and sectioning. (approx. X 400.)

Figure 5 - 26

Section of two 100 day old Hymenolepis microstoma in the bile duct of a mouse showing a broad layer of specific IgA fluorescence on the worm surface and nonspecific fluorescence on the eggs (arrows); tissue fixed in methanol after freezing and sectioning. (approx. X 500.)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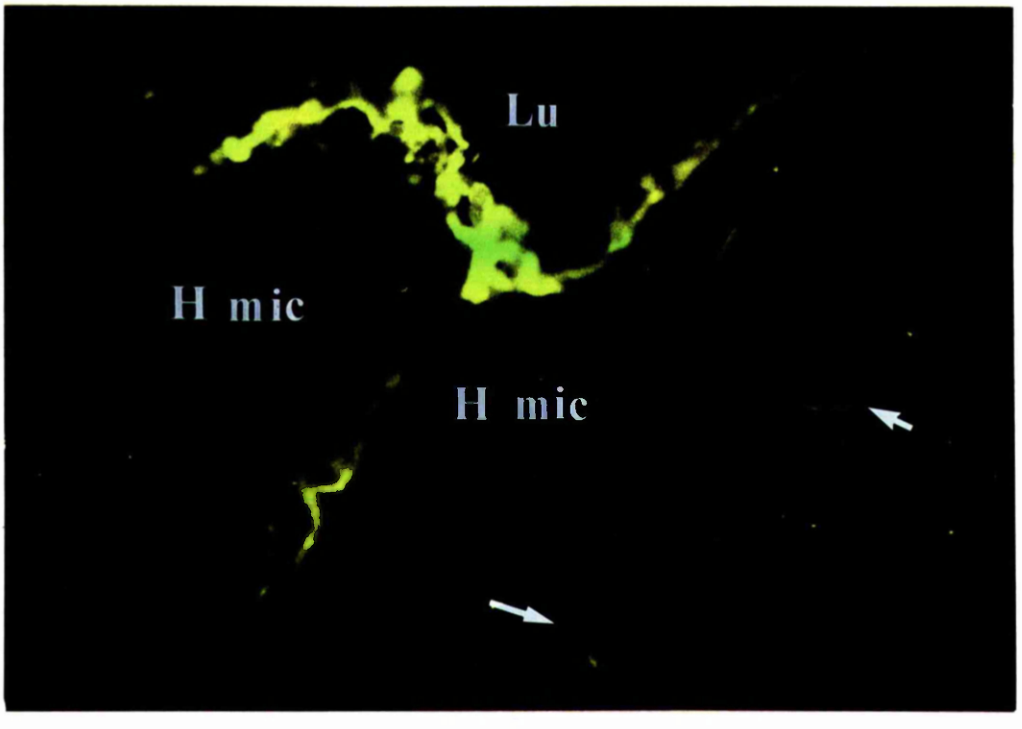
Lu
H mic
H mic

relative abundance of Igs on H. microstoma are summarized in Table 5 - 5.

i) IgA

In Exp. 29 specific fluorescence occurred on about 5% of the tegument of H. microstoma in the bile duct on day 8 post infection but in Exp. 26 no fluorescence occurred on this day (Table 5 - 5). On day 12 in both experiments 10-15% of the worm surface stained. By day 25 (Exp. 29) the worm surface was completely covered with fluorescence and remained so thereafter in both experiments (records are incomplete for day 20 Exp. 26). The intensity of fluorescence seemed to increase with the duration of the infection. Eosinophils were abundant in the subepithelial connective tissue of the bile duct, which was beginning to hypertrophy by day 8, but no specific fluorescence occurred. On day 12, however, some IgA containing cells were detected in the subepithelial tissue and the numbers increased by days 20-25. Such cells were numerous (Fig. 5 - 27) in the wall of the hypertrophic bile duct on all subsequent days studied. In addition to intracellular fluorescence, intercellular fluorescence of variable intensity was scattered throughout the wall of the bile duct by days 20 and 25 and thereafter (Fig. 5 - 27), and as with fluorescence on the worm surface, this intercellular fluorescence appeared to increase in intensity with the duration of the infection.

IgA was detected on the strobila in the intestine by day 12 (only in Exp. 26) whereas in the bile duct it occurred on some worms by day 8, suggesting that there was a delay in the appearance of IgA on the

Table 5 - 5

The time of appearance and relative abundance of immunoglobulins (Igs) on the tegument of Hymenolepis microstoma

Igs	Habitat	Age of worms (days)									
		8	12	20	25	34	40	48	52	79	100
IgA	bile duct	-, +*	+		+5	+5	+5	+5	+5	+5	+5
	intestine	-	-, +		+4	+5	+5	+5	+5	+5	+5
IgM	bile duct	-	-	+	+	+	+2	+3	+2	+3	+4
	intestine	-	-		+	+	+2		+3	+2	+3
IgG_1	bile duct	-	-	+3	+2	+3	+5	+3	+3	+4	+5
	intestine	-	-				+4		+5	+5	+5
IgG_2	bile duct	-	-	+2	+	+2	+4	+3	+3		+3
	intestine		-		+2			+	+3		+3

* Relative abundance of Igs estimated as: - negative, + 1-25% of worm surface with fluorescence, +2 26-50% positive, +3 51-75% positive, +4 76-99% positive, +5 100% positive; a blank indicates that records are incomplete.

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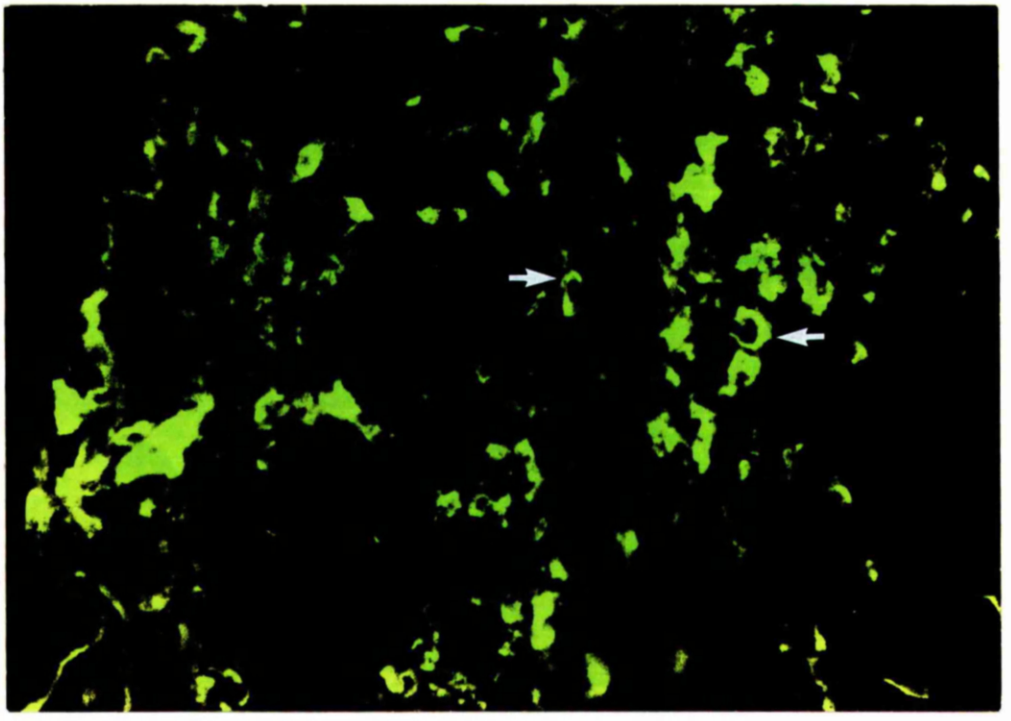
Figure 5 - 27

Section of the hypertrophic bile duct of a mouse infected with five Hymenolepis microstoma for 266 days showing specific IgA fluorescence in plasma cells (arrows) and throughout the intercellular spaces of the subepithelium; tissue fixed in formalin prior to freezing and sectioning. (approx. X 500.)



portions of the worms in the intestine. In accordance with this suggestion, by day 25, when in the bile duct the entire tegumental surface fluoresced, only 75-80% of the worm surface in the intestine fluoresced. On all days subsequently studied the entire worm surface stained. What appeared to be the outer coat (terminology of Rybicka, 1966) of the eggs *in utero* fluoresced on all days studied and with all the antisera used, but this was found to be nonspecific fluorescence (Fig. 5 - 26). No counts were conducted on IgA containing cells in villus-crypt units because after day 20 partial villous atrophy (see Fig. 5 - 19) occurred as with *H. diminuta* infections. IgA containing cells were abundant in these villi and subjective evaluations suggested their numbers exceeded those of uninfected mice. In addition to partial villous atrophy, hypertrophy of the circular and longitudinal muscles occurred in the intestinal wall where worms were present in the lumen. The earliest this was recorded was day 34 post infection, but it may have occurred earlier, albeit less obviously. After day 20 the distribution of other specific fluorescence in the intestinal wall exceeded that in uninfected mice. In particular fluorescence on the serosal and mucosal surfaces of the muscle layer, on the basement lamina of cryptal and villous lamina propria and within the lumen was more extensive and intensive.

With tissue fixed in methanol after sectioning, fluorescence on the worm surface (Fig. 5 - 26) was similar in its time of appearance and relative abundance to fluorescence on worms fixed in formalin before sectioning.

ii) IgM

Specific IgM fluorescence, covering <25% of the surface of the worm in the bile duct, first occurred days 20-25 post infection (Table 5 - 5). On day 40 and thereafter the fluorescence varied in extent from about 35 to 75% of the worm surface. In general the extent and intensity of fluorescence increased with time and by day 100 >75% of the worm surface fluoresced. The pattern of appearance and distribution of IgM on the worms in the intestine was similar to that on the worms in the bile duct in Exp. 29. However, in Exp. 26 IgM was detected only on days 79 and 100 on the worms in the intestine and occupied 26-50% of the surface (records for day 48 do not include IgM), although in the bile duct tegumental fluorescence occurred as early as day 20 (see above). In summary, IgM occurs on H. microstoma in both the bile duct and intestine but takes longer to appear and is never as abundant as IgA. Unlike infections of H. diminuta where IgM was not detected on the worm surface when the tissue was fixed after sectioning, with H. microstoma specific fluorescence occurred by day 23 post infection. On subsequent days studied with this method the extent of this fluorescence increased and in general was similar to that detected on worms fixed before sectioning.

Intercellular fluorescence occurred in the entire wall of the bile duct by day 8 post infection, considerably earlier than for IgA, and in general increased in intensity with the duration of the infection. IgM containing cells were detected in the subepithelial connective tissue by day 12 and increased in number by

days 20-25 but were always obviously less numerous than IgA containing cells. Fluorescence in the intestinal wall of infected mice was more extensive than in uninfected animals after day 20.

iii) IgG_1 and IgG_2

Specific IgG_1 and IgG_2 fluorescence on the surface of worms in the bile duct first occurred days 20 and 25 post infection as did IgM, but the fluorescence induced by the IgG antisera was more extensive (Table 5 - 5). On 5 of the 7 days studied thereafter IgG_1 was more extensive than IgG_2 fluorescence, covering respectively 51-100% and 26-99% of the worm surface. Data on worms in the intestine is less complete than on worms in the bile duct; where there is information, however, no consistent differences occurred between the two habitats. As with IgM, when tissue was fixed after sectioning specific fluorescence occurred on the worm surface and its extensity increased with the duration of infection.

Fluorescence was scattered throughout the subepithelium of the hypertrophic bile duct but it occurred in uninfected animals as well (see above). Only on days 40 and 79 was the intensity of this fluorescence clearly greater in the infected than in the uninfected mice. Cells containing IgG_1 and IgG_2 rarely were seen but as the subepithelial fluorescence was intense, particularly with IgG_1, cells with cytoplasmic fluorescence could have been masked. In the wall of the intestine beginning on days 20 and 25 the fluorescence was more extensive and intensive in infected

than in uninfected mice and the differences became greater with time.

iv) C_3

H. microstoma was tested for C_3 on its surface using Nordic antiserum and as early as 20 days post infection fluorescence occurred on about 15% of the worm surface from sections of a hypertrophic bile duct fixed before sectioning. In distinct contrast to fluorescence induced by C_3 antisera on the surface of similarly fixed *H. diminuta*, however, the fluorescence was weak and linear rather than intense and lumpy bumpy. On subsequent days studied the presence and relative abundance of C_3 fluorescence on *H. microstoma* was unpredictable, ranging from being absent to covering about 75% of the surface. Blocking controls were attempted and they successfully removed the worm surface fluorescence, although they were less successful in depressing the fluorescence in the wall of the bile duct. As stated above C_3 fluorescence occurred in the wall of the bile duct, but it was indistinguishable from that in uninfected animals.

DISCUSSION

In summary of the major aspects of the above RESULTS, no precipitating anti-worm antibodies were detected in either the serum or gut contents of infected animals. Further, using DID and SRID the only Ig detected in the gut contents was IgA and its presence was independent of the history of tapeworm infection of the mice. No differences were detected in the concentrations of intestinal IgA or serum IgA, IgG_1, IgG_2 and IgM between mice infected with H. diminuta or uninfected mice. Direct anti-globulin immunofluorescent studies on H. diminuta in situ showed that, contrary to the results of immunodiffusion studies, all Igs studied can be detected in the intestinal lumen, specifically on the worm tegument, when the tissue is fixed before sectioning. Differences occur in the time of appearance and relative abundance of the different Igs (Table 5 - 3); specific IgA fluorescence can be detected first and is most abundant. This is most notable when the tissue is fixed after sectioning, as then only IgA can be detected on H. diminuta. Often the distribution and abundance of the various Igs in the intestinal wall of mice infected with H. diminuta exceeded that in uninfected mice but these differences were not without inconsistencies. Fluorescence induced by C_3 antisera also occurred on the tegument of H. diminuta but, although it appeared lumpy bumpy as has been reported for specific C_3 fluorescence elsewhere, the blocking control with one antiserum was

unsuccessful and the other altered but did not suppress the fluorescence.

Studies on H. microstoma in the bile duct and intestine showed that on this worm which, unlike H. diminuta, survives well in mice, IgA, IgG_1, IgG_2 and IgM can be detected independent of what fixation method is used. These Igs appear more slowly on H. microstoma than on H. diminuta but as with the latter, specific IgA fluorescence appears first and is most abundant (Table 5 - 5). The increase in the distribution and abundance of Igs which occurred often in the intestine of mice infected with H. diminuta occurred more consistently in the bile duct and intestine of mice infected with H. microstoma. In addition to the well known hypertrophy of the bile duct, partial villous atrophy and hypertrophy of the intestinal muscle layers are other characteristics of H. microstoma infection. Fluorescence induced by C_3 antiserum occurred on H. microstoma but was unpredictable in occurrence and linear, not lumpy bumpy as on H. diminuta.

In response to intestinal microbial infections there are increases in the concentrations of intestinal and serum Igs (see INTRODUCTION). Apparently with helminth and protozoan infections the only report on intestinal Ig concentrations is by Crandall, Crandall & Franco (1974), although there are many on changes in serum Ig concentrations (e.g. Crandall & Crandall, 1971, 1972; Abioye, Lewis & McFarlane, 1972; Cappuccinelli, Martinetto, Frentzel-Beyme & Sena, 1973; Kellermeyer, Warren, Waldmann, Cook & Jordan, 1973). The lack of consistent alterations in the concentrations of serum Igs

in infections with H. diminuta is perhaps at first sight surprising, but on closer examination all infections, with the possible exception of the protozoan Giardia lamblia, studied by the above authors are systemic or where intestinal, cause considerable mucosal damage. H. diminuta on the other hand is confined to the intestinal lumen and apart from partial villous atrophy causes no obvious mucosal damage. The suggestion that 'systemic' parasites induce raised levels of serum IgE but intestinal parasites restricted to the lumen throughout their life in the host do not (Radermecker, Bekhti, Poncelet & Salmon, 1974), may apply to other Igs also. Moreover, SRID employed herein does not detect antibodies but only levels of Ig and therefore, although anti-worm antibody may occur, it may be insufficient to detectably alter the level of Ig.

The Crandalls in their three papers listed above concluded that increases in the concentrations of various serum Igs occur in response to Ascaris suum, Trichinella spiralis and Heligmosomoides polygyrus (= Nematospiroides dubius) infections. Some of the increases are impressively large (e.g. IgM in A. suum infections) and probably are real elevations resulting from infection but unfortunately no uninfected control animals were studied concurrently. Hence, their results are inconclusive as the present studies show that elevations can occur with time regardless of infection with the test worm. It is well known that the exposure of a young animal to antigenic stimulation is essential for the maturation of lymphoid tissue (Crabbé, Nash, Bazin, Eyssen & Heremans, 1970), and hence the acquisition of serum Ig levels

characteristic of adults. Accordingly elevations seem particularly likely when relatively young animals (≤6 weeks old) are purchased from high grade colonies, subjected to the rigours of travel and then housed in environments where they are exposed to microbial, protozoan and helminthic infections they have not experienced previously. A priori young (about 6 week old) homebred animals raised in relatively rich antigenic surroundings should be more competent than mice of the same age raised in high grade (less antigenic stimulation) commercial environments. This would explain the observations with immunofluorescence that homebred animals are more rapidly responsive than those purchased from suppliers. Unquestionably for SRID studies animals not infected with the test worm are essential as controls; certainly in clinical studies carefully matched controls are used (Kellermeyer et al. 1973).

The low coefficient of variation (2.5%) in the present study provides confidence in the results, as does the fact that the ranges of concentrations of IgM and IgG_1 in the serum of the mice studied by Crandall & Crandall (1971) overlap the ranges obtained herein. Although the range of concentration of serum IgA falls within the range presented by Crabbé, Bazin, Eyssen & Heremans (1968), the absolute concentrations presented here may be in error. Tomasi & Grey (1972) and Samson et al. (1973) have discussed the difficulties that IgA with its diversity of molecular sizes, presents when SRID studies are conducted. One problem is that the antiserum dispersed in the agar should be specific for the molecular size predominant in the sample. Fortunately the

commercial anti-IgA serum was against an 11S molecule; mouse serum IgA is predominantly 11S (INTRODUCTION) as is intestinal IgA, albeit the latter contains the additional component, SC. Another problem is that the sample should be compared with a reference which is of the same molecular size, as larger molecules are less mobile in agar than smaller ones, and if, say, a sample of a human secretion with predominantly 11S IgA was compared with a reference containing largely 7S IgA the concentration in the sample could be underestimated by 3-10 fold (Samson et al. 1973). The reference IgA used in the present study was a 7S molecule (MATERIALS AND METHODS), and hence the IgA concentrations calculated for the serum and intestinal contents are probably underestimates of unknown degree. Other difficulties discussed by Tomasi & Grey (1972) and Samson et al. (1973) include variations in the rate of fluid secretion in the intestine and in methods of storage of samples. By starving all animals prior to killing, and freezing the samples with only short term thawing on two occasions attempts were made to minimize variation due to these problems.

As shown above it is not difficult to rationalize the lack of a difference in serum Ig concentrations between uninfected and infected mice but why, as shown by Crandall et al. (1974) for H. polygyrus, no consistent difference was detected in levels of intestinal IgA is unknown. The surface area of H. diminuta is large and as in 6c infections the entire worm surface is covered by specific IgA fluorescence by day 9 post infection (Table 5 - 3) a significant portion of the IgA released into the intestine must be on the worm

surface. Given the procedure employed for the collection of intestinal contents it is likely that IgA on the worm surface was not collected in the supernatant studied by SRID, and hence the values for IgA concentrations in the intestine of infected mice are low. This difficulty could be solved by eluting the IgA from the worm surface and summing the amount of IgA in the lumen and on the worm to estimate the amount in the intestine of infected mice. The possibility of eluting Igs from the worm surface will be discussed more fully below.

In support of the suggestion that there is an increase in IgA in the intestine of mice infected with Hymenolepis sp., Bazin et al. (1973) reported that, in otherwise axenic mice, H. nana infection for 30 days stimulates an increase in the number of IgA plasma cells comparable with the increase which would occur in mice of the same age but kept under conventional, rather than germ free, conditions for 30 days. Preliminary studies reported herein showed no differences between infected and uninfected mice in the numbers of plasma cells, perhaps because of the large variation encountered. In part this variation is due to the method of sectioning employed which made routine acquisition of satisfactory villus-crypt units for counts difficult. Variation in the immunological history of individual mice must also influence the numbers of plasma cells. Axenic mice as used by Bazin et al. (1973) have few plasma cells and there is little variation in number. Therefore, these mice are particularly well suited for studies on plasma cell numbers as increases are more easily detected in

monocontaminated mice than in conventional mice, where changes in cell numbers in response to worm infection may be masked by responses to other organisms.

Changes in plasma cell numbers were not detected when the majority of the cell counts were from mice given two H. diminuta, but as 6c infections are more immunogenic (SECTION 3) changes might be detected with these heavier infections. With chronic infections of H. microstoma perhaps increases would be easily detected. However, the unforeseen difficulty of partial villous atrophy arose in infections with H. microstoma, and also with H. diminuta, especially in mice treated with cortisone. Atrophy was correlated with the presence of large worms and may be a result of the pressure of the worms. Recently Ferguson & Jarrett (1975) showed that partial villous atrophy which occurs in rats infected with N. brasiliensis did not occur when the rats were deprived of T cells, a fact which suggests that it may be a worm induced autoimmune phenomenon. That atrophy occurred in mice infected with H. diminuta and treated with cortisone indicates that it has a different etiology in H. diminuta than in N. brasiliensis infections.

Intercellular fluorescence occurred in the intestinal lamina propria and in the subepithelium of the bile duct. There were no differences in the distribution of intercellular IgA fluorescence in uninfected or mice infected with H. diminuta, but with IgG_1, IgG_2 and IgM increases occurred in many, albeit not all, infected animals. This increase must be due to leakage from the circulation rather than local production, as particularly with IgG_1 and IgG_2 there are few local plasma cells and

in most cases cortisone, which is known to depress vascular permeability in parasitic infections (Rose & Long, 1969; Murray, Jarrett & Jennings, 1971), depressed the increase. In the hypertrophic bile duct of mice infected with H. microstoma increases occurred in IgM, by day 8, and IgA by day 20 post infection, and their abundance increased further with time. IgG_1 and IgG_2 were abundant in the normal bile duct and increases were difficult to identify objectively but occurred on some of the days studied after prolonged infection. The increase in IgM occurred prior to the appearance of IgM plasma cells in the bile duct indicating that the Ig was of systemic origin as was the IgG_1 and IgG_2 there. As the appearance of intercellular IgA correlated with the presence of IgA plasma cells this Ig was probably of predominantly local origin. With the appearance of IgM plasma cells in the bile duct local production probably accounted for a portion of this Ig present. The origins of Igs in the intestinal wall were probably similar to those in the bile duct. The increased abundance of Igs, particularly at the site of a chronic inflammatory response such as in the bile duct of mice infected with H. microstoma, is a phenomenon called "pathotopic potentiation" (see Brandtzaeg, 1973).

The two methods of tissue preparation employed in the present immunofluorescent studies, namely formalin fixation prior to freezing and sectioning, and methanol fixation after freezing and sectioning, have been used commonly. Consistent with the results of other studies (e.g. Saint-Marie, 1962; Eidelman & Berschauer, 1969) plasma cells in tissue which is fixed prior to freezing

are preserved well, stain intensely and, apart from the exception of tissue with severe pathotopic potentiation, are easily distinguished from the limited background fluorescence. In tissue fixed post sectioning there has been diffusion of Ig from plasma cells into intercellular spaces making it difficult to distinguish the cells from the background fluorescence. Furthermore as Saint-Marie (1962) stated of fixation in ethanol prior to sectioning, in tissue fixed in formalin before sectioning the immunofluorescent technique is more sensitive than in tissue fixed in methanol after sectioning. This was clearly demonstrated by the detection of all Igs on H. diminuta fixed prior to sectioning but only IgA on H. diminuta fixed after sectioning. Accordingly, as both methods detected all Igs on H. microstoma, their concentrations must be greater than on H. diminuta. Why the diffuse coat of IgA on the epithelium was detected in tissue fixed post sectioning but not in tissue fixed before sectioning is unknown. Perhaps formalin alters the antigenicity of the IgA in this coat, as is known for some other antigens (Deng & Beutner, 1974; Gatti, Östborn & Fagraeus, 1974). Apparently, even incubation with 30% sucrose does not restore the antigenicity, although it does in other cases (Deng & Beutner, 1974). In contrast, with both methods IgA was detected on the cryptal basement lamina and, particularly with tissue fixed after sectioning, in the apical regions of cryptal epithelial cells. Fluorescence was never detected in epithelial cells of the villi, as reported by Poger & Lamm (1974), and seldomly on their basement lamina. These observations suggest that selective transport of

IgA (maybe IgM also, see INTRODUCTION) into the lumen occurs largely in the cryptal region. The association of SC with the cryptal epithelium but rarely near the tips of the villi supports this suggestion (Brandtzaeg, 1974 a).

In mice infected with H. nana anti-worm antibodies have been detected by indirect hemagglutination (Coleman & DeSa, 1964), by passive transfer of protection (Weinmann, 1966) and by micro-complement fixation (Coleman, Carty & Graziadei, 1968). Moss (1971) demonstrated IgE and IgG_1 (he called IgA) antibodies against H. microstoma in mice by passive cutaneous anaphylaxis (PCA) tests. Goodall (1973) confirmed the findings of Moss and added that IgG_{2a} antibodies occur. With H. diminuta infections of rats anti-worm antibodies have been detected by micro-complement fixation (Coleman et al. 1968) and by PCA (IgE and IgG_1) and indirect immunofluorescence tests (Harris & Turton, 1973). In mice however, antibody against H. diminuta has not been detected even though strong protective immunity exists, unlike in H. diminuta infections of rats or H. microstoma infections of mice.

DID is not the most sensitive test for antibody and it is not surprising that attempts at finding anti-H. diminuta antibodies have been unsuccessful. But in addition, preliminary indirect immunofluorescent studies were unsuccessful (personal observation), even though the antigen of Harris & Turton (1973), which was successful in detecting antibody in rats, was used. Furthermore, Goodall (personal communication) did not detect antibody in mice using PCA tests even though his similar studies

with H. microstoma in mice were successful. Attempts to passively transfer protection against H. diminuta to naive mice using large volumes (6 ml) of serum failed too (Hopkins, personal communication). Why all these attempts have been unsuccessful is not known; technical problems cannot be excluded but the evidence suggests that the level of circulating antibody, if any exists, against H. diminuta is low in resistant mice. It is not improbable that the majority of antibody occurs locally and not systemically, therefore the intestinal contents and specifically the Ig on the worm tegument must be investigated thoroughly.

There is no direct evidence that the Igs on the worm tegument are specific antibodies; this will only be determined when they have been eluted from the worm and subjected to tests used to demonstrate specific antibody. Indeed, the in vitro binding of IgA, IgG and IgM to the cuticle of various nematodes (Coombs, Pout & Soulsby, 1965; Hogarth-Scott, 1968) which may be nonspecific (Ogilvie, 1974 b, p.92), suggests the alternative, namely that they are not specific antibodies. If the Igs on the tegument of H. diminuta and H. microstoma are not specific antibodies then:

1) their occurrence on the tegument would depend on the availability of the Ig; with IgA which is abundant in the gut of mice the entire worm would be covered rapidly and there would be no gradual accumulation,
2) they would appear on H. diminuta and H. microstoma at about the same time post infection,
3) their time of appearance on the tegument would be independent of the intensity of infection,

particularly for IgA which is abundant in the gut,

4) they would cover the entire exposed surface of the worms including the tegument and pores of the genital and protonephridial ducts.

However, the results (Tables 5 - 3 & 5 - 5) show that IgA does not appear immediately on the worm but once it begins to appear it increases its distribution with time, suggesting that it is specific antibody combining with the antigen(s) as it is being produced. Moreover, the Igs appear on H. diminuta more quickly than on H. microstoma and with the former, 6c infections which are more immunogenic (SECTION 3) induce a more rapid appearance of Igs on the tegument than 1c infections. Igs were never observed in any ducts approaching the worm surface, although sections of such structures, e.g. cirrus pouch, were studied; this suggests that binding to the tegument is specific.

Apparently all the observations on binding of Ig to nematode cuticle have been in in vitro systems rather than in vivo binding as shown here. The nematode cuticle is chemically, physically and biologically different from the cestode tegument (Lee, 1966) and there is no reason to assume that binding, if nonspecific to nematode cuticle, should be so on cestode tegument. In fact it is widely held that host immunity is directed against worm metabolism, inhibiting feeding (e.g. Thorson, 1963) and as the cestode tegument is the digestive-absorptive surface it is where one would expect to find host antibody interacting with the worm, unlike in nematodes where the digestive tract and gland openings would be sites of attack. It is interesting that

Coleman (1961), Coleman & Fotorny (1962) and Coleman et al. (1963) stated that in infected mice specific antibody binds to H. nana. Unfortunately where the binding sites are was not given, and Fig. 1 (Coleman & Fotorny, 1962), which has no explanation, shows what is apparently fluorescence on the eggs in utero and on the seminal receptacles; such egg fluorescence is nonspecific in H. microstoma (RESULTS) and in H. diminuta (G. Harris, personal communication). Fluorescence of the seminal receptacles has not been observed herein, but Duwe (1967) stated that the reproductive organs of Moniezia expansa show autofluorescence. He demonstrated, however, that FITC conjugated rabbit antiserum to homogenized M. expansa produced fluorescence on the tegument of sections of the worm, similar to what is reported above for H. diminuta and H. microstoma.

Individually the arguments presented in the preceding two paragraphs provide limited support for the hypothesis that specific antibody covers the tegument of Hymenolepis spp. but collectively they make it almost certain. Henceforth, the Igs will be considered specific anti-worm antibody. What is the function of this antibody; is it, as is common with serum antibody, to identify as a marker molecule the antigen for some other component of the immune response?

In this context the fluorescence on the tegument of H. diminuta induced by anti-C_3 sera is potentially important. As summarized briefly in the INTRODUCTION it is considered that complement fixation does not occur in the gut because, although complement is present in all tissues and cells (Colten, 1974, p.183)

and in mucous secretions (Cluff, 1971, p.482), the intestinal contents are in *in vitro* studies anticomplementary (Fubara & Freter, 1972 b) and for *in vivo*, one sees undocumented statements such as "the conditions of pH and salt concentrations are strongly anticomplementary" (South, 1971 b, p.6). Furthermore, unaltered IgA does not fix complement, although recently it has been shown that IgA aggregates do (Boackle *et al.* 1974). Other Igs apparently are not thought important in the gut as possible activators of complement because of their apparent short survival time in the gut. The RESULTS show however, that IgG and IgM occur on the worm and accumulate with time suggesting that once on or near the tegument they are relatively insusceptible to proteolysis. Fubara & Freter (1972 a) showed that indigenous bacteria reduced proteolysis of intestinal IgG and IgM, presumably by altering enzymic activity. *H. diminuta* inactivates intestinal trypsin and chymotrypsin (Pappas & Read, 1972 a & b) thereby probably reducing proteolytic breakdown of intestinal antibody and complement and perhaps resulting in the fixation of complement on the tegument.

However, is the evidence that suggests C_3 occurs on the worm conclusive? The repeated failure of the blocking control when Nordic, but not Cappel, antisera were used makes the observation inconclusive. Furthermore, preconceptions from the literature (see above) about complement in the gut make one suspicious of the result. *A priori* assuming C_3 occurs on *H. diminuta* in mice and is a component of protective immunity, then in cortisone treated and athymic mice and in rats, all

of which do not reject H. diminuta, C_3 would not be expected to occur on the worms. C_3 induced fluorescence occurred, however, on all H. diminuta studied. On the other hand evidence upholding the suggestion that C_3 occurs on H. diminuta includes the fact that three different commercial antisera, namely Nordic and Cappel (direct immunofluorescence) and Mercia-Dako (indirect immunofluorescence) (see MATERIALS AND METHODS), produced the fluorescence, and with Mercia-Dako antisera C_3 was localized on the tegumental glycocalyx of H. diminuta by indirect immunoperoxidase ultrastructural studies (Befus & Threadgold, unpublished). Furthermore, the fluorescence induced by Nordic and Cappel antisera on H. diminuta from mice and rats was lumpy bumpy, a characteristic of specific C_3 fluorescence in immune complex diseases of the kidney (Turk, 1969; Churg & Grishman, 1972; Peters, 1974) and lung (Brentjens, O'Connell, Pawlowski, Hsu & Andres, 1974; Turner-Warwick, 1974). Lumpy bumpy fluorescence never occurred on H. microstoma, although weak linear fluorescence occurred occasionally suggesting that C_3 occurred near H. microstoma but was not fixed into complexes and probably was broken down quickly. If it be true that C_3 is fixed to antigen-antibody complexes on the tegument of H. diminuta then these complexes are formed in cortisone treated and athymic mice as well as in rats and hence something other than the formation of such complexes is essential for rejection of worms.

Within the intestinal environment what could be the function of fixed C_3? Complement dependent lysis requires fixation of components 1-9 whereas opsonization,

immune adherence, production of anaphylatoxin and complement dependent histamine release require only components 1, 4, 2 and 3 of the complement sequence (Weiser, Myrvik & Pearsall, 1969, p.117). The existence of C_3 on the worm does not imply that other components of the sequence occur and hence further discussions of its possible function would be highly speculative. The most attractive possible function of intestinal antibody against an helminth, and one which would not necessarily require complement, is the neutralization of some essential physiological process in the worm. With cestodes this is particularly easily visualized as the tegument is the digestive-absorptive interface and is covered by antibody. In primary infections the worms initially grow free of host neutralizing antibody, but once covered by the newly produced antibody they destrobilate and/or are expelled. Whether expulsion requires a different mechanism than destrobilation as suggested in the ADDENDUM to SECTION 2 or whether it represents simply a further summation of units of a single mechanism is unknown. In secondary infections H. diminuta is severely stunted (SECTION 3) probably because it is covered by neutralizing antibody at a much earlier age than in primary infections.

With the evidence presented herein and from other studies on Hymenolepis spp. (quoted throughout this thesis) and studies on Raillietina cesticillus by Gray (1973), clearly parasitologists must accept that intestinal tapeworms evoke host immune responses, and they must progress to the question of how most tapeworms survive despite these responses. Mechanisms of survival

of other parasites have been reviewed recently (Parasites in the Immunized Host: mechanisms of survival, 1974) and tapeworms most likely utilize some of these mechanisms for survival. The antibody coat on H. microstoma in mice and H. diminuta in rats could be a component of the survival mechanism of these worms. Perhaps the antibodies on these worms are specific to different, 'less important' antigens than antibodies on H. diminuta in mice. That it takes longer for antibody to coat H. microstoma than H. diminuta in mice may be of significance by for example, allowing H. microstoma to 'adapt' prior to strong immune attack. One could hypothesize that a similar delay occurs in the appearance of antibody on H. diminuta in rats.

The different classes of antibody on the worms most likely do not have the same specific functions and as discussed in the INTRODUCTION one of the major functions of antibody is immunoregulatory, it is easy to visualize that some of the antibody may be relatively host protective, whereas other antibody relatively worm protective. With Herpes simplex it was shown by Centifanto & Kaufman (1971) that, although IgA antibodies could neutralize the virus, if it was previously "sensitized" by IgG antibodies, it was insusceptible to IgA neutralization. Witz (1973) reviewed concepts on the biological significance of tumour-bound Igs many of which could apply to tapeworm-bound antibody. The possible complexity of interactions between various classes of antibodies on Hymenolepis spp. was shown by Rubinstein, DeCary & Streun (1974) who enhanced or inhibited tumour growth depending on the class, combination of classes or

concentration of antibody transferred to mice. Although with Herpes simplex, IgA antibody was host protective, many authors (see INTRODUCTION) have suggested that as IgA coats the indigenous flora (Brandtzaeg et al. 1968 b) and probably food antigens, it may frequently act as a 'blocking antibody'. Perhaps, as recently proposed by Bartlett & Ball (1974) for Heligmosomoides polygyrus, the strategy of tapeworms and other helminths is to mask important antigens with IgA blocking antibody. Alternately IgA antibodies may interfere with uptake of tapeworm antigens (Walker & Isselbacher, 1974), inducing a partially tolerant or 'negative memory' state in the host (see INTRODUCTION).

One aspect of helminthic infection of the intestine which recently has received considerable attention is alterations in the intestinal microcosm induced by infection viz. pathophysiology (e.g. Mettrick & Podesta, 1974). These changes may be due to excretory or secretory products of the worms, or to immunological and physiological responses of the host. A strategy of the worms may be to release products which depress components of host immunity, as has been demonstrated for some bacteria; e.g. Streptococcus sanguis releases an enzyme which specifically cleaves IgA (Plaut, Genco & Tomasi, 1974) and Pseudomonas aeruginosa releases an elastase which inactivates some complement components (Schultz & Miller, 1974). The lower pH in the intestine of rats infected with H. diminuta than in uninfected rats (Mettrick, 1971; Podesta & Mettrick, 1974 b), which is probably due to H^+ secretion by the worms (Podesta & Mettrick, 1974 c), may depress

the immunological potential of the host. In those helminthic infections where the worms are expelled, the pathophysiological alterations induced by both physiological and immunological responses of the host must be important in protective immunity. Castro, Cotter, Ferguson & Gorden (1973) suggested that localized reduction of pH in areas of inflammation may be an essential condition in the expulsion of adult T. spiralis. The striking rise in levels of intestinal phospholipase shown by Ottolenghi (1973) and Larsh, Ottolenghi & Weatherly (1974) in infections with H. nana and T. spiralis respectively, may indicate that this enzyme is involved in protective immunity. Similarly, 10 fold increases in peroxidase activity in extracts of laminal proprial cells from mice infected with adult T. spiralis indicate that this enzyme may be important (Castro et al. 1974), particularly as it occurs in polymorphonuclear leucocytes, monocytes and macrophages where it is known to have lethal activity against micro-organisms (quoted by Castro et al. 1974). It is clear that for a proper understanding of the survival or rejection of parasites, the influence of host and parasite induced pathophysiological alterations in the intestinal microcosm on host immune responses must be understood.

In conclusion, immune responses of the intestine and particularly the involvement of IgA in helminthic infections warrant further study. With tapeworm infections where the worms survive, as well as those where they are rejected, the tegument is covered by antibody of both local and systemic origin. The biological significance of this tapeworm-bound antibody

may be either, depending upon the host-parasite relationship, in worm rejection or survival. If the suggestion that C_3 is fixed to the tegument of H. diminuta in vivo is true, it will be a significant contribution to our understanding of intestinal immunity.

SUMMARY

1. A précis of the characteristics of intestinal immunological responses is given. Despite the vast amount known about microbial infections, helminthologists know little of intestinal immune responses to worms. The prime objective of the present research was to determine if local responses and predominantly IgA antibody warranted further study.

2. No anti-*H. diminuta* antibody was detected by double immunodiffusion in the serum or gut contents of infected mice.

3. No differences in the levels of serum or intestinal Igs were detected between infected and uninfected mice using single radial immunodiffusion.

4. Immunofluorescence studies showed that in mice which reject *H. diminuta* the worm tegument is covered by IgA, IgG_1, IgG_2 and IgM. *H. microstoma*, however, which survives well in mice is also covered by these Igs, albeit they appear more slowly on this worm than on *H. diminuta*.

5. It is argued that these Igs on the worms are specific antibodies.

6. Fluorescence induced by C_3 antisera occurred on H. diminuta also and, although one of the controls was unsuccessful, there is sufficient evidence to suggest that C_3 is in fact fixed to the tegument of H. diminuta.

7. In the intestinal wall and bile duct of infected mice there were increases in the distribution and abundance of the various Igs relative to that in control animals.

8. These and other points are discussed in relation to the rejection and survival of adult tapeworms.

9. It is concluded that intestinal immune responses and the IgA system warrant further study in helminthic infections.

SECTION 6

POSSIBLE IMMUNOLOGICAL DAMAGE TO THE TEGUMENT OF *HYMENOLEPIS DIMINUTA* IN MICE AND RATS

Possible immunological damage to the tegument of *Hymenolepis diminuta* in mice and rats

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SUMMARY

Opaque or darkened areas (DA) of variable size and position occur on Hymenolepis diminuta in mice and rats. In mice DA normally first appear in the neck region of the worm but subsequently they appear elsewhere and increase in number until destrobilation or worm expulsion. The posterior of destrobilated worms is often darkened. In the more immunogenic six cysticercoid infections there are more DA per worm than in one cysticercoid infections. DA are areas of the tegument with a homogeneous increase in electron density; abnormal mitochondria; reduced granular endoplasmic reticulum, Golgi complexes and discoidal secretory bodies; and the accumulation of lipid droplets. DA disappear from worms maintained for up to 4 hours in Hanks' balanced salt solution and can be induced by mechanical damage to the worms.

As the numbers of DA increase with the duration and intensity of infection and have similarities with types of cell injury, they are probably sites of immunologically induced worm pathology.

INTRODUCTION

In the intestine of its definitive host a tapeworm strobilates and matures into an egg producing adult which often lives for considerable time in equilibrium between loss and production of proglottids. This equilibrium can be disrupted, resulting in partial or complete loss of the strobila from the scolex, by unfavourable conditions such as anthelmintic treatment (Walley, 1966; Hopkins, Grant & Stallard, 1973), host starvation (Reid, 1942), hibernation (Ford, 1972) or immunity (Hopkins, Subramanian & Stallard, 1972 a & b; Gray, 1973; Andreassen, Hindsbo & Hesselberg, 1974); when the stress is removed the strobila regrows. In immunologically induced destrobilation regrowth occurs when the destrobilated worm is transplanted from the resistant into a naive host (Hopkins et al. 1972 a; Gray, 1973) or when resistant hosts are treated with the immunodepressant, cortisone (Hopkins & Stallard, 1975).

Immunologically induced structural degeneration comparable to destrobilation in cestodes has been described in the nematodes Nippostrongylus brasiliensis by Ogilvie & Hockley (1968) and Lee (1969), Haemonchus placei by Harness, Smith & Bland (1973) and in the trematode Schistosoma mansoni by Hockley & Smithers (1970) and Perez & Terry (1973). Ogilvie & Jones (1973) reviewed these and other examples of immunologically mediated structural damage to helminths and suggested that knowledge of the underlying metabolic changes causing the

structural changes will increase our understanding of host-parasite relationships. With N. brasiliensis changes in ^{32}P uptake during infection (Henney, MacLean & Mulligan, 1971) and in the worm acetylcholinesterase (Edwards, Burt & Ogilvie, 1971; Sanderson, Jenkins & Phillipson, 1972) are metabolic alterations correlated with host immunity. Destrobilation in some tapeworms is a result of immunological damage but there is no information on the site of initial damage or what metabolic changes lead to destrobilation. As host immunity is generally thought to act by altering worm metabolism, the tapeworm tegument, which has both digestive and absorptive functions in these animals which lack a gut, would seem to be a highly probable site for immunological attack.

Hymenolepis diminuta destrobilates and is expelled from mice by host immunity (Hopkins et al. 1972 a & b) and in rats when high intensities of infection occur, there is similar rejection (Andreassen et al. 1974). This report describes some morphological changes in the tegument of H. diminuta which are probably induced by host immunity and lead to destrobilation, and discusses what, in general terms, they may represent.

MATERIALS AND METHODS

CFLP and CD-1 outbred male mice 6-12 weeks old at infection and CFY male and female rats of various ages reared in the laboratory were used for studies on the morphological changes of *H. diminuta*. For studies on the occurrence of these changes on worms throughout infections, CFLP and NIH (inbred) male mice 6 ± 1 week old supplied by Anglia Laboratory Animals (formerly Carworth Europe) were used; for grading of animal quality see Befus (1975). Animal maintenance, infection and autopsy has been described previously (Hopkins *et al.* 1972 *a*; Befus & Featherston, 1974).

Approximately 0.5 cm portions of *H. diminuta* from mice and rats were fixed for 24 hours (refrigerated or room temperature) in 5% glutaraldehyde in Millonig buffer, 120 mM, pH 7.2, plus 3% sucrose and 0.5 mM $CaCl_2$. The tissue was then washed in the Millonig buffer plus 5% sucrose and 0.5 mM $CaCl_2$, dehydrated in ethanol, and embedded in Araldite. Sections were cut on an LKB III ultratome, mounted on bare copper grids and double stained in ethanol uranyl acetate and lead citrate. An AEI EM 801 was used for viewing and photographs taken at X 2,000-25,000 magnification.

The occurrence of the morphological changes was determined by observing live worms in modified Hanks' saline (see Hopkins & Stallard, 1974 for description) using the X6 or X12 magnification of a dissecting microscope with transmitted light. For reasons elaborated

later the data were grouped for analysis. The nonparametric Wilcoxon rank sum test (with a significance level of P = 0.05) was used for analysis (Remington & Schork, 1970).

RESULTS

When H. diminuta is removed from rats or mice and immediately placed in Hanks' saline, areas which do not transmit light as well as the surrounding tissue can be seen (Pl. 1). These opaque or darkened areas (DA) are variable in size, ranging from being barely visible using a dissecting microscope to occupying at least 1 cm of the strobila. There may be none to many DA per worm; in mice at least, the number depends upon the duration and intensity of a primary infection (see below). In mice when there are few (1-5) DA they occur generally in the neck and immature proglottid regions of the strobila (Pl. 1A), but when there are more DA their distribution on the strobila increases. Occasionally the scolex may be darkened and swollen (Pl. 1B). DA may occupy the entire width of the strobila or be restricted to a small area, often a lateral margin (Pl. 1C). The posterior ends of the small worms remaining after destrobilation frequently are darkened also (Pl. 1D) and at least superficially this darkening resembles the DA described above. The ontogeny of DA has not been studied on worms from rats as extensively as from mice, but it is known that long established rat worms have DA which are variable in number and position.

When worms with DA were incubated at 37°C in Hanks' saline some DA, particularly smaller ones, disappeared within 5-10 minutes. Larger DA got progressively smaller with time and disappeared within 1-4

hours. On worms which were placed in Hanks' and refrigerated (about 4-8°C) the DA: a) did not disappear or b) became smaller more slowly than on worms kept at 37°C. After placing these refrigerated worms at 37°C the DA disappeared as quickly as on worms never refrigerated. DA did not appear spontaneously on any worms after up to 6 hours incubation at 37°C in Hanks', but if such worms were pinched with forceps DA appeared within about 1 hour in these areas of mechanical damage. DA induced by mechanical damage did not disappear during subsequent observation.

In contrast to the distal (Pl. 2A) and perinuclear cytoplasm (Pl. 2B) of normal tegumental cells, in DA the distal (Pl. 3) and perinuclear cytoplasm (Pl. 4) has an increased electron density. This darkening is more pronounced in the distal cytoplasm of the scolex and neck region (Pl. 3A) than in the proglottids (Pl. 3B) but in all cases the discoidal secretory bodies appear similar to or lighter than background, whereas normally they are darker. The density in the distal cytoplasm cannot be attributed to accumulation of organelles but is an uniform increase in the cytoplasmic density between organelles. Other changes in the distal cytoplasm include the presence of basally situated lipid droplets (Pl. 3A), and elongate vacuoles (Pl. 5C) varying in size; both of these changes are more common in DA of the scolex and neck than in the proglottids. Mitochondria are uncommon in the distal cytoplasm of DA in the scolex and neck, but more numerous in the proglottids. In all regions, however, they are morphologically abnormal (compare Pl. 5A with Pl. 5B), having light, rather than dark, matrices containing granules and granular masses

and somewhat disorganized cristae. Some mitochondria are more degenerate than others, consisting of a light matrix without cristae, a few granules and one or two limiting membranes.

The increased density in the perinuclear cytoplasm appears to be due to an increased abundance of granules about the size of ribosomes (Pl. 4A & B) when compared with normal perinuclear cytoplasm (Pl. 2B). It is difficult to identify cell organelles in this dense matrix but the granular endoplasmic reticulum, Golgi complex and discoidal secretory bodies are greatly reduced or absent (Pl. 4). Mitochondria have a normal morphology with a moderately dense matrix, although some have unusual shapes. Small vacuoles, with a double membrane and an intermembranous space, which resemble autophagosomes are present in most perinuclear areas, and one to many lipid droplets also occur (Pl. 4).

The ultrastructure of DA at the posterior of destrobilated worms has not been studied but at the light level these DA appear to contain more droplets (presumably lipid) than other DA.

DA were observed as early as day 4 in a primary infection of CD-1 mice. Thereafter the variation in size and location, and particularly the transient nature of DA, made accurate counts difficult, hence the data were grouped into four categories for analysis, namely 0, 1-5, 6-10, and >10 DA per worm. The mean number of DA per worm increased from days 8 to 14-16 in one (1c) and six (6c) cysticercoid infections of CFLP mice (Fig. 1). With each intensity of infection the numbers on days 14-16 were

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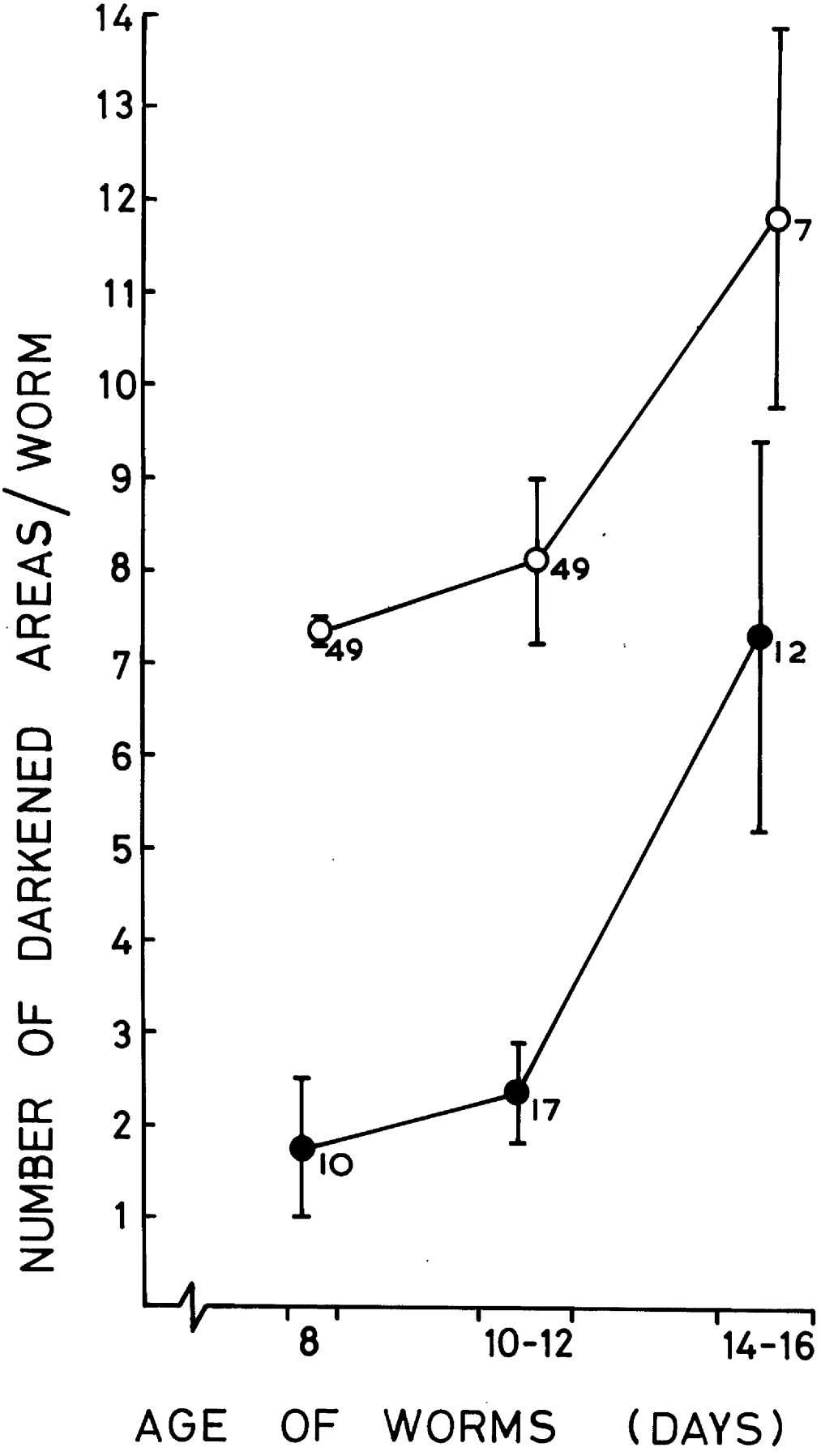
Figure 1

Changes in the mean number of darkened areas on Hymenolepis diminuta throughout one (●—●) and six (o—o) cysticercoid primary infections of CFLP male mice; on day 8 n = 10 mice/group, thereafter n = 20 mice/group. Destrobilated worms (after day 8 all worms <0.1 mg dry weight) not included; numbers adjacent to the points are the number of strobilated worms surviving, bars show ± standard errors.


NUMBER OF DARKENED AREAS/WORM
14
13
12
11
10
9
8
7
6
5
4
3
2
1
49
49
7
10
17
12
8
10-12
14-16
AGE OF WORMS (DAYS)

significantly greater than on day 8. On all days some worms had none and some had many DA; in general the variation (expressed as standard error) in the number of DA per worm increased as worm loss began (see numbers adjacent the points), and was greatest on days 14-16 when worm recoveries had fallen from 82% (6c) and 100% (1c) on day 8 to 6% and 60% respectively. Correlated with the more rapid rejection of 6c than 1c infections (reported previously, Befus, 1975) was that the mean number of DA per worm in the more immunogenic infection was consistently greater than in the 1c infection. The differences were significant except for on days 14-16 when variation was great.

In an experiment with NIH mice (Fig. 2) the patterns of occurrence of DA on worms were essentially similar to those shown above, although in the 6c infection the numbers had fallen by days 14-16. This decrease does not necessarily mean that the mean number of DA per worm is being reduced, but probably only that the surviving worms on these days had fewer DA on average than the large number of worms about to be rejected 2-6 days earlier. In accordance with the results from the CFLP mice, the mean number of DA per worm on day 8 was significantly more in 6c than in the 1c infections, and in the 1c infections the mean number of DA per worm on days 14-16 was significantly greater than on day 8. Fig. 2 is shown in addition to Fig. 1 to show the notable differences which occurred between experiments for, although there were more DA in both 6c and 1c infections in CFLP mice than in NIH mice, these differences cannot be attributed solely to the differences in mouse strain, as

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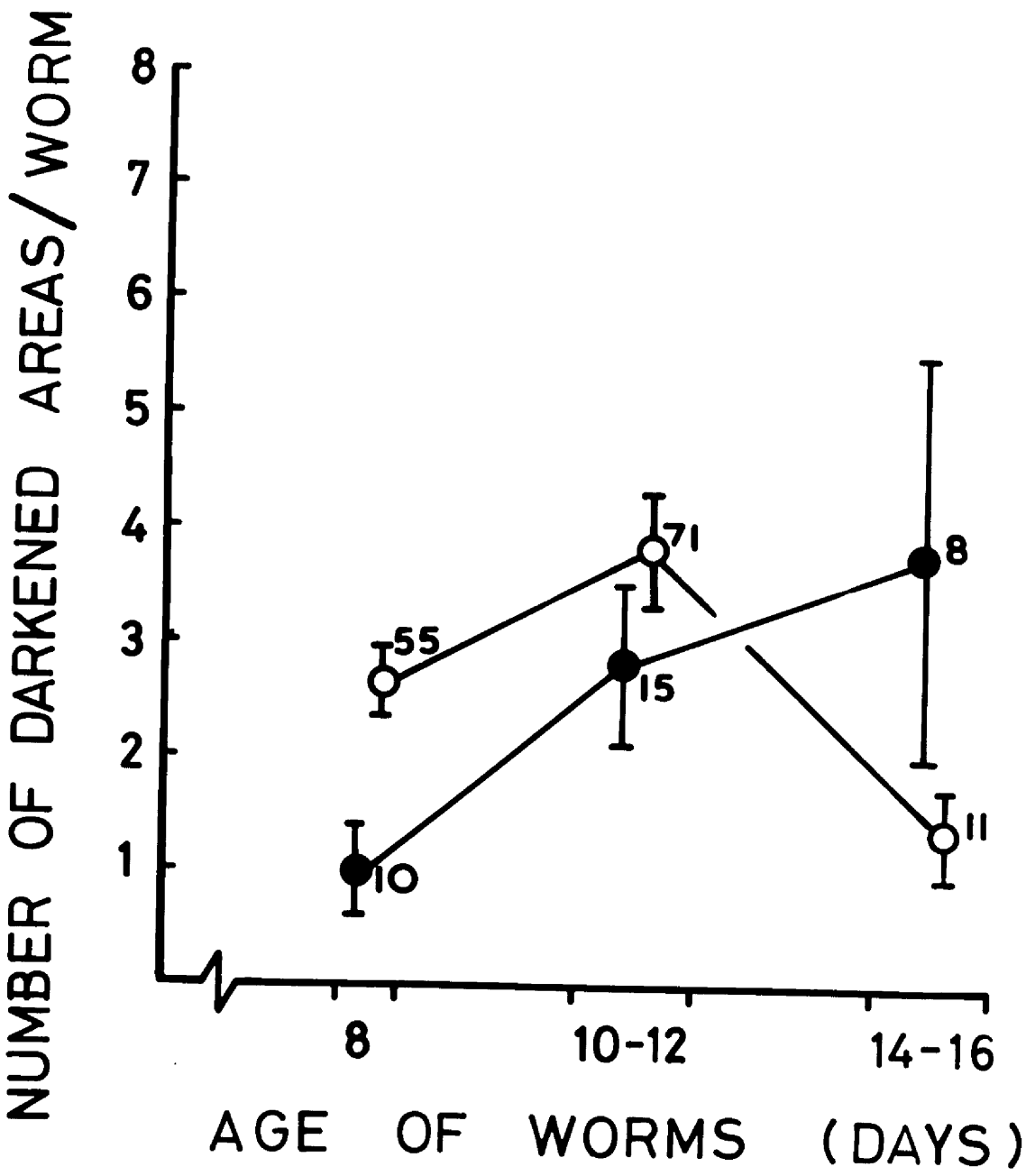
Figure 2

Changes in the mean number of darkened areas on Hymenolepis diminuta throughout one (●—●) and six (o—o) cysticercoid primary infections of NIH male mice; on day 8 n = 10 mice/group, thereafter n = 20 mice/group. Destrobilated worms (after day 8 all worms <0.1 mg dry weight) not included; numbers adjacent to the points are the number of strobilated worms surviving, bars show ± standard errors.


NUMBER OF DARKENED AREAS / WORM
8
7
6
5
4
3
2
1
55
71
8
15
10
11
8
10-12
14-16
AGE OF WORMS (DAYS)

in many experiments with CFLP mice (unpublished) similar differences occurred between experiments.

In support of the hypothesis that DA are immunologically induced, they occurred on worms in 1c and 6c secondary infections. For reasons discussed below, however, DA on these worms did not increase in number with increasing intensity or duration of infection.

DISCUSSION

This report describes darkened areas (DA) in the tegument of H. diminuta in mice and rats which to our knowledge have not been described previously. In mice DA occur as early as day 4 after a primary infection and increase in number until destrobilation and worm expulsion occurs. In 6c infections, which are destrobilated and expelled faster than 1c infections (Befus, 1975), there are more DA per worm. However, these patterns of occurrence of DA in primary infections are not characteristic of secondary infections because secondary worms are severely stunted by host immunity (Befus, 1975) and therefore, seldom have more than one or two DA each. Ultrastructurally most DA are areas of the tegument with extreme electron density; abnormal mitochondria; reduced granular endoplasmic reticulum, Golgi complexes and discoidal secretory bodies; and the accumulation of lipid droplets. When worms are kept in Hanks' salt solution DA disappear at a rate which can be reduced by low temperatures. DA can be produced by mechanical damage to the worms.

Information on the morphology and occurrence of DA has been presented but precisely what are they and what causes them? The high electron density in the distal cytoplasm cannot be attributed to the accumulation of organelles, whereas in the perinuclear regions it appears to be due to the accumulation of ribosome-like particles. Perhaps the reduction of granular endoplasmic reticulum

and general synthetic machinery in the perinuclear regions results in a release and packing of ribosomes free in the cytoplasm. Trump & Arstila (1971, p.67) stated that "diffuse increase in density producing so-called dark cells ... has been observed in many nonspecific types of injury", for example, in coagulative necrosis cells become opaque due to denaturation of intracellular protein (Dixon, 1970, p.25,28) and in glomerulonephritis electron dense deposits in the glomerular and tubular basement laminae are common (e.g. Churg & Grishman, 1972; Cochrane & Koffler, 1973; Peters, 1974). These deposits are immune complexes of antigen, antibody and complement trapped by the filtering mechanism of the basement laminae. They regress given appropriate chemotherapy or removal of the antigenic source.

It is unlikely that the DA in the tegument of H. diminuta are denaturated protein as they can disappear quickly. Although the immune complex deposits in glomerulonephritis can regress albeit probably much more slowly than DA, these areas of extreme electron density in H. diminuta tegument are not immune complexes. Immunoglobulin, one component of an immune complex, is associated with the tegument of H. diminuta (Befus, 1974) but is solely on the glycocalyx of the plasmalemma (Befus & Threadgold, unpublished) and not, like the electron density of DA, within the tegument. As DA are probably proteinaceous, but are unlikely to be denatured protein or immune complexes, then they must represent relatively high concentrations of material due to one or more of the following: a) increased synthesis or uptake, b) decreased dispersal or metabolism or c) dehydration in selective

portions of the tegument. It is impossible to decide which of these mechanisms is the cause, but Trump & Arstila (1971, p.67) suggested that dehydration is a common cause of increased electron density in other situations.

In support of the dehydration mechanism as a cause of DA, the tegument in DA appears 'thinner' than normal tegument, and the vacuoles in the basal region of the distal cytoplasm could be due either to: a) dehydration and shrinkage of the distal cytoplasm separating the basement lamina and the interstitial tissue or b) by the accumulation of fluid, from the dehydrated distal cytoplasm, in invaginations of the basal plasma membrane or in multitubular complexes (Threadgold & Read, 1970). In either case these vacuoles imply a change in tegumental function producing a net efflux of fluid from the cytoplasm. It is interesting that Hockley (1973) showed that in both hypotonic and hypertonic media the tegumental cytoplasm of schistosomes becomes very electron dense and vacuoles, apparently formed from swollen basal plasma membrane invaginations, appear; both characteristics of DA in H. diminuta.

The accumulation of lipid droplets especially prominent in the perinuclear cytoplasm of DA is characteristic of degenerative changes associated with cell injury (Dixon, 1970, p.25.29; Abraham & Robb-Smith, 1970, p.417). Lee (1969; 1971) showed similar lipid accumulation in immunologically damaged Nippostrongylus brasiliensis from rats and Harness et al. (1973) reported similar changes in Haemonchus placei from calves. Perhaps lipid accumulation results from a metabolic fault in the mitochondria which are known to be morphologically abnormal (Ogilvie & Hockley, 1968; present study).

Increase in the number of DA with the duration and intensity of infection and their similarity to types of cell injury suggest that they are sites of immunologically induced worm pathology. That H. nana incubated an immune mucosal extracts became "darkened and granular" (Weinmann, 1966) and that the teguments of schistosomes from an hyperimmune monkey became dense and vacuolated (Hockley & Smithers, 1970) supports this hypothesis. In the mouse, which rejects H. diminuta quickly, DA are visible by day 4 and accumulate until destrobilation and expulsion. The worms probably have some ability to repair the damage, hence in vivo, as in vitro, DA may disappear but they reappear and gradually accumulate until worm destrobilation or expulsion. In the rat, however, where H. diminuta survives well at moderate intensities of infection, an equilibrium must occur between this host induced damage and its repair by the worms. At higher intensities of infection in rats (discussed previously, Befus, 1975) this equilibrium must be destroyed resulting in worm loss.

As cestodes lack a gut the tegument serves as the digestive-absorptive organ which Ogilvie & Jones (1973) concluded in a review is probably antigenic and will be affected by host immunity. Befus (1974) and Befus & Threadgold (unpublished) have shown that the tegument and specifically the glycocalyx is the in vivo immunoglobulin binding site in H. diminuta from mice; the present communication describes changes in the tegument probably induced by host immunity and this bound immunoglobulin. At least superficially similar DA occur on Hymenolepis spp. in in vitro culture (Turton, 1968; Evans, 1973) and their

appearance signals the approaching death of the worms (Hopkins, personal communication). This suggests that DA are nonspecific responses to physiological stress induced *in vitro* by improper culture media and *in vivo* by host immune responses.

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REFERENCES

ABRAHAM, E.P. & ROBB-SMITH, A.H.T. (1970). Degenerative changes and some of their consequences. In General Pathology, 4th edition (ed. H.W. Florey), pp. 413-30. London: Lloyd-Luke Ltd.

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, Third International Congress of Parasitology 2, 1056-7.

BEFUS, A.D. (1974). The immunoglobulin coat on Hymenolepis spp. Transactions of the Roval Society of Tropical Medicine and Hygiene 68, 273.

BEFUS, A.D. (1975). Secondary infections of Hymenolepis diminuta in mice: effects of varying worm burdens in primary and secondary infections. Parasitology, in press.

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

CHURG, J. & GRISHMAN, E. (1972). Ultrastructure of immune deposits in renal glomeruli. Annals of Medicine 76, 479-86.

COCHRANE, C.G. & KOFFLER, D. (1973). Immune complex disease in experimental animals and man. In Advances in Immunology, vol. 16 (ed. F.J. Dixon and H.G. Kunkel), pp. 185-264. New York, London: Academic Press.

DIXON, K.C. (1970). Disorders of the cell. In Companion to Medical Studies, vol. 2 (ed. R. Passmore and J.S. Robson), pp. 25.1-25.44. Oxford, Edinburgh: Blackwell Scientific Publications Ltd.

EDWARDS, A.J., BURT, J.S. & OGILVIE, B.M. (1971). The effect of immunity upon some enzymes of the parasitic nematode Nippostrongylus brasiliensis. Parasitology 62, 339-47.

EVANS, W.S. (1973). The in vitro cultivation of Hymenolepis nana and H. microstoma. Ph.D. thesis, University of Glasgow, Scotland.

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

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

HARNESS, E., SMITH, K. & BLAND, P. (1973). Structural changes in the bovine nematode Haemonchus placei, that may be associated with host immune responses. Parasitology 66, 199-205.

HENNEY, R.W., MACLEAN, J.M. & MULLIGAN, W. (1971). The effect of host immunity on the metabolism of Nippostrongylus brasiliensis. Immunology 21, 711-8.

HOCKLEY, D.J. (1973). Ultrastructure of the tegument of Schistosoma. In Advances in Parasitology, vol. 11 (ed. B. Dawes), pp. 233-305. London, New York: Academic Press.

HOCKLEY, D.J. & SMITHERS, S.R. (1970). Damage to adult Schistosoma mansoni after transfer to a hyperimmune host. Parasitology 61, 95-100.

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. (1975). The effect of cortisone on the survival of Hymenolepis diminuta in mice. Submitted for publication.

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

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

HOPKINS, C.A., SUBRAMANIAN, G. & STALLARD, H. (1972 b). The effect of immunosuppressants on the development of Hymenolepis diminuta in mice. Parasitology 65, 111-20.

LEE, D.L. (1969). Changes in adult Nippostrongylus brasiliensis during the development of immunity to this nematode in rats. 1. Changes in ultrastructure. Parasitology 59, 29-39.

LEE, D.L. (1971). Changes in adult Nippostrongylus brasiliensis during the development of immunity to this nematode in rats. 2. Total lipids and neutral lipids. Parasitology 63, 271-4.

OGILVIE, B.M. & HOCKLEY, D.J. (1968). Effects of immunity on Nippostrongylus brasiliensis adult worms: reversible and irreversible changes in infectivity, reproduction, and morphology. The Journal of Parasitology 54, 1073-84.

OGILVIE, B.M. & JONES, V.E. (1973). Immunity in the parasitic relationship between helminths and hosts. In Progress in Allergy, vol. 17 (ed. P. Kallos, B.H. Waksman and A. de Weck), pp. 93-144. Basel, München, Paris, London, New York, Sydney: S. Karger.

PEREZ, H. & TERRY, R.J. (1973). The killing of adult Schistosoma mansoni in vitro in the presence of antisera to host antigenic determinants and peritoneal cells. International Journal for Parasitology 3, 499-503.

PETERS, D.K. (1974). The immunological basis of glomerulonephritis. Proceedings of the Royal Society of Medicine 67, 557-62.

REID, W.M. (1942). Certain nutritional requirements of the fowl cestode Raillietina cesticillus (Molin) as demonstrated by short periods of starvation of the host. The Journal of Parasitology 28, 319-40.

REMINGTON, R.D. & SCHORK, M.A. (1970). Statistics with Applications to the Biological and Health Sciences. New Jersey: Prentice-Hill Inc.

SANDERSON, B.E., JENKINS, D.C. & PHILLIPSON, R.F. (1972). Nippostrongylus brasiliensis: relation between immune damage and acetylcholinesterase levels. International Journal for Parasitology 2, 227-32.

THREADGOLD, L.T. & READ, C.P. (1970). Hymenolepis diminuta: ultrastructure of a unique membrane specialisation in tegument. Experimental Parasitology 28, 246-52.

TRUMP, B.J. & ARSTILA, A.U. (1971). Cell injury and cell death. In Principles of Pathobiology (ed. M.F. La Via and R.B. Hill Jr.), pp. 9-95. New York, London, Toronto: Oxford University Press.

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, Scotland.

WALLEY, J.K. (1966). Oxyclozanide (3, 3', 5, 5', 6-pentachloro - 2, 2' - dihydroxy-benzanilide - "Zanil") in the treatment of the liver fluke Fasciola hepatica in sheep and cattle. The Veterinary Record 78, 267-76.

WEINMANN, C.J. (1966). Immunity mechanisms in cestode infections. In Biology of Parasites (ed. E.J.L. Soulsby), pp. 301-20. New York, London: Academic Press.

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PLATE 1

A. Undestrobilated Hymenolepis diminuta showing a DA in the neck regions. (approx. X 75.)

B. Undestrobilated Hymenolepis diminuta showing a DA on the scolex. (approx. X 100.)

C. DA on the lateral margin of the strobila of Hymenolepis diminuta. (approx. X 25.)

D. Destrobilated Hymenolepis diminuta with a DA at the posterior end. (approx. X 100.)

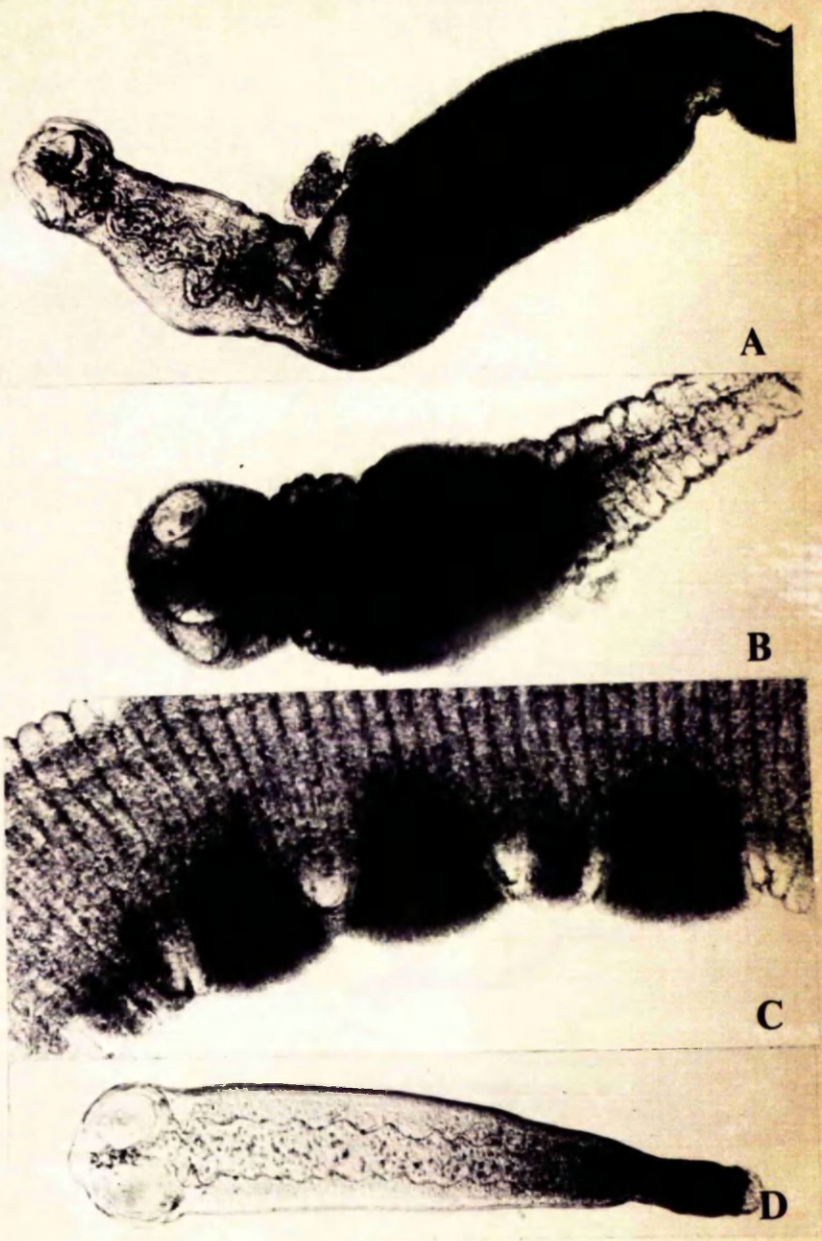
A
B
C
D

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PLATE 2

A. Distal cytoplasm of normal tegument from a mature proglottid. (X 25 200.)

B. Perinuclear cytoplasm of normal tegument from a mature proglottid. (X 40 000.)

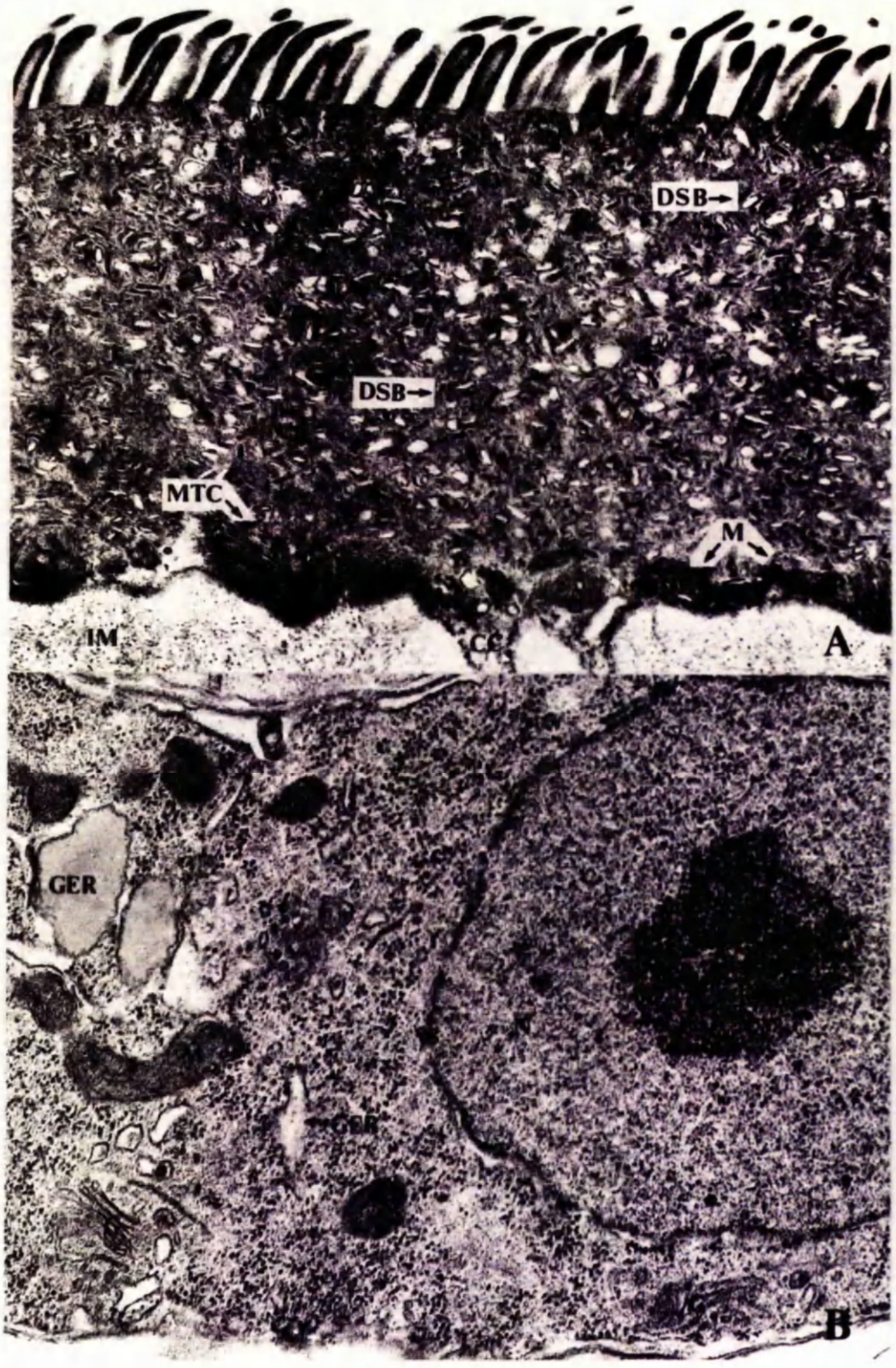
DSB
DSB
MTC
M
IM
CC
A
GER
GER
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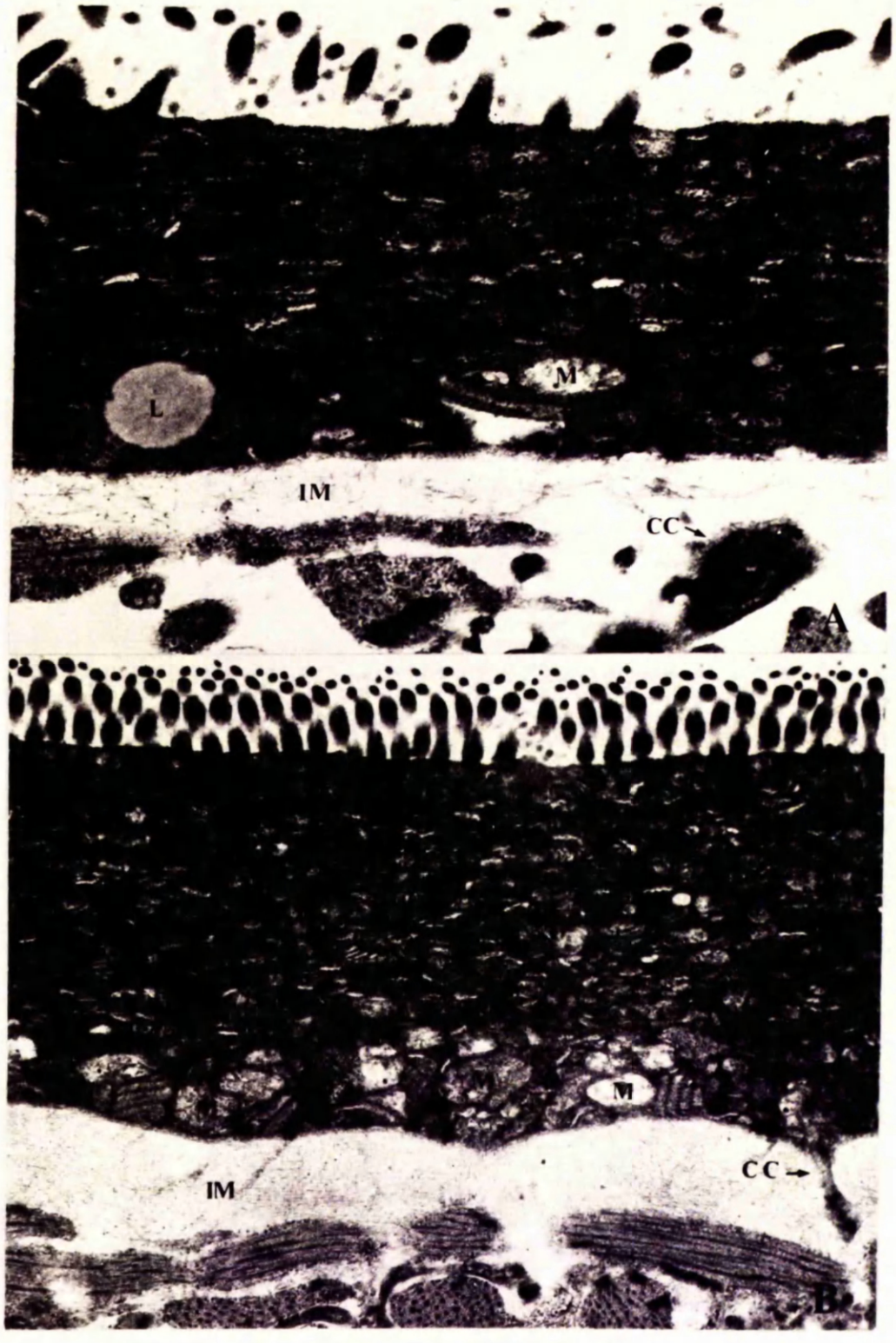
PLATE 3

A. Distal cytoplasm of the tegument from a DA on the scolex. It is dense throughout and contains abnormal mitochondria and a lipid droplet. (X 40 000.)

B. Distal cytoplasm of the tegument from a DA on a mature proglottid. The tegument is dense and contains abnormal mitochondria. (X 20 000.)


L
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CC
A
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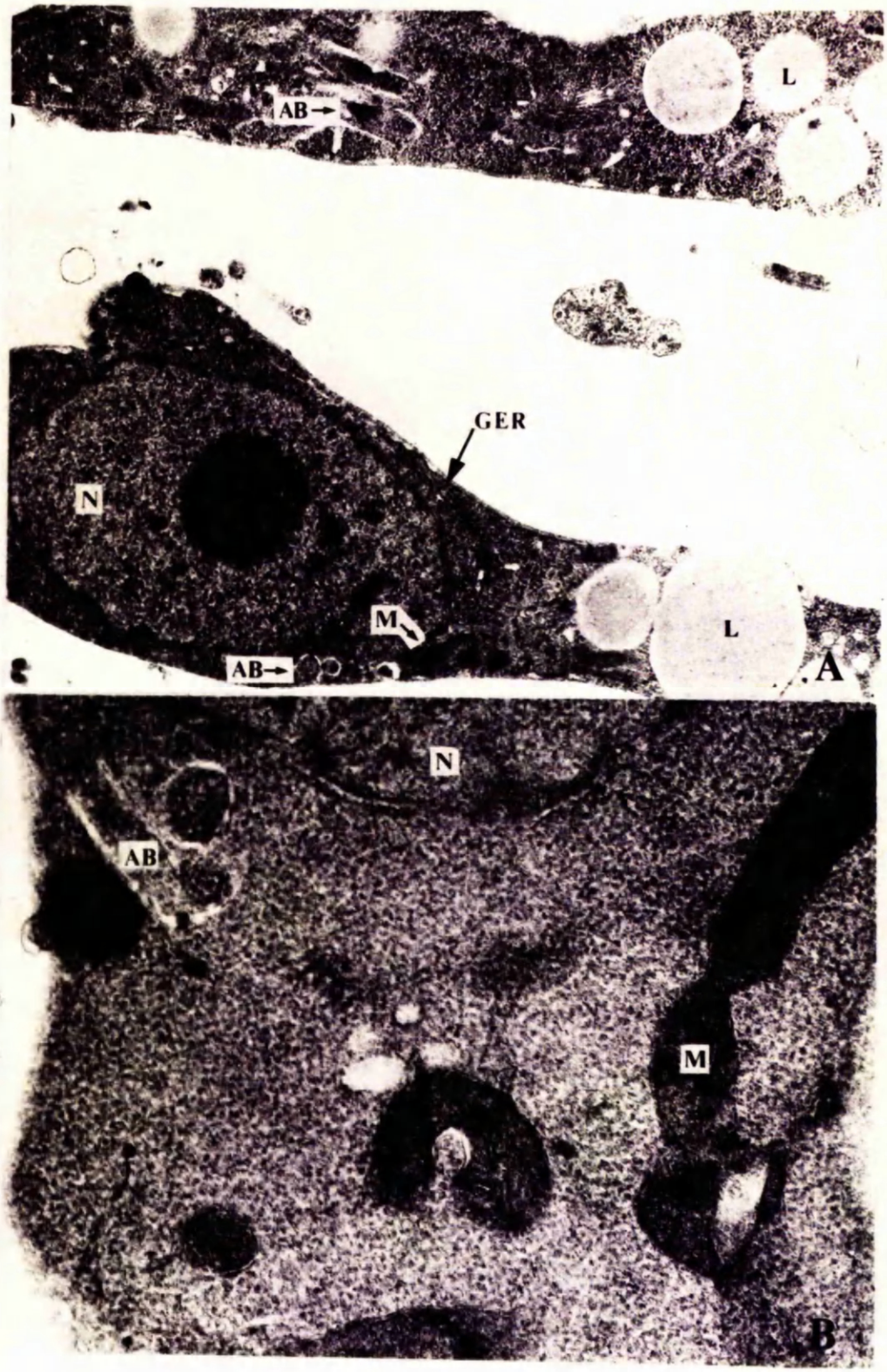
PLATE 4

A. Perinuclear cytoplasm of the tegument from a DA on the scolex. The cells contain normal mitochondria in a dense cytoplasm with little granular endoplasmic reticulum but many lipid droplets. Autophagic-like bodies with double limiting membranes occur in both cells. (X 16 000.)

B. Perinuclear cytoplasm of the tegument from a DA on a mature proglottid. The nucleus appears normal, but granular endoplasmic reticulum and Golgi complexes are absent. The granular cytoplasm contains apparently normal, albeit unusually shaped, mitochondria and some autophagic-like bodies. (X 40 000.)


AB
L
GER
N
M
AB
L
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N
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M
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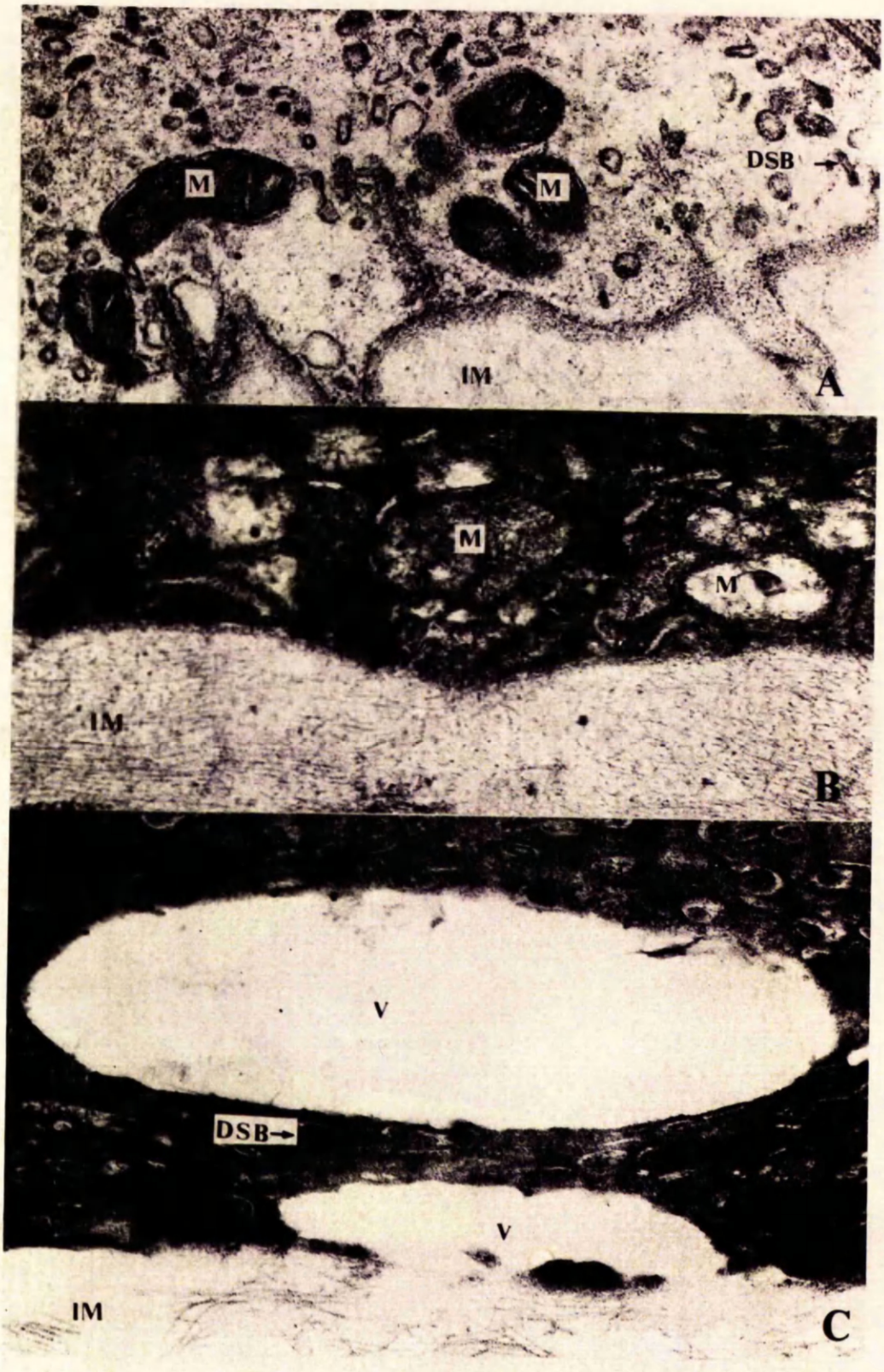
Facing page 232

PLATE 5

A. Basal region of the distal cytoplasm of normal tegument from a mature proglottid showing normal mitochondria. (X 56 000.)

B. Basal region of the distal cytoplasm of the tegument from a DA on a mature proglottid showing abnormal mitochondria. (X 40 000.)

C. Basal region of the distal cytoplasm of the tegument from a DA on a mature proglottid showing vacuoles. (X 50 000.)


M
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DSB
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V
DSB
V
IM
C

KEY TO LETTERING OF PLATES

AB	Autophagic-like body
CC	Cytoplasmic connection
DSB	Discoidal secretory body
G	Golgi complex
GER	Granular endoplasmic reticulum
IM	Interstitial material
L	Lipid droplet
M	Mitochondrion
MTC	Multitubular complex
N	Nucleus
V	Vacuole

EPILOGUE

The results presented above characterize more fully the dynamics of infection of Hymenolepis diminuta in mice, a delineation which is a prerequisite to the proper design of experiments to study the sequence of immunological events which ultimately lead to worm rejection. Analysis of the events of rejection was begun by studying intestinal immune responses to infection, an approach which in the past largely has been neglected by helminth immunologists. Additional efforts along the lines initiated herein and discussed below will contribute to our understanding of immunity to adult tapeworms and the role of immune responses in endosymbioses.

There is considerable variation between the results in different experiments but with careful experimental design the variation can be minimized. Unfortunately because of such variation and the fact that for so many years people have thought that most adult tapeworms are not immunogenic it may take a considerable time before there is widespread acceptance of immunity to adult cestodes. Furthermore, H. diminuta has been extensively studied by parasite physiologists who, with equivocal evidence, have attributed the phenomenon of crowding solely to inter-worm competition for a limiting resource. Hence, when six worm infections are used to minimize variability in the time of rejection many workers may argue that one is measuring the additional component of inter-worm competition rather than potentiated host immunity. Indeed it may take several

years to separate the importance of these two components but until then the probable major importance of the immunological component must be recognized. Obviously, to stimulate more workers to investigate immunological aspects of adult tapeworm infections there must be convincing evidence that in a model system, H. diminuta in the mouse, worm loss is due to an immunological mechanism. For those versed in helminth immunology the evidence presented by Hopkins, Subramanian & Stallard (1972 a & b) was virtually unequivocal, but those less well versed in helminth immunology remain sceptical. What then has the present study contributed to convince sceptics that there is immunity to adult cestodes?

Adult mice immunologically expel H. diminuta, but what of neonatal mice? Relative immunological unresponsiveness of neonatal animals to a variety of antigens is well known and at least for many animals there is a gradually increasing responsiveness until well past sexual maturation. With nematode infections unresponsiveness of the young has been reported many times but the report herein is the first documentation of neonatal unresponsiveness to adult cestodes. There is no absolute unresponsiveness to H. diminuta as even the youngest mice studied rejected their worms, albeit more slowly than older mice. Worm growth is best in mice 4 weeks old at infection and it is clear that the pattern of growth and survival of H. diminuta in mice 2-7 weeks old is governed by a combination of the immunological and physiological maturation of the intestine. The immunological deficiency in the young in this and other helminth-host systems is quantitative rather than

qualitative and probably involves most, if not all, components of the immune system. Hence, it is cautioned that care be taken before statements of the specific underlying cause of unresponsiveness be made. Furthermore, when experiments are conducted comparing mechanisms of expulsion in young and older animals it is essential to consider that the worms may be more capable of adapting to host immune responses in the young where they are immunologically assaulted more slowly and less intensely than in older animals where they are attacked more quickly and intensely. In all these considerations the intensity of infection is important as more immunogenic infections can potentiate the host immune responses, thereby erasing the relative unresponsiveness.

In addition to demonstrating that immunological unresponsiveness occurs to H. diminuta infection, the present study has shown that there is an effective anamnestic response which severely stunts the growth of worms in challenge infections. This response can be detected following an immunizing infection which is terminated after only 3 days but its effectiveness increases with the duration and intensity of the immunizing infection. Primary and secondary infections of six worms are more immunogenic than single worm infections and, of considerable practical importance in experimental design, minimize variation in the results. However, as stated above some workers would argue that with multiple worm infections there is the difficulty of inter-worm competition, but it is clearly of minor importance as six worm secondary infections are more immunogenic than one worm secondary infections despite the

fact that the worms are severely stunted, eliminating the possibility of inter-worm crowding.

Unlike in nematode systems where normally worm survival, not growth, is studied, it is concluded in SECTION 3 that in many types of experiments with H. diminuta infections, worm growth, not survival, is a more sensitive measure of the immune responses. Certainly, for experiments to establish more completely the importance of the duration of the immunizing infection on the effectiveness of the anamnestic response, worm growth is an essential parameter to measure. Recent studies with rejection of H. diminuta from rats have employed both growth and survival data (Andreassen, Hindsbo & Hesselberg, 1974; Pike & Chappell, personal communication). However, there are those types of experiments where data on worm survival would be adequate. For example, if one were to X-irradiate mice, data on worm survival would probably be adequate to compare normal and treated mice; further, worm survival could be used to assess the effectiveness of reconstitution procedures in X-irradiated mice. In fact, now that there is a considerable amount of information on the dynamics of infection perhaps the time is ripe to move on to experiments, with either mice or rats, which are designed such that differences between groups are sufficiently large that only worm survival need be studied (at least initially). It is inevitable that someone will utilize this approach as it is well tried with nematodes and would reduce the number of animals required, as kills would be done on only 2 or 3 days, to determine initial worm establishment and subsequent survival. Moreover, survival is a less

variable parameter than growth as the latter can be more easily influenced by various conditions; minimal variability is a prerequisite of any system. Initially, studies such as those conducted by Wakelin (1975 b) on the nematode Trichuris muris would provide fundamental information for the development of the system.

The demonstration of immunological damage to the tegument of H. diminuta, probably induced by antibody bound to the glycocalyx, provides a useful tool for further studies. However, this tool has some restrictions, as the tegument, which is known to have a rapid turnover of protein and carbohydrate containing macromolecules (Lumsden, 1966; Oaks & Lumsden, 1971), can, at least in vitro, rapidly repair the damage. Furthermore, transplantation of damaged H. diminuta presents a problem as by the time the worms are extensively damaged they are often too large to be transplanted, and the alternative of removing the damaged strobila and transplanting only the scolex defeats the benefit of having a damaged strobila. The tremendous powers of regrowth of a strobila and tegumental turnover in cestodes, and the latter in Schistosoma mansoni, as opposed to cell constancy and apparently limited powers for the repair of immunological damage in nematodes, differentiate the platyhelminthes from the nematodes. These distinctions may be expressed in, as yet unknown, strategies of members of the two groups for survival in immunized hosts. Damage in H. diminuta requires further characterization: what is it's ontogeny in worms in rats; how does it differ in immunodepressed hosts from in untreated hosts? Undoubtedly the most important question

is what are the underlying metabolic alterations induced by host immunity which produce the damage observed? With the answer to this question will come an increased understanding of how the host immune responses lead to worm rejection. Attempts must be made, using a variety of techniques, to demonstrate metabolic differences between damaged and 'normal' areas of tegument. Such studies could include organelle isolation (e.g. Harlow & Byram, 1971; Oaks & Knowles, 1974) and subsequent characterization, and autoradiographic studies on nutrient uptake and macromolecular turnover (Lumsden, 1966; Oaks & Lumsden, 1971). The considerable background of physiological information on H. diminuta and the abundance of workers interested in this helminth suggests that there is a bright future for these approaches.

The results from the immunofluorescent studies described in SECTION 5 have opened the way for many interesting lines of investigation. It has been established that: 1) antibody of a variety of Ig classes, not just s-IgA, enters the intestine and is bound to the glycocalyx of both H. diminuta and H. microstoma, 2) worm antigens occur on the tegument, and 3) there are local immunological changes in the intestinal wall such as pathotopic potentiation. These changes require further study as to their role in protective immunity. One apparent change in the intestine, namely partial villous atrophy, needs to be confirmed by comparing villus:crypt ratios (villus height/crypt depth) in normal and infected animals. To ascertain that the apparent villous atrophy is not simply an artefact of the

difficulty in producing transverse sections of villi using cryostat prepared tissue, the tissue must be carefully oriented during fixation and subsequent embedding in paraffin using the method of Murray, Miller, Sanford & Jarrett (1971). If the measurements from tissue prepared in this manner demonstrate that the apparent partial villous atrophy is an artefact, then plasma cell counts could be conducted on tissue oriented during fixation according to Murray et al. (1971), and washed and prepared for immunofluorescence according to Brandtzaeg (1974 a). However, if partial villous atrophy be true, then its cause will be of considerable interest (see Ferguson & Jarrett, 1975).

One of the difficulties with work on adult cestodes has been the crude nature of antigen preparations, which often are nothing more than homogenized whole worm. Identifying protective antigens in such a complex mixture would be difficult. Therefore, it is particularly significant that this study has localised antigen to the tegumental glycocalyx. Using the procedure of Oaks & Knowles (1974), which is a modification of that described by Kusel (1972) for studies on the tegument of S. mansoni, it is possible to isolate the distal cytoplasm of the tegument of H. diminuta and to fractionate it into the various organelles and a soluble component. This should provide a useful tool for the precise localization and ultimate identification of protective antigens in adult cestodes.

Of all the potentially fruitful studies on immunity to adult cestodes, investigation of the tegumental bound antibody is undoubtedly the most exciting because of

the apparent similarities to tumour bound Ig (see Witz, 1973). Many types of tumours are covered with Ig, much of which is antitumour antibody and is almost exclusively IgG_2, unlike worm bound antibody which is of many classes. This antitumour IgG_2 enhances tumour growth and survival despite also fixing complement to its surface. This parallels closely the excellent survival of H. diminuta in rats despite bound antibody, and apparently fixed complement, on the tegument. Why superficially similar bound antibody and complement on H. diminuta in the mouse leads to rejection of the worm is not known but its elucidation may be of significance in immunotherapy of neoplasms.

To study the role of bound antibody and complement it is essential to develop an experimental system where the worm is devoid of these components, but where they can be added at will. It seems highly probable that the methods for the elution of bound Ig from living tumour cells, which allow both the eluted Ig and the cells to be studied subsequently (Phillips & Lewis, 1971; Witz, 1973), could be readily adopted for work with Hymenolepis spp. If the elution is successful the nature and functions of the worm bound antibodies could be systemically studied. In vivo studies using passive transfer of the entire antibody mixture or of only purified antibody classes or combinations of classes (see Rubinstein, DeCary & Streun, 1974) may distinguish host protective antibody from worm enhancing antibody. Metabolic studies could be conducted in vitro comparing characteristics of worms devoid of antibody with those of worms covered with antibody of various

classes. In addition to antibody, other components such as complement and lysozyme (IgA, complement and lysozyme together are bacteriolytic: Adinolfi, Glynn, Lindsay & Milne, 1966; Burdon, 1973; Hill & Porter, 1974) could be added to the in vitro system. This approach should provide information on how the bound antibody affects nutrient transport in the worm, and hence perhaps influence comparisons between tegumental and epithelial transport systems (Mettrick & Podesta, 1974). Moreover, in this in vitro system attempts could be made to approximate in vivo pathophysiological changes induced by infection which may influence the efficacy of host immune responses.

The results presented in this thesis provide considerable evidence that immune responses are important in host-tapeworm relationships. Within essentially the last decade concepts have progressed from the naive ideas about the necessity of mucosal damage to the point where it is inevitable that knowledge of immune mechanisms in tapeworm infections will have fundamental medical significance.

REFERENCES

ABIOYE, A.A., LEWIS, E.A. & MCFARLANE, H. (1972). Clinical evaluation of serum immunoglobulins in amoebiasis. Immunology 23, 937-46.

ABRAHAM, E.P. & ROBB-SMITH, A.H.T. (1970). Degenerative changes and some of their consequences. In General Pathology (ed. H.W. Florey), pp. 413-30. London: Lloyd-Luke Ltd.

ADINOLFI, M., GLYNN, A.A., LINDSAY, M. & MILNE, C.M. (1966). Serological properties of γA antibodies to Escherichia coli present in human colostrum. Immunology 10, 517-26.

AGUS, S.G., FALCHUK, Z.M., SESSOMS, C.S., WYATT, R.G. & DOLIN, R. (1974). Increased jejunal IgA synthesis in vitro during acute infectious nonbacterial gastroenteritis. The American Journal of Digestive Diseases 19, 127-31.

ALLEN, W.D. & PORTER, P. (1973). The relative distribution of IgM and IgA cells in intestinal mucosa and lymphoid tissues of the young unweaned pig and their significance in ontogenesis of secretory immunity. Immunology 24, 493-501.

ALLEN, W.D., SMITH, C.G. & PORTER, P. (1973). Localization of intracellular immunoglobulin A in porcine intestinal mucosa using enzyme-labelled antibody. Immunology 25, 55-70.

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, Third International Congress of Parasitology 2, 1056-7.

ARRENBRECHT, S. (1973). Normal development of the thymus-dependent limb of humoral immune responses in mice. European Journal of Immunology 3, 506-11.

AUERBACH, R. (1972). Studies on the development of immunity: the response to sheep red blood cells. In Current Topics in Developmental Biology, vol. 7 (ed. A.A. Moscona and A. Monroy), pp. 257-80. New York, London: Academic Press.

BARTLETT, A. & BALL, P.A.J. (1974). The immune response of the mouse to larvae and adults of Nematospiroides dubius. International Journal for Parasitology 4, 463-70.

BAZIN, H., ANDRÉ, C. & HEREMANS, J.F. (1973). Résponses immunologiques induites par voie orale. Annales d'Immunologie (Collection des "Annales de L'Institut Pasteur") 124C, 253-72.

BEALE, A.J., DOUGLAS, A.P., PARISH, W.E. & HOBBS, J.R. (1971). Impaired IgA responses in coeliac disease. The Lancet 1, 1198-1200.

BEFUS, A.D. (1974). The immunoglobulin coat on Hymenolepis spp. Transactions of the Royal Society of Tropical Medicine and Hygiene 68, 273.

BENACERRAF, B. (1974). The genetic mechanisms that control the immune response and antigen recognition. Annales d'Immunologie (Collection des "Annales de L'Institut Pasteur") 125C, 143-64.

BENACERRAF, B. & DORF, M.E. (1974). Genetic control of specific immune responses. In Progress in Immunology II, vol. 2 (ed. L. Brent and J. Holborow), pp. 181-90. Amsterdam, Oxford: North-Holland Publishing Co.

BERG, R.D. & SAVAGE, D.C. (1972). Immunological responses and microorganisms indigenous to the gastrointestinal tract. The American Journal of Clinical Nutrition 25, 1364-71.

BERNSTEIN, I.D. & OVARY, Z. (1968). Absorption of antigens from the gastrointestinal tract. International Archives of Allergy and Applied Immunology 33, 521-7.

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

BIENENSTOCK, J., GAULDIE, J. & PEREY, D.Y.E. (1973). Synthesis of IgG, IgA, IgM by chicken tissues: immunofluorescent and ^{14}C amino acid incorporation studies. The Journal of Immunology 111, 1112-8.

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. The Journal of Experimental Medicine 140, 333-48.

BOACKLE, R.J., PRUITT, K.M. & MESTECKY, J. (1974). The interactions of human complement with interfacially aggregated preparations of human secretory IgA. Immunochemistry 11, 543-8.

BOCKMAN, D.E. & COOPER, M.D. (1973). Pinocytosis by epithelium associated with lymphoid follicles in the bursa of Fabricius, appendix, and Peyer's patches. An electron microscopic study. The American Journal of Anatomy 136, 455-78.

BOGITSH, B.J. (1966). Histochemical studies on Hymenolepis microstoma (Cestoda: Hymenolepididae). III. Effects on the bile duct of the mouse. Transactions of the American Microscopical Society 85, 372-7.

BOSMA, M.J., MAKINODAN, T. & WALBURG, H.E., Jr. (1967). Development of immunologic competence in germfree and conventional mice. The Journal of Immunology 99, 420-30.

BRAMBELL, F.W.R. (1970). The transmission of passive immunity from mother to young. In Frontiers of Biology, vol. 18 (ed. A. Neuberger and E.L. Tatum). Amsterdam, London: North-Holland Publishing Co.

BRANDTZAEG, P. (1973). Structure, synthesis and external transfer of mucosal immunoglobulins. Annales d'Immunologie (Collection des "Annales de L'Institut Pasteur") 124C, 417-38.

BRANDTZAEG, P. (1974 a). Mucosal and glandular distribution of immunoglobulin components: immunohistochemistry with a cold ethanol-fixation technique. Immunology 26, 1101-14.

BRANDTZAEG, P. (1974 b). Mucosal and glandular distribution of immunoglobulin components: differential localization of free and bound SC in secretory epithelial cells. The Journal of Immunology 112, 1553-9.

BRANDTZAEG, P. (1974 c). Secretory immunoglobulins. In Progress in Immunology II, vol. 1 (ed. L. Brent and J. Holborow), pp. 238-43. Amsterdam, Oxford: North-Holland Publishing Co.

BRANDTZAEG, P., FJELLANGER, I. & GJERULDSEN, S.T. (1968 a). Immunoglobulin M: local synthesis and selective secretion in patients with immunoglobulin A deficiency. Science 160, 789-91.

BRANDTZAEG, P., FJELLANGER, I. & GJERULDSEN, S.T. (1968 b). Absorption of immunoglobulin A onto oral bacteria in vivo. The Journal of Bacteriology 96, 242-9.

BRANDTZAEG, P., FJELLANGER, I. & GJERULDSEN, S.T. (1970). Human secretory immunoglobulins. I. Salivary secretions from individuals with normal or low levels of serum immunoglobulins. Scandinavian Journal of Hematology Supplementum No. 12, 83 pp.

BRENTJENS, J.R., O'CONNELL, D.W., PAWLOWSKI, I.B., HSU, K.C. & ANDRES, G.A. (1974). Experimental immune complex disease of the lung. The pathogenesis of a laboratory model resembling certain human interstitial lung diseases. The Journal of Experimental Medicine 140, 105-25.

BURDON, D.W. (1973). The bactericidal action of immunoglobulin A. Journal of Medical Microbiology 6, 131-9.

CAMPBELL, W.C., HARTMAN, R.K. & CUCKLER, A.C. (1963). Induction of immunity to trichinosis in mice by means of chemically abbreviated infections. Experimental Parasitology 14, 29-36.

CAPPUCCINELLI, P., MARTINETTO, P., FRENTZEL-BEYME, R.R. & SENA, L. (1973). Immunoglobulins and parasitic diseases. I. Levels of IgG, IgA, IgM and IgD in different protozoal and helminthic infections in man. Giornale di Batteriologia Virologia ed Immunologia 64, 155-61.

CARTER, B.G. & RECTOR, E.S. (1972). The generation of antigen sensitive cells in the newborn mouse. The Journal of Immunology 109, 1345-51.

CARTER, P.B. & COLLINS, F.M. (1974). The route of enteric infection in normal mice. The Journal of Experimental Medicine 139, 1189-1203.

CASTRO, G.A., ROY, S.A. & STOCKSTILL, R.D. (1974). Trichinella spiralis: peroxidase activity in isolated cells from the rat intestine. Experimental Parasitology 36, 307-15.

CASTRO, G.A., COTTER, M.V., FERGUSON, J.D. & GORDEN, C.W. (1973). Trichinosis: physiologic factors possibly altering the course of infection. The Journal of Parasitology 59, 268-76.

CEBRA, J.J., CRAIG, S.W. & JONES, P.P. (1974). Cell types contributing to the biosynthesis of sIgA. In The Immunoglobulin A System (ed. J. Mestecky and A.R. Lawton), pp. 23-33. New York, London: Plenum Press.

CENTIFANTO, Y.M. & KAUFMAN, H.E. (1971). The development of tear antibodies and Herpes simplex keratitis. In The Secretory Immunologic System (ed. D.H. Dayton Jr., P.A. Small Jr., R.M. Chanock, H.E. Kaufman and T.B. Tomasi Jr.), pp. 331-40. Washington, D.C.: United States Government Printing Office.

CHANANA, A.D., SCHAEDELI, J., HESS, M.W. & COTTIER, H. (1973). Predominance of theta-positive lymphocytes in gut-associated and peripheral lymphoid tissues of newborn mice. The Journal of Immunology 110, 283-5.

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

CHANDLER, A.C. (1953). Immunity in parasitic diseases. The Journal of the Egyptian Medical Association 36, 811-35.

CHANDLER, A.C. (1955). Introduction to Parasitology. New York: John Wiley & Sons, Inc.

CHAPMAN, H.A., JOHNSON, J.S. & COOPER, M.D. (1974). Ontogeny of Peyer's patches and immunoglobulin-containing cells in pigs. The Journal of Immunology 112, 555-63.

CHAPPELL, L.H. & READ, C.P. (1973). Studies on the free pool of amino acids of the cestode Hymenolepis diminuta. Parasitology 67, 289-305.

CHENG, T.C. (1964). The Biology of Animal Parasites. Philadelphia, London: W.B. Saunders Co.

CHURG, J. & GRISHMAN, E. (1972). Ultrastructure of immune deposits in renal glomeruli. Annals of Medicine 76, 479-86.

CLUFF, L.E. (1971). Secretory immunological system: a perspective summation. In The Secretory Immunologic System (ed. D.H. Dayton Jr., P.A. Small Jr., R.M. Chanock, H.E. Kaufman and T.B. Tomasi Jr.), pp. 479-84. Washington, D.C.: United States Government Printing Office.

COCHRANE, C.G. & KOFFLER, D. (1973). Immune complex disease in experimental animals and man. In Advances in Immunology, vol. 16 (ed. F.J. Dixon and H.G. Kunkel), pp. 185-264. New York, London: Academic Press.

COLEMAN, R.M. (1961). In vivo antibody binding sites in Hymenolepis nana as demonstrated by direct and indirect immunofluorescent staining. The Journal of Parasitology 47 (suppl.), 54.

COLEMAN, R.M. & DESA, L.M. (1964). Immunological response to implanted adult dwarf tapeworms. Nature 204, 397-8.

COLEMAN, R.M. & FOTORNY, N.M. (1962). In vivo isolation of Hymenolepis nana and antibody-binding sites. Nature 195, 920-1.

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 Eighth International Congress of Malaria and Tropical Medicine, 107.

COLQUHOUN, D. (1971). Lectures on Biostatistics: An Introduction to Statistics with Applications in Biology and Medicine. Oxford: Clarendon Press.

COLTEN, H.R. (1974). Biosynthesis of serum complement. In Progress in Immunology II, vol. 1 (ed. L. Brent and J. Holborow), pp. 183-90. Amsterdam, Oxford: North-Holland Publishing Co.

COLTEN, H.R. & BIENENSTOCK, J. (1974). Lack of C_3 activation through classical or alternate pathways by human secretory IgA anti blood group A antibody. In The Immunoglobulin A System (ed. J. Mestecky and and A.R. Lawton), pp. 305-8. New York, London: Plenum Press.

COMOGLIO, P.M. & GUGLIELMONE, R. (1973). Immunohistochemical study of IgA transepithelial transfer into digestive tract secretions in the mouse. Immunology 25, 71-80.

CONNAN, R.M. (1974). The distribution of Nippostrongylus brasiliensis in prolonged infections in lactating and neonatally infected rats. Parasitology 68, 347-54.

COOMBS, R.R.A., POUT, D.D. & SOULSBY, E.J.L. (1965). Globulin, possibly of antibody nature, combining with the cuticle of live Turbatrix aceti. Experimental Parasitology 16, 311-7.

COOPER, M.D. & LAWTON, A.R. (1972). The mammalian "bursa equivalent": does lymphoid differentiation along plasma cell lines begin in the gut-associated lymphoepithelial tissues (GALT) of mammals? In Contemporary Topics in Immunobiology, vol. 1 (ed. M.G. Hanna Jr.), pp. 49-68. New York, London: Plenum Press.

COOPER, M.D., KINCADE, P.W., BOCKMAN, D.E. & LAWTON, A.R. (1974). Origin, distribution and differentiation of IgA-producing cells. In The Immunoglobulin A System (ed. J. Mestecky and A.R. Lawton), pp. 13-22. New York, London: Plenum Press.

CRABBÉ, P.A., BAZIN, H., EYSSEN, H. & HEREMANS, J.F. (1968). The normal microbial flora as a major stimulus for proliferation of plasma cells synthesizing IgA in the gut. International Archives of Allergy and Applied Immunology 34, 362-75.

CRABBÉ, P.A., NASH, D.R., BAZIN, H., EYSSEN, H. & HEREMANS, J.F. (1969). Antibodies of the IgA type in intestinal plasma cells of germfree mice after oral or parenteral immunization with ferritin. The Journal of Experimental Medicine 130, 723-44.

CRABBÉ, 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, 448-57.

CRANDALL, C.A. & CRANDALL, R.B. (1971). Ascaris suum: immunoglobulin responses in mice. Experimental Parasitology 30, 426-37.

CRANDALL, R.B. & CRANDALL, C.A. (1972). Trichinella spiralis: immunologic response to infection in mice. Experimental Parasicology 31, 378-98.

CRANDALL, R.B., CEBRA, J.J. & CRANDALL, C.A. (1967). 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 responses to infection in mice. Experimental Parasitology 35, 275-87.

CUNNINGHAM, A.J. & SERCARZ, E.E. (1971). The asynchronous development of immunological memory in helper (T) and precursor (B) cell lines. European Journal of Immunology 1, 413-21.

CURLIN, G.T. & CARPENTER, C.C.J., Jr. (1970). Antitoxic immunity to cholera in isolated perfused canine ileal segments. The Journal of Infectious Diseases 121 (suppl.), 132-6.

DAMIAN, R.T. (1970). Ouchterlony technique. In Experiments and Techniques in Parasitology (ed. A.J. MacInnis and M. Voge), pp. 205-9. San Francisco: W.H. Freeman and Co.

DAWSON, R.M.C., ELLIOTT, D.C., ELLIOTT, W.H. & JONES, K.M. (1969). Data for Biochemical Research. London: Oxford University Press.

DAYTON, D.H., Jr., SMALL, P.A., Jr., CHANOCK, R.M., KAUFMAN, H.E. & TOMASI, T.B., Jr. (1971). The Secretory Immunologic System. Washington, D.C.: United States Government Printing Office.

DENG, J.S. & BEUTNER, E.H. (1974). Effect of formaldehyde, glutaraldehyde and sucrose on the tissue antigenicity. International Archives of Allergy and Applied Immunology 47, 562-9.

DINEEN, J.K. & KELLY, J.D. (1973 a). Expulsion of Nippostrongylus brasiliensis from the intestine of rats: the role of a cellular component derived from bone marrow. International Archives of Allergy and Applied Immunology 45, 759-66.

DINEEN, J.K. & KELLY, J.D. (1973 b). 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., OGILVIE, B.M. & KELLY, J.D. (1973). Expulsion of Nippostrongylus brasiliensis from the intestine of rats: collaboration between humoral and cellular components of the immune response. Immunology 24, 467-75.

DINEEN, J.K., RONAI, P.M. & WAGLAND, B.M. (1968). The cellular transfer of immunity to Trichostrongylus colubriformis in an isogenic strain of guinea-pig. IV. The localization of immune lymphocytes in small intestine in infected and non-infected guinea-pigs. Immunology 15, 671-9.

DINEEN, J.K., WAGLAND, B.M. & RONAI, P.M. (1968). The cellular transfer of immunity to Trichostrongylus colubriformis in an isogenic strain of guinea-pig. III. The localization and functional activity of immune lymph node cells following syngeneic and allogeneic transfer. Immunology 15, 335-41.

DINEEN, J.K., KELLY, J.D., GOODRICH, B.S. & SMITH, I.D. (1974). Expulsion of Nippostrongylus brasiliensis from the small intestine of the rat by prostaglandin-like factors from ram semen. International Archives of Allergy and Applied Immunology 46, 360-74.

DIXON, K.C. (1970). Disorders of the cell. In Comparison to Medical Studies, vol. 2 (ed. R. Passmore and J.S. Robson), pp. 25.1-25.44. Oxford, Edinburgh: Blackwell Scientific Publications Ltd.

DOBSON, C. (1967). Changes in the protein content of the serum and intestinal mucus of sheep with reference to the histology of the gut and immunological response to Oesophagostomum columbianum infections. Parasitology 57, 201-19.

DOBSON, C. & SOULSBY, E.J.L. (1974). Lymphoid cell kinetics in guinea pigs infected with Trichostrongylus colubriformis: tritiated thymidine uptake in gut and allied lymphoid tissue, humoral IgE and hemagglutinating antibody responses, delayed hypersensitivity reactions, and in vitro lymphocyte transformations during primary infections. Experimental Parasitology 35, 16-34.

DOE, W.F. (1972). The secretory immune system of the intestine. Gut 13, 572-8.

DOUVRES, F.W. (1962). The in vitro cultivation of Oesophagostomum radiatum, the nodular worm of cattle. II. The use of this technique to study immune responses of host tissue extracts against the developing nematode. The Journal of Parasitology 48, 852-64.

DUWE, A.E. (1967). Antigens of Moniezia expansa: fluorescent antibody localization. Transactions of the American Microscopical Society 86, 126-31.

DVORAK, J.A., JONES, A.W. & KUHLMAN, H.H. (1961). Studies on the biology of Hymenolepis microstoma (Dujardin, 1845). The Journal of Parasitology 47, 833-8.

EDWARDS, A.J., BURT, J.S. & OGILVIE, B.M. (1971). The effect of immunity upon some enzymes of the parasitic nematode Nippostrongylus brasiliensis. Parasitology 62, 339-47.

EIDELMAN, S. & BERSCHAUER, J.A. (1969). A method for immunocytochemical study of human gastrointestinal suction biopsies. Stain Technology 44, 43-4.

ESKELAND, T. & BRANDTZAEG, P. (1974). Does J chain mediate the combination of 19S IgM and dimeric IgA with the secretory component rather than being necessary for their polymerization. Immunochemistry 11, 161-3.

EVANS, W.S. (1973). The in vitro cultivation of Hymenolepis nana and H. microstoma. Ph.D. thesis, University of Glasgow.

FAHEY, K.J. (1974). Immunological reactivity in the foetus and the structure of foetal lymphoid tissues. In Progress in Immunology II, vol. 3 (ed. L. Brent and J. Holborow), pp. 49-60. Amsterdam, Oxford: North-Holland Publishing Co.

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

FERGUSON, A. & JARRETT, E.E.E. (1975). Hypersensitivity reactions in the small intestine. I. Thymus dependence of experimental 'partial villous atrophy'. Gut 16, 114-7.

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 and Experimental Immunology 12, 477-88.

FICHTELIUS, K.E. (1968). The gut epithelium - a first level lymphoid organ? Experimental Cell Research 49, 87-104.

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

FREEMAN, R.S. (1973). Ontogeny of cestodes and its bearing on their phylogeny and systematics. In Advances in Parasitology, vol. 11 (ed. B. Dawes), pp. 481-557. London, New York: Academic Press.

FRETER, R. (1972). Parameters affecting the association of vibrios with the intestinal surface in experimental cholera. Infection and Immunity 6, 134-41.

FUBARA, E.S. & FRETER, R. (1972 a). Source and protective function of coproantibodies in intestinal disease. The American Journal of Clinical Nutrition 25, 1357-63.

FUBARA, E.S. & FRETER, R. (1972 b). Availability of locally synthesized and systemic antibodies in the intestine. Infection and Immunity 6, 965-81.

FUBARA, E.S. & FRETER, R. (1973). Protection against enteric bacterial infection by secretory IgA antibodies. The Journal of Immunology 111, 395-403.

GATTI, R.A., ÖSTBORN, A. & FAGRAEUS, A. (1974). Selective impairment of cell antigenicity by fixation. The Journal of Immunology 113, 1361-8.

GAZDAR, A.F., BEITZEL, W. & TALAL, N. (1971). The age related responses of New Zealand mice to murine sarcoma virus. Clinical and Experimental Immunology 8, 501-9.

GEMMELL, M.A. & MACNAMARA, F.N. (1972). Immune response to tissue parasites. II. Cestodes. In Immunity to Animal Parasites (ed. E.J.L. Soulsby), pp. 235-72. New York, London: Academic Press.

GENCO, R.J., EVANS, R.T. & TAUBMAN, M.A. (1974). Specificity of antibodies to Streptococcus mutans; significance in inhibition of adherence. In The Immunoglobulin A System (ed. J. Mestecky and A.R. Lawton), pp. 327-36. New York, London: Plenum Press.

GERBRANDY, J.L.F. & VAN DURA, E.A. (1972). Anamnestic secretory antibody response in respiratory secretions of intranasally immunized mice. The Journal of Immunology 109, 1146-8.

GERRIS, J. & DE RYCKE, P.H. (1972). Excystation of Hymenolepis microstoma. I. Influence of age, population density and pre-treatment of the cysticercoids. Zeitschrift für Parasitenkunde 39, 277-83.

GIBBONS, R.J. (1974). Bacterial adherence to mucosal surfaces and its inhibition by secretory antibodies. In The Immunoglobulin A System (ed. J. Mestecky and A.R. Lawton), pp. 315-25. New York, London: Plenum Press.

GINSBERG, A.L. (1971). Alterations in immunologic mechanisms in diseases of the gastrointestinal tract. The American Journal of Digestive Diseases 16, 61-80.

GLAISTER, J.R. (1973 a). Factors affecting the lymphoid cells in the small intestinal epithelium of the mouse. International Archives of Allergy and Applied Immunology 45, 719-30.

GLAISTER, J.R. (1973 b). Light, fluorescence and electron microscopic studies of lymphoid cells in the small intestinal epithelium of mice. International Archives of Allergy and Applied Immunology 45, 854-67.

GODFREY, J.F. & COULOMBE, L.S. (1970). Immunological methods and equipment. In Experiments and Techniques in Parasitology (ed. A.J. MacInnis and M. Voge), pp. 209-13. San Francisco: W.H. Freeman and Co.

GOLDMAN, M. (1968). Fluorescent Antibody Methods. New York, London: Academic Press.

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

GOODCHILD, C.G. & MOORE, T.L., Jr. (1963). Development of Hymenolepis diminuta (Rudolphi, 1819) in mice made obese by aurothioglucose. The Journal of Parasitology 49, 398-402.

GRAY, J.S. (1972). Studies on the course of infection of the poultry cestode Raillietina cesticillus (Molin, 1858) in the definitive host. Parasitology 65, 243-50.

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

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, 435-43.

HANNA, M.G., Jr. & PETERS, L.C. (1971). Requirement for continuous antigenic stimulation in the development and differentiation of antibody-forming cells: effect of antigen dose. Immunology 20, 707-18.

HARLOW, D.R. & BYRAM, J.E. (1971). Isolation and morphology of the mitochondrion of the cestode, Hymenolepis diminuta. The Journal of Parasitology 57, 559-65.

HARNESS, E., SMITH, K. & BLAND, P. (1973). Structural changes in the bovine nematode Haemonchus placei, that may be associated with host immune responses. Parasitology 66, 199-205.

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

HEARIN, J.T. (1941). Studies on acquired immunity to the dwarf tapeworm, Hymenolepis nana var. fraterna, in the mouse host. American Journal of Hygiene 33, 71-87.

HENNEY, R.W., MACLEAN, J.M. & MULLIGAN, W. (1971). The effect of host immunity on the metabolism of Nippostrongylus brasiliensis. Immunology 21, 711-8.

HEREMANS, J.F. (1974). The IgA system in connection with local and systemic immunity. In The Immunoglobulin A System (ed. J. Mestecky and A.R. Lawton), pp. 3-11. New York, London: Plenum Press.

HEYNEMAN, D. (1962 a). Studies on helminth immunity: I. Comparison between lumenal and tissue phases of infection in the white mouse by Hymenolepis nana (Cestoda: Hymenolepididae). American Journal of Tropical Medicine and Hygiene 11, 46-63.

HEYNEMAN, D. (1962 b). 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.

HILL, I.R. & PORTER, P. (1974). Studies of bactericidal activity to Escherichia coli of porcine serum and colostral immunoglobulins and the role of lysozyme with secretory IgA. Immunology 26, 1239-50.

HOBBS, J.R. (1970). Simplified radial immunodiffusion. Association of Clinical Pathologists, Broadsheet 68, 8 pp.

HOBBS, J.R. (1971). Immunological disturbances in the pathogenesis of malabsorption. Journal of Clinical Pathology 24 (suppl. 5), 146-50.

HOCKLEY, D.J. (1973). Ultrastructure of the tegument of Schistosoma. In Advances in Parasitology, vol. 11 (ed. B. Dawes), pp. 233-305. London, New York: Academic Press.

HOCKLEY, D.J. & SMITHERS, S.R. (1970). Damage to adult Schistosoma mansoni after transfer to a hyperimmune host. Parasitology 61, 95-100.

HOGARTH-SCOTT, R.S. (1968). Naturally occurring antibodies to the cuticle of nematodes. Parasitology 58, 221-6.

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. (1975). The effect of cortisone on the survival of Hymenolepis diminuta in mice. Parasitology, submitted for publication.

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

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

HOPKINS, C.A., SUBRAMANIAN, G. & STALLARD, H. (1972 b). The effect of immunosuppressants on the development of Hymenolepis diminuta in mice. Parasitology 65, 111-20.

HUNNINEN, A.V. (1935). Studies on the life history and host-parasite relations of Hymenolepis fraterna (H. nana var. fraterna Stiles) in white mice. American Journal of Hygiene 22, 414-43.

ISMAIL, M.M. & TANNER, C.E. (1972). Trichinella spiralis: peripheral blood, intestinal, and bone-marrow eosinophilia in rats and its relationship to the inoculating dose of larvae, antibody response and parasitism. Experimental Parasitology 31, 262-72.

ISHIZAKA, K., ISHIZAKA, T., TADA, T. & NEWCOMB, R.W. (1971). Site of synthesis and function of gamma-E. In The Secretory Immunologic System (ed. D.H. Dayton Jr., P.A. Small Jr., R.M. Chanock, H.E. Kaufman and T.B. Tomasi Jr.), pp. 71-80. Washington, D.C.: United States Government Printing Office.

JARRETT, E.E.E. & URQUHART, G.M. (1971). The immune response to nematode infections. In International Review of Tropical Medicine, vol. 4 (ed. A.W. Woodruff and D.R. Lincicome), pp. 53-96. New York, London: Academic Press.

JARRETT, E.E.E., JARRETT, W.F.H. & URQUHART, G.M. (1966). Immunological unresponsiveness in adult rats to the nematode Nippostrongylus brasiliensis induced by infection in early life. Nature 211, 1310-1.

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, E.E.E., URQUHART, G.M. & DOUTHWAITE, R.M. (1969). Immunological unresponsiveness to helminth parasites. II. The antibody response and the hypersensitivity reaction associated with Nippostrongylus brasiliensis infection in young rats. Experimental Parasitology 24, 270-8.

JARRETT, W.F.H., JARRETT, E.E.E., MILLER, H.R.P. & URQUHART, G.M. (1968). Quantitative studies on the mechanism of self cure in Nippostrongylus brasiliensis infections. In Reaction of the Host to Parasitism (ed. E.J.L. Soulsby), pp. 191-8. Lahn, Germany: Elwert, Marburg.

JENKINS, D.C. (1972). Nippostrongylus brasiliensis: observations on the comparative immunogenicity of adult worms from primary and immune-adapted infections. Parasitology 65, 547-50.

JENKINS, D.C. (1973). Observations on the distribution of an immune-adapted population of Nippostrongylus brasiliensis within the small intestine of rats given repeated small challenge infections. Zeitschrift für Parasitenkunde 41, 73-82.

JENKINS, D.C. (1974 a). Nippostrongylus brasiliensis: observations on factors affecting the establishment of secondary worm populations in rats. Parasitology 68, 13-7.

JENKINS, D.C. (1974 b). Nippostrongylus brasiliensis: the distribution of primary worm populations within the small intestine of neonatal rats. Parasitology 68, 339-45.

JERNE, N.K. (1973). The immune system. Scientific American 229, 52-60.

JERNE, N.K. (1974). Towards a network theory of the immune system. Annales d'Immunologie (Collection des "Annales de L'Institut Pasteur") 125C, 373-89.

JOHNSON, G.D. & HOLBOROW, E.J. (1973). Immunofluorescence. In Handbook of Experimental Immunology, vol. 1 (ed. D.M. Weir), pp. 18.1-18.20. Oxford, London, Edinburgh, Melbourne: Blackwell Scientific Publications.

JOHNSTON, N.W. & BIENENSTOCK, J. (1974). Abolition of non-specific fluorescent staining of eosinophils. Journal of Immunological Methods 4, 189-94.

JONES, A.W. & TAN, B.G. (1971). Effect of crowding upon growth and fecundity in the mouse bile duct tapeworm, Hymenolepis microstoma. The Journal of Parasitology 57, 88-93.

JONES, E.A. (1972). Immunoglobulins and the gut. Gut 13, 825-35.

JONES, V.E. & OGILVIE, B.M. (1971). Protective immunity to Nippostrongylus brasiliensis: the sequence of events which expels worms from the rat intestine. Immunology 20, 549-61.

JONES, V.E., EDWARDS, A.J. & OGILVIE, B.M. (1970). The circulating immunoglobulins involved in protective immunity to the intestinal stage of Nippostrongylus brasiliensis in the rat. Immunology 18, 621-33.

KELLER, R. (1971). Nippostrongylus brasiliensis in the rat: failure to relate intestinal histamine and mast cell levels with worm expulsion. Parasitology 63, 473-81.

KELLER, R. & KEIST, R. (1972). Protective immunity to Nippostrongylus brasiliensis in the rat: central role of the lymphocyte in worm expulsion. Immunology 22, 767-73.

KELLERMEYER, R.W., WARREN, K.S., WALDMANN, T.S., COOK, J.A. & JORDAN, P. (1973). Concentration of serum immunoglobulins in St. Lucians with schistosomiasis mansoni compared with matched uninfected St. Vincentians. The Journal of Infectious Diseases 127, 557-62.

KELLY, J.D. (1973). Mechanisms of immunity to intestinal helminths. Australian Veterinary Journal 49, 91-7.

KELLY, J.D., DINEEN, J.K. & LOVE, R.J. (1973). Expulsion of Nippostrongylus brasiliensis from the intestine of rats: evidence for a third component in the rejection mechanism. International Archives of Allergy and Applied Immunology 45, 767-79.

KILBOURNE, E.D., BUTLER, W.T. & ROSSEN, R.D. (1973). Specific immunity in influenza - summary of influenza workshop III. The Journal of Infectious Diseases 127, 220-36.

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

KUHN, T.S. (1970). The Structure of Scientific Revolutions. Chicago, London: The University of Chicago Press.

KUSEL, J.R. (1972). Protein composition and protein synthesis in the surface membranes of Schistosoma mansoni. Parasitology 65, 55-69.

LARSH, J.E., Jr. (1951). Host-parasite relationships in cestode infections, with emphasis on host resistance. The Journal of Parasitology 37, 343-52.

LARSH, J.E., Jr. & WEATHERLY, N.F. (1974). Cell-mediated immunity in certain parasitic infections. In Current Topics in Microbiology and Immunology, vol. 67 (ed. W. Arber et al.), pp. 113-37. Berlin, Heidleberg, New York: Springer-Verlag.

LARSH, J.E., Jr., OTTOLENGHI, A. & WEATHERLY, N.F. (1974). Trichinella spiralis: phospholipase in challenged mice and rats. Experimental Parasitology 36, 299-306.

LEE, D.L. (1966). The structure and composition of the helminth cuticle. In Advances in Parasitology, vol. 4 (ed. B. Dawes), pp. 187-254. London, New York: Academic Press.

LEE, D.L. (1969). Changes in adult Nippostrongylus brasiliensis during the development of immunity to this nematode in rats. 1. Changes in ultrastructure. Parasitology 59, 29-39.

LEE, D.L. (1971). Changes in adult Nippostrongylus brasiliensis during the development of immunity to this nematode in rats. 2. Total lipids and neutral lipids. Parasitology 63, 271-4.

LEID, R.W. & WILLIAMS, J.F. (1974 a). The immunological response of the rat to infection with Taenia taeniaeformis. I. Immunoglobulin classes involved in passive transfer of resistance. Immunology 27, 195-208.

LEID, R.W. & WILLIAMS, J.F. (1974 b). The immunological response of the rat to infection with Taenia taeniaeformis. II. Characterization of reaginic antibody and an allergen associated with the larval stage. Immunology 27, 209-25.

LOVE, R.J. & OGILVIE, B.M. (1974). The immune deficiency responsible for the persistance of infection in neonatal rats infected with Nippostrongylus brasiliensis. Parasitology 69, xxi.

LUMSDEN, R.D. (1966). Cytological studies on the absorptive surfaces of cestodes. II. The synthesis and intracellular transport of protein in the strobilar integument of Hymenolepis diminuta. Zeitschrift für Parasitenkunde 28, 1-13.

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

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2, 235-54.

MAYER, M.M. (1973). The complement system. Scientific American 229, 54-66.

MCCLELLAND, D.B.L., WARWICK, R.R.G. & SHEARMAN, D.J.C. (1973). Letters to the editor: IgA concentration. The American Journal of Digestive Diseases 18, 347-8.

MCCLELLAND, D.B.L., PARKIN, D.M., HEADING, R.C., BARNETSON, R.ST.C., WARWICK, R.R.G. & SHEARMAN, D.J.C. (1972). Small-intestinal immunoglobulin levels in dermatitis herpetiformis. The Lancet 2, 1108-10.

MESTECKY, J. & LAWTON, A.R. (1974). The Immunoglobulin A System. New York, London: Plenum Press.

METTRICK, D.F. (1971). *Hymenolepis diminuta*: pH changes in rat intestinal contents and worm migration. Experimental Parasitology 29, 386-401.

METTRICK, D.F. & PODESTA, R.B. (1974). Ecological and physiological aspects of helminth-host interactions in the mammalian gastrointestinal canal. In Advances in Parasitology, vol. 12 (ed. B. Dawes), pp. 183-278. London, New York: Academic Press.

MILLER, J.F.A.P. & RAJEWSKY, K. (1974). Cell co-operation: mechanism of the immune response. In Progress in Immunology II, vol. 3 (ed. L. Brent and J. Holborow), pp. 61-120. Amsterdam, Oxford: North-Holland Publishing Co.

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

MOSS, G.D. (1972). The effect of cortisone acetate treatment on the growth of *Hymenolepis microstoma* in mice. Parasitology 64, 311-20.

MURRAY, M. (1972 a). Secretory antibody and local immunity: effector mechanisms of immediate hypersensitivity at mucosal surfaces. Proceedings of the Royal Society of Medicine 65, 659-62.

MURRAY, M. (1972 b). Immediate hypersensitivity effector mechanisms. II. *In vivo* reactions. In Immunity to Animal Parasites (ed. E.J.L. Soulsby), pp. 155-90. New York, London: Academic Press.

MURRAY, M., JARRETT, W.F.H. & JENNINGS, F.W. (1971). Mast cells and macromolecular leak in intestinal immunological reactions. The influence of sex of rats infected with Nippostrongylus brasiliensis. Immunology 21, 17-31.

MURRAY, M., MILLER, H.R.P., SANFORD, J. & JARRETT, W.F.H. (1971). 5-Hydroxytryptamine in intestinal immunological reactions. Its relationship to mast cell activity and worm expulsion in rats infected with Nippostrongylus brasiliensis. International Archives of Allergy and Applied Immunology 40, 236-47.

NORTHRUP, R.S. & HOSSAIN, S.A. (1970). Immunoglobulins and antibody activity in the intestine and serum in cholera. II. Measurement of antibody activity in jejunal aspirates and sera of cholera patients by radioimmunodiffusion. The Journal of Infectious Diseases 121 (suppl.), 142-6.

NOSSAL, G.J.V. & ADA, G.L. (1971). Antigens, Lymphoid Cells and the Immune Response. New York, London: Academic Press.

OAKS, J.A. & KNOWLES, W. (1974). Isolation and partial characterization of fractions derived from the tegument of Hymenolepis diminuta. The American Society of Parasitologists, Program and Abstracts 49th Annual Meeting, 37 p.

OAKS, J.A. & LUMSDEN, R.D. (1971). Cytological studies on the absorptive surfaces of cestodes. V. Incorporation of carbohydrate-containing macromolecules into tegument membranes. The Journal of Parasitology 57, 1256-68.

OGILVIE, B.M. (1964). Reagin-like antibodies in animals immune to helminth parasites. Nature, 204, 91-2.

OGILVIE, B.M. (1969). Immunity to Nippostrongylus brasiliensis. In Nippostrongylus and Toxoplasma, vol. 7 Symposia of British Society for Parasitology (ed. A.E.R. Taylor), pp. 31-40. Oxford, Edinburgh: Blackwell Scientific Publications.

OGILVIE, B.M. (1970). Immunoglobulin responses in parasitic infections. The Journal of Parasitology 56 Section II, 525-34.

OGILVIE, B.M. (1972). Protective immunity to Nippostrongylus brasiliensis in the rat. II. Adaptation by worms. Immunology 22, 111-8.

OGILVIE, B.M. (1974 a). Immunity to parasites (helminths and arthropods). In Progress in Immunology II, vol. 4 (ed. L. Brent and J. Holborow), pp. 127-35. Amsterdam, Oxford: North-Holland Publishing Co.

OGILVIE, B.M. (1974 b). Antigenic variation in the nematode Nippostrongylus brasiliensis. In Parasites in the Immunized Host: mechanisms of survival, Ciba Foundation Symposium 25 (ed. R. Porter and J. Knight), pp. 81-100. Amsterdam, London, New York: Associated Scientific Publishers.

OGILVIE, B.M. & HOCKLEY, D.J. (1968). Effects of immunity on Nippostrongylus brasiliensis adult worms: reversible and irreversible changes in infectivity, reproduction, and morphology. The Journal of Parasitology 54, 1073-84.

OGILVIE, B.M. & JONES, V.E. (1967). Reaginic antibodies and immunity to Nippostrongylus brasiliensis in the rat. I. The effect of thymectomy, neonatal infections and splenectomy. Parasitology 57, 335-49.

OGILVIE, B.M. & JONES, V.E. (1971). Nippostrongylus brasiliensis: A review of immunity and the host/parasite relationship in the rat. Experimental Parasitology 29, 138-77.

OGILVIE, B.M. & JONES, V.E. (1973). Immunity in the parasitic relationship between helminths and hosts. In Progress in Allergy, vol. 17 (ed. P. Kallos, B.H. Waksman and A. de Weck), pp. 93-144. Basel, München, Paris, London, New York, Sydney: S. Karger.

OGILVIE, B.M. & LOVE, R.J. (1974). Co-operation between antibodies and cells in immunity to a nematode parasite. Transplantation Reviews 19, 147-68.

OGRA, P.L., WALLACE, R.B., UMANA, G., OGRA, S.S., GRANT, D.K. & MORAG, A. (1974). Implications of secretory immune system in viral infections. In The Immunoglobulin A System (ed. J. Mestecky and A.R. Lawton), pp. 271-82. New York, London: Plenum Press.

OTTOLENGHI, A. (1973). High phospholipase content of intestines of mice infected with Hymenolepis nana. Lipids 8, 426-8.

OUDIN, J. & WILLIAMS, C.A. (1971). Precipitation analysis by diffusion in gels. In Methods in Immunology and Immunochemistry, vol. III (ed. C.A. Williams and M.W. Chase), pp. 103-374. New York, London: Academic Press.

PAPPAS, P.W. & READ, C.P. (1972 a). Trypsin inactivation by intact Hymenolepis diminuta. The Journal of Parasitology 58, 864-71.

PAPPAS, P.W. & READ, C.P. (1972 b). Inactivation of α- and β-chymotrypsin by intact Hymenolepis diminuta (Cestoda). Biological Bulletin 143, 605-16.

PARROTT, D.M.V. & FERGUSON, A. (1974). Selective migration of lymphocytes within the mouse small intestine. Immunology 26, 571-88.

PEREZ, H. & TERRY, R.J. (1973). The killing of adult Schistosoma mansoni in vitro in the presence of antisera to host antigenic determinants and peritoneal cells. International Journal for Parasitology 3, 499-503.

PETERS, D.K. (1974). The immunological basis of glomerulonephritis. Proceedings of the Royal Society of Medicine 67, 557-62.

PHILLIPS, T.M. & LEWIS, M.G. (1971). A method for elution of immunoglobulin from the surface of living cells. European Journal of Clinical and Biological Research 16, 1052-5.

PIERCE, N.F. & REYNOLDS, H.Y. (1974). Immunity to experimental cholera. I. Protective effect of humoral IgG antitoxin demonstrated by passive immunization. The Journal of Immunology 113, 1017-23.

PLAUT, A.G. & KEONIL, P. (1969). Immunoglobulins in human small intestinal fluid. Gastroenterology 56, 522-30.

PLAUT, A.G., GENCO, R.J. & TOMASI, T.B., Jr. (1974). Isolation of an enzyme from Streptococcus sanguis which specifically cleaves IgA. The Journal of Immunology 113, 289-91.

PODESTA, R.B. & METTRICK, D.F. (1974 a). Components of glucose transport in the host-parasite system, Hymenolepis diminuta (Cestoda) and the rat intestine. Canadian Journal of Physiology and Pharmacology 52, 183-97.

PODESTA, R.B. & METTRICK, D.F. (1974 b). Pathophysiology of cestode infections: effect of Hymenolepis diminuta on oxygen tensions, pH and gastrointestinal function. International Journal for Parasitology 4, 277-92.

PODESTA, R.B. & METTRICK, D.F. (1974 c). The effect of bicarbonate and acidification on water and electrolyte absorption by the intestine of normal and infected (Hymenolepis diminuta: Cestoda) rats. The American Journal of Digestive Diseases 19, 725-35.

POGER, M.E. & LAMM, M.E. (1974). Localization of free and bound secretory component in human intestinal epithelial cells. The Journal of Experimental Medicine 139, 629-42.

PORTER, P. (1973). The functional role of secretory antibody systems in early development of the pig and the calf. Proceedings of the Nutrition Society 32, 217-22.

PORTER, P., KENWORTHY, R. & ALLEN, W.D. (1974). Effect of oral immunisation with E. coli antigens on post weaning enteric infection in the young pig. The Veterinary Record 95, 99-104.

PORTER, P., KENWORTHY, R., NOAKES, D.E. & ALLEN, W.D. (1974). Intestinal antibody secretion in the young pig in response to oral immunization with Escherichia coli. Immunology 27, 841-53.

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

RADERMECKER, M., BEKHTI, A., PONCELET, E. & SALMON, J. (1974). Serum IgE levels in protozoal and helminthic infections. International Archives of Allergy and Applied Immunology 47, 285-95.

RADL, J., SCHUIT, H.R.E., MESTECKY, J. & HIJMANS, W. (1974). The origin of monomeric and polymeric forms of IgA in man. In The Immunoglobulin A System (ed. J. Mestecky and A.R. Lawton), pp. 57-65. New York, London: Plenum Press.

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

READ, C.P. & SIMMONS, J.E., Jr. (1963). Biochemistry and physiology of tapeworms. Physiological Reviews 43, 263-305.

READ, C.P. & VOGE, M. (1954). The size attained by Hymenolepis diminuta in different host species. The Journal of Parasitology 40, 88-9.

READ, C.P., ROTHMAN, A.H. & SIMMONS, J.E., Jr. (1963). Studies on membrane transport, with special reference to parasite-host integration. Annals of the New York Academy of Sciences 113, 154-205.

RECTOR, E.S. & CARTER, B.G. (1973). Age-dependent changes in sensitivity to antigen in the mouse. The Journal of Immunology 110, 1591-8.

REED, W.P. & WILLIAMS, R.C. (1971). Intestinal immunoglobulins in shigellosis. Gastroenterology 61, 35-45.

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

REID, W.M. (1942). Certain nutritional requirements of the fowl cestode Raillietina cesticillus (Molin) as demonstrated by short periods of starvation of the host. The Journal of Parasitology 28, 319-40.

REMINGTON, R.D. & SCHORK, M.A. (1970). Statistics with Applications to the Biological and Health Sciences. New Jersey: Prentice-Hill, Inc.

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

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

ROITT, I. (1974). Essential Immunology. Oxford, London, Edinburgh, Melbourne: Blackwell Scientific Publications.

ROSE, M.E. (1972). Immune response to intracellular parasites. II. Coccidia. In Immunity to Animal Parasites (ed. E.J.L. Soulsby), pp. 365-88. New York, London: Academic Press.

ROSE, M.E. (1974). The early development of immunity to Eimeria maxima in comparison with that to Eimeria tenella. Parasitology 68, 35-45.

ROSE, M.E. & LONG, P.L. (1969). Immunity to coccidiosis: gut permeability changes in response to sporozoite invasion. Experientia 25, 183-4.

ROTHBERG, R.M., KRAFT, S.C. & MICHALEK, S.M. (1973). Systemic immunity after local antigenic stimulation of the lymphoid tissue of the gastrointestinal tract. The Journal of Immunology 111, 1906-13.

ROTHBERG, R.M., KRAFT, S.C. & MICHALEK, S.M. (1974). The establishment of systemic immunity following antigenic stimulation of the lymphoid tissue of the gastrointestinal mucosa. In The Immunoglobulin A System (ed. J. Mestecky and A.R. Lawton), pp. 473-8. New York, London: Plenum Press.

ROTHWELL, T.L.W. & MERRITT, G.C. (1974). Acetylcholinesterase secretion by parasitic nematodes. IV. Antibodies against the enzyme in Trichostrongylus colubriformis infected sheep. International Journal for Parasitology 4, 63-71.

ROTHWELL, T.L.W., PRICHARD, R.K. & LOVE, R.J. (1974). Studies on the role of histamine and 5-hydroxytryptamine in immunity against the nematode Trichostrongylus colubriformis. I. In vivo and in vitro effects of the amines. International Archives of Allergy and Applied Immunology 46, 1-13.

RUBIN, W., FAUCI, A.S., SLEISENGER, M.H. & JEFFRIES, G.H. (1965). Immunofluorescent studies in adult celiac disease. The Journal of Clinical Investigation 44, 475-85.

RUBINSTEIN, P., DECARY, F. & STREUN, E.W. (1974). Quantitative studies on tumor enhancement in mice. I. Enhancement of sarcoma I induced by IgM, IgG_1 and IgG_2. The Journal of Experimental Medicine 140, 591-6.

RUDZIK, O. & BIENENSTOCK, J. (1974). Isolation and characteristics of gut mucosal lymphocytes. Laboratory Investigation 30, 260-6.

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

SAMSON, R.R., MCCLELLAND, D.B.L. & SHEARMAN, D.J.C. (1973). Studies on the quantitation of immunoglobulin in human intestinal secretions. Gut 14, 616-26.

SANDERSON, B.E., JENKINS, D.C. & PHILLIPSON, R.F. (1972). Nippostrongylus brasiliensis: relation between immune damage and acetycholinesterase levels. International Journal for Parasitology 2, 227-32.

SAVAGE, D.C. (1970). Associations of indigenous microorganisms with gastrointestinal mucosal epithelia. The American Journal of Clinical Nutrition 23, 1495-501.

*RYBICKA, K. see p.294.

SAVILAHTI, E. (1972). Intestinal immunoglobulins in children with coeliac disease. Gut 13, 958-64.

SCHNEIDER, G.B. (1973). The ontogeny of cell-mediated immunity: the response of the mouse to dinitrofluorobenzene (DNFB). The American Journal of Anatomy 137, 257-80.

SCHULTZ, D.R. & MILLER, K.D. (1974). Elastase of Pseudomonas aeruginosa: inactivation of complement components and complement-derived chemotactic and phagocytic factors. Infection and Immunity 10, 128-35.

SELBY, G.R. & WAKELIN, D. (1973). Transfer of immunity against Trichuris muris in the mouse by serum and cells. International Journal for Parasitology 3, 717-22.

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

SHIMAMURA, T. (1972). Immune response in germfree mice orally immunized with Vibrio cholerae. Keio Journal of Medicine 21, 113-26.

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

SMYTH, J.D. (1972). Changes in the digestive-absorptive surface of cestodes during larval/adult differentiation. In Functional Aspects of Parasite Surfaces, vol. 10 Symposia of the British Society for Parasitology (ed. A.E.R. Taylor and R. Muller), pp. 41-70. Oxford, London, Edinburgh, Melbourne: Blackwell Scientific Publications.

SMYTH, J.D. & DAVIES, Z. (1974). Occurrence of physiological strains of Echinococcus granulosus demonstrated by in vitro culture of protoscoleces from sheep and horse hydatid cysts. International Journal for Parasitology 4, 443-5.

SMYTH, J.D. & HEATH, D.D. (1970). Pathogenesis of larval cestodes in mammals. Helminthological Abstracts 39, 1-23.

SOLOMON, J.B. (1971). Fetal and neonatal immunology. In Frontiers of Biology, vol. 20 (ed. A. Neuberger and E.L. Tatum). Amsterdam, London: North-Holland Publishing Co.

SÖLTOFT, J., BINDER, V. & GUDMAND-HÖYER, E. (1973). Intestinal immunoglobulins in ulcerative colitis. Scandinavian Journal of Gastroenterology 8, 293-300.

SOULSBY, E.J.L. (1972). Immunity to Animal Parasites. New York, London: Academic Press.

SOULSBY, E.J.L. (1974). Immunity to worms. In Progress in Immunology II, vol. 4 (ed. L. Brent and J. Holborow), pp. 376-80. Amsterdam, Oxford: North-Holland Publishing Co.

SOUTH, M.A. (1971 a). IgA in neonatal immunity. Annals of the New York Academy of Sciences 176, 40-8.

SOUTH, M.A. (1971 b). Enteropathogenic Escherichia coli disease: new developments and perspectives. The Journal of Pediatrics 79, 1-11.

ŠTERZL, J. & SILVERSTEIN, A.M. (1967). Developmental aspects of immunity. In Advances in Immunology, vol. 6 (ed. F.J. Dixon and J.H. Humphrey), pp. 337-459. New York, London: Academic Press.

SVANVIK, J. (1973). Mucosal blood circulation and its influence on passive absorption in the small intestine. An experimental study in the cat. Acta Physiologica Scandinavica Supplementum 385, 44 pp.

TALIAFERRO, W.H. (1940 a). The mechanism of immunity to metazoan parasites. American Journal of Tropical Medicine 20, 149-61.

TALIAFERRO, W.H. (1940 b). The mechanism of acquired immunity in infections with parasitic worms. Physiological Reviews 20, 469-92.

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

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

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

THORSON, R.E. (1963). Seminar on immunity to parasitic helminths. II. Physiology of immunity to helminth infections. Experimental Parasitology 13, 3-12.

THREADGOLD, L.T. (1967). The Ultrastructure of the Animal Cell. Oxford, London, Edinburgh, New York, Toronto, Sydney, Paris, Braunschweig: Pergamon Press.

THREADGOLD, L.T. & READ, C.P. (1970). Hymenolepis diminuta: ultrastructure of a unique membrane specialisation in tegument. Experimental Parasitology 28, 246-52.

TIDBALL, C.S. (1971). The nature of the intestinal epithelial barrier. The American Journal of Digestive Diseases 16, 745-67.

TOMASI, T.B., Jr. (1971). The concept of local immunity and the secretory system. In The Secretory Immunological System (ed. D.H. Dayton Jr., P.A. Small Jr., R.M. Chanock, H.E. Kaufman and T.B. Tomasi Jr.), pp. 3-10. Washington, D.C.: United States Government Printing Office.

TOMASI, T.B., Jr. & BIENENSTOCK, J. (1968). Secretory immunoglobulins. In Advances in Immunology, vol. 9 (ed. F.J. Dixon Jr. and H.G. Kunkel), pp. 1-96. New York, London: Academic Press.

TOMASI, T.B. & GREY, H.M. (1972). Structure and function of immunoglobulin A. In Progress in Allergy, vol. 16 (ed. P. Kallos, B.H. Waksman and A. de Weck), pp. 81-213. Basel, München, Paris, London, New York, Sydney: S. Karger.

TRUMP, B.J. & ARSTILA, A.U. (1971). Cell injury and cell death. In Principles of Pathobiology (ed. M.F. La Via and R.B. Hill Jr.), pp. 9-95. New York, London, Toronto: Oxford University Press.

TURK, J.L. (1969). Immunology in Clinical Medicine. London: William Heinemann Medical Books, Ltd.

TURNER-WARWICK, M. (1974). Immunological aspects of systemic diseases of the lungs. Proceedings of the Royal Society of Medicine 67, 541-7.

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 für Parasitenkunde 37, 315-29.

URQUHART, G.M. (1970). Immunological unresponsiveness in parasitic infections. The Journal of Parasitology 56 Section II, 547-51.

VAERMAN, J.P., ANDRÉ, C., BAZIN, H. & HEREMANS, J.F. (1973). Mesenteric lymph as a major source of serum IgA in guinea pigs and rats. European Journal of Immunology 3, 580-4.

VALDIVIESO, D. & TAMSITT, J.R. (1970). A review of the immunology of parasitic nematodes. Revista de Biologia Tropical 17, 1-25.

VELDKAMP, J., VAN DER GAAG, R. & WILLERS, J.M.N. (1973). The role of 'Peyer's patch cells in antibody formation. Immunology 25, 761-71.

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. (1974). Genetic control of the immune response of mice to the nematode Trichuris muris. Transactions of the Royal Society of Tropical Medicine and Hygiene 68, 277.

WAKELIN, D. (1975 a). Immune expulsion of Trichuris muris from mice during a primary infection. Analysis of the components involved. Parasitology, 71, in press.

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

WAKSMAN, B.H. (1973). The homing pattern of thymus-derived lymphocytes in calf and neonatal mouse Peyer's patches. The Journal of Immunology 111, 878-84.

WAKSMAN, B.H., OZER, H. & BLYTHMAN, H.E. (1973). Appendix and γM-antibody formation. VI. The functional anatomy of the rabbit appendix. Laboratory Investigation 28, 614-26.

WALDMAN, R.H. & GANGULY, R. (1974). The role of the secretory immune system in protection against agents which infect the respiratory tract. In The Immunoglobulin A System (ed. J. Mestecky and A.R. Lawton), pp. 283-94. New York, London: Plenum Press.

WALDMAN, R.H., VIRCHOW, C. & ROWE, D.S. (1973). IgE levels in external secretions. International Archives of Allergy and Applied Immunology 44, 242-8.

WALDMAN, R.H., BENČIĆ, Z., SAKAZAKI, R., SINHA, R., GANGULY, R., DEB, B.C. & MUKERJEE, S. (1971). Cholera immunology. I. Immunoglobulin levels in serum, fluid from the small intestine, and feces from patients with cholera and noncholeraic diarrhea during illness and convalescence. The Journal of Infectious Diseases 123, 579-86.

WALDMAN, R.H., BENČIĆ, Z., SINHA, R., DEB, B.C., SAKAZAKI, R., TAMURA, K., MUKERJEE, S. & GANGULY, R. (1972). Cholera immunology. II. Serum and intestinal secretion antibody response after naturally occurring cholera. The Journal of Infectious Diseases 126, 401-7.

WALKER, W.A. & HONG, R. (1973 a). Immunology of the gastrointestinal tract: part I. The Journal of Pediatrics 83, 517-30.

WALKER, W.A. & HONG, R. (1973 b). Immunology of the gastrointestinal tract. Part II. The Journal of Pediatrics 83, 711-20.

WALKER, W.A. & ISSELBACHER, K.J. (1974). Uptake and transport of macromolecules by the intestine: possible role in clinical disorders. Gastroenterology 67, 531-50.

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 and A.R. Lawton), pp. 295-303. New York, London: Plenum Press.

WALLEY, J.K. (1966). Oxyclozanide (3, 3', 5, 5', 6-pentachloro - 2, 2'-dihydroxy-benzanilide-"Zanil") in the treatment of the liver fluke Fasciola hepatica in sheep and cattle. The Veterinary Record 78, 267-76.

WARDLAW, A.C. & WATT, P.J. (1974). Immunity in mucosal infections. In Progress in Immunology II, vol. 4 (ed. L. Brent and J. Holborow), pp. 341-4. Amsterdam, Oxford: North-Holland Publishing Co.

WARSHAW, A.L., WALKER, W.A. & ISSELBACHER, K.J. (1974). Protein uptake by the intestine: evidence for absorption of intact macromolecules. Gastroenterology 66, 987-92.

WASSOM, D.L., DEWITT, C.W. & GRUNDMANN, A.W. (1974). Immunity to Hymenolepis citelli by Peromyscus maniculatus: genetic control and ecological implications. The 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. The Journal of Parasitology 59, 117-21.

WEINMANN, C.J. (1966). Immunity mechanisms in cestode infections. In Biology of Parasites (ed. E.J.L. Soulsby), pp. 301-20. New York, London: Academic Press.

WEINMANN, C.J. (1970). Cestodes and Acanthocephala. In Immunity to Parasitic Animals, vol. 2 (ed. G.J. Jackson, R. Herman and I. Singer), pp. 1021-59. New York: Appleton.

WEISER, R.S., MYRVIK, Q.N. & PEARSALL, N.N. (1969). Fundamentals of Immunology for Students of Medicine and Related Sciences. Philadelphia: Lea & Febiger.

WILLIAMS, R.C. & GIBBONS, R.J. (1972). Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science 177, 697-9.

WITZ, I.P. (1973). The biological significance of tumor-bound immunoglobulins. In Current Topics in Microbiology and Immunology, vol. 61 (ed. W. Arber et al.), pp. 151-71. Berlin, Heidelberg, New York: Springer-Verlag.

ZINKERNAGEL, R.M., BLANDEN, R.V. & LANGMAN, R.E. (1974). Early appearance of sensitized lymphocytes in mice infected with Listeria monocytogenes. The Journal of Immunology 112, 496-501.

ZINNEMAN, H.H. & KAPLAN, A.P. (1972). The association of giardiasis with reduced intestinal secretory immunoglobulin A. The American Journal of Digestive Diseases 17, 793-7.

RYBICKA, K. (1966). Embryogenesis in cestodes. In Advances in Parasitology, vol. 4 (ed. B. Dawes), pp. 107-86. London, New York: Academic Press.