IMMUNITY TO *NIPPOSTRONGYLUS BRASILIENSIS* INFECTION IN THE RAT

DISSERTATION FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY
IN THE FACULTY OF MEDICINE

BY

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PREFACE

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TABLE OF CONTENTS

GENERAL INTRODUCTION

A REVIEW OF THE LITERATURE

At the present time it is difficult to generalise about the mode of action of the immune response of man and animals in helminth infections. This is due to a number of factors such as the zoological diversity of the parasites, the variety of habitats in the host, and not least by the scarcity of workers in this field. Such information as is available has been well summarized in recent reviews (Soulsby, 1962, 1966; Urquhart et al, 1962) and in this introduction attention will be focussed primarily on the present state of knowledge of immune mechanisms in gastro-intestinal nematode infections of domestic and laboratory animals.

Manifestations of immunity

In general the effects of immunity are exhibited in four ways.

The first is the self-cure phenomenon, a reaction which results in the expulsion of a worm population from the stomach or intestine of an infected animal. It may terminate both primary or subsequent infections and has been recorded both in the field and in the laboratory. That the elimination of the primary infection is due to an immune response was shown for Nippostrongylus brasiliensis by the re-establishment and continued egg production which occurred after adult worms were surgically transferred from rats about to self-cure, to normal rats (Chandler, 1936a; Ogilvie, 1962; Mulligan et al, 1965). Other evidence is provided by the ability of cortisone (Coker, 1955; Weinstein, 1955; Campbell, 1963; Ogilvie, 1965; Urquhart et al, 1965) and the whole body irradiation of the

host (Yarinsky, 1962) to prevent the expulsion of the worms during the primary infection. The self-cure reaction is reviewed in some detail in the introductions to Section 1 and 2.

Increased resistance as a result of previous infection, demonstrated by a significant reduction in the number of adult worms developing from a challenge infection, is a frequently recorded manifestation of acquired immunity. The reduction may be caused by the death of developing worms or by the inhibition of larval stages. The former mechanism has been shown to operate in infections of N. brasiliensis and Trichinella spiralis in the rat (Taliaferro and Sarles, 1939; McCoy, 1940), Trichostrongylus retortaeformis in the rabbit (Michel, 1953) and Cooperia punctata and Dictyocaulus viviparus in cattle (Stewart and Gordon, 1958; Jarrett et al, 1959a).

In some helminth infections the development of a large proportion of the parasites may become inhibited at a particular stage of the life cycle. This phenomenon of inhibition is sometimes associated with resistance following previous infection. Developing stages of Oesophagostomum columbianum become inhibited and may remain viable for many months in the mucosal nodules of previously infected sheep (Monnig, 1949; Gordon, 1950). Reinfections of T. retortaeformis in the rabbit become inhibited at the third larval stage (Michel, 1952) and reinfections of sheep with Haemonchus contortus may become inhibited at the fourth and fifth larval stages (Silverman and Patterson, 1960).

Inhibition has also been described under circumstances which seem to preclude the agency of any immune response. It has been shown to occur after the administration of a single and usually large dose of infective larvae in both Oesophagostomum circumcincta (Sommerville, 1954; Dunsmore, 1960) and Haemonchus placei infections (Roberts, 1957). Recent experiments have suggested that inhibition of Ostertagia ostertagi in cattle is a genetically determined trait, and is triggered by seasonal physiological changes in larvae on the pasture (Anderson et al, 1965; Armour et al, 1967). Inhibited larvae may be subsequently thrown off, walled off and destroyed or they may mature at some later time. Such subsequent maturation of inhibited larvae constitutes an important phase in the complex of bovine ostertagiasis (Martin et al, 1957; Anderson et al, 1965).

Worms which survive the larval hazards may become adult but remain stunted in size and have a reduced egg laying capacity. This has been shown to occur after reinfections of N. brasiliensis (Chandler, 1932, 1938; Taliaferro and Sarles, 1939; Hurley, 1959), T. colubriformis (Stewart and Gordon, 1958), O. ostertagi (Michel, 1963) and H. contortus (Roberts, 1957). This type of immune reaction has been passively transferred to chickens infected with Ascaridia galli (Sadun, 1949).

The antigens

An individual nematode larva, passing through various developmental stages, sheds antigens not only as successive larval cuticles but also as

a variety of other excreted and secreted substances. In addition any nematodes which die *in situ* expose the host to a large number of somatic antigens. The central problem of the antigens lies in the definition and isolation of those which stimulate the phenomena associated with resistance, as opposed to the numerous incidental immune responses.

It has been the general experience that in order to produce good immunity it is necessary to infect the host with the living parasite, and conversely that non-viable parasitic materials generally produce only a low degree of immunity. Vaccination with antigens made from dead whole worm material while sometimes having some protective influence has been on the whole disappointing and even when successful required large amounts of parasitic material (Chandler, 1932; McCoy, 1935; Kerr, 1938; Culbertson, 1942; Oliver Gonzalez, 1956; Soulsby, 1957a; Jarrett *et al*, 1960).

In contrast good immunity can in many cases be obtained by administration of infective larvae, e.g. against *Ascaris lumbricoides* (Sprent and Chen, 1949; Oliver Gonzalez, 1956), *D. viviparus* (Rubin and Lucker, 1956; Jarrett *et al*, 1957; Jarrett *et al*, 1959b) and *N. brasiliensis* (Taliaferro and Sarles, 1939).

The stages in the parasitic life cycle responsible for the stimulation of immunity vary in different host-parasite systems. With some species of nematode, host resistance can be stimulated with larval stages alone. Using X-irradiated larvae, thereby preventing development of most of the parasites beyond the final larval moult, it was possible to stimulate resistance to *T. spiralis* in the rat (Levin and Evans, 1942; Gould *et al*, 1955), to *D.*

viviparus in cattle (Jarrett et al, 1959b), Uncinaria stenocephala and Ancylostoma caninum in dogs (Dow et al, 1961; Miller, 1967) and H. contortus in sheep (Jarrett et al, 1961). On the other hand immunity can be stimulated by adult stages alone in N. brasiliensis (Chandler, 1936b; Spindler, 1936; Ogilvie, 1962), H. placei (Ross, 1963) and Oesophagostomum radiatum infections (Roberts et al, 1963).

The first evidence of the possible importance of the secretions of worms in the immune response of the host was the observation of precipitates formed around and at the orifices of N. brasiliensis larvae both when migrating in the host (Sarles and Taliaferro, 1936) and when immersed in immune serum in vitro (Sarles, 1938). Similar precipitates have been observed with T. spiralis (Oliver Gonzalez, 1940), A. caninum (Otto, 1940), A. lumbricoides (Soulsby, 1957a) and O. radiatum (Douvres, 1962). It remains a dubious point whether these antigen-antibody interactions constitute an embarrassment to the worm. Oliver Gonzalez (1940) reported shortened in vitro survival times of T. spiralis larvae on which precipitates had formed. However, although similar precipitates were found by Jackson (1959) parasiticidal activity was not confirmed. The fluorescent antibody technique employed by Jackson in his experiments showed that the antigen-antibody reaction was localized on the oral and vulvar orifices of the adult worms, and in section, on the digestive and reproductive tracts. Together with the desirability of using live worms for the stimulation of immunity, this evidence drew attention to the soluble materials released by the worm in life.

Attempts have been made to locate such secreted antigens in solutions of materials collected from living worms. Thorson (1953) showed that vaccination with culture fluid in which N. brasiliensis larvae had been incubated conferred a degree of protection to subsequent infection. The same author (1954a) was further able to show that while larval somatic antigens could not remove the protective capacity of immune rat serum, solutions of metabolites could. Similar results have been obtained with the excretions and secretions of T. spiralis (Campbell, 1955), T. colubriformis, D. viviparus and Strongyloides papillosus (Silverman et al, 1962).

It was first suggested by Chandler (1935, 1953) that the parasitic secretions and excretions might contain enzymes which are important for digestion and metabolism, or for penetration of the host's tissues during migration. This theory received support through the work of Thorson (1953) who showed that the metabolic solution which was successful in protecting rats against N. brasiliensis contained a lipase, and that antibodies were formed to this during infection. The same author (1956) using A. caninum showed that oesophageal extracts contained proteolytic enzymes whose action could be inhibited by immune, though not normal, dog serum. Other antigenic enzymes now demonstrated in parasitic helminths are those associated with invasiveness of migrating forms (Lewert and Lee, 1954; Lee and Lewert, 1957), with exsheathment of infective larvae (Sommerville, 1957; Rogers and Sommerville, 1957) and others, probably associated with digestion and respiration (Lee, 1962a,b; Tromba and Baisden, 1964). It was suggested

that these antigens could induce antibodies which, acting as antienzymes, might interfere with parasitic development.

Helminth antigens have been reviewed by Soulsby (1962, 1963a), Kent (1963) and Thorson (1963).

The mechanisms of the immune reaction

There is no evidence to suppose that immune mechanisms differing from those described in other immunological systems operate in helminth immunity; however, the precise implication of a particular antibody in a defined protective role is hampered by the heterogeneity of both antibody and response.

In some parasitic systems, e.g. N. brasiliensis, Ascaridia galli, T. spiralis and D. viviparus, the effect of a naturally acquired resistance can be passively transferred by serum (Sarles and Taliaferro, 1936; Sadun, 1949; Culbertson, 1942; Jarrett et al, 1955).

Further evidence of the effect of humoral antibody is provided by the work of Crandal and Arean (1964) who placed 2nd stage A. lumbricoides larvae in Millipore diffusion chambers in the peritoneal cavity of mice. Cessation of growth of the parasite was demonstrated when the chambers were placed in immune mice with subsequent resumption of growth when the parasites were returned to normal mice. Since the chambers excluded cells but not serum proteins the observed effect was attributed to conventional antibodies. Despite the evidence of the efficacy of antibodies in helminth immunity,

resource to the conventional serological techniques (such as complement fixation, precipitation, agglutination and haemaglutination), while often proving of diagnostic interest, has failed to provide a method for titrating protective capacity (Stewart, 1950a,b,c; Stewart, 1951; Soulsby, 1956, 1957b, 1960; Michel and Cornwall, 1959; Taffs, 1964a,b).

It has also proved extremely difficult to demonstrate any deleterious effect of immune serum on helminths incubated in vitro. Schwabe (1957) found that the oxygen consumption rate of the free living but not the parasitic 3rd stage larvae of N. brasiliensis was reduced during incubation in immune serum. The formation of precipitates on various parasites incubated in immune serum has already been mentioned. Attempts to demonstrate the fixing of antibody onto the cuticle of Ascaris larvae previously exposed to immune serum led Soulsby (1963a) to develop two cell adhesion tests. One involved white cells (more specifically eosinophils and monocytes) which were found to adhere to larvae exposed to immune though not normal serum. The second reaction requiring complement involved human red cells which were likewise found to adhere to larvae exposed to immune serum.

The failure of immune serum to reduce in vitro survival time may be due to the fact that parasites cannot be kept alive for any prolonged period in vitro in any medium, and development of methods for axenic culture may in future greatly influence such tests. It may also be that factors other than those present in host immune serum are required to cause death of the parasite.

Complex cellular reactions, which appear to be directed against the invading parasites, are frequently observed in the tissues of infected animals. The first detailed description of such a reaction in N. brasiliensis infected rats was by Taliaferro and Sarles (1939). These authors reached the conclusion that the cellular foci which they observed were formed after the parasites had already been immobilized by antibody and that their prime functions were to wall off and to phagocytose the parasite. A cellular response to D. viviparus has been described in detail by Jarrett and Sharp (1963). They described, in response to the presence of dying worms, the development of a peribronchial focus of antibody producing cells (haemacytoblasts, immature and mature plasma cells) in the lungs of calves within 5 days of re-infection. The accumulation of similar cells probably producing antibody and situated in the superficial intestinal mucosa, together with the formation of lymphoreticular follicles with active germ centres in the lower part of the mucosa and submucosa has been described in O. ostertagi infections in calves (Armour, 1967). Further evidence of local antibody production is provided by Crandall et al (1967) in experiments using fluorescent antibody techniques. A relative increase in IgM containing cells is described in the intestinal mucosa after infection of rabbits with T. spiralis followed by an apparent increase in cells with IgG late in infection and after hyperinfection. It is not yet certain whether these antibody producing sites play any part in resistance, or whether they are merely concerned in the production of anti-bodies against helminth somatic antigens which may have no protective function.

Eosinophil leucocytes are also frequently observed at the site of parasitic infection; the function of these cells remains obscure although several interesting hypotheses have been advanced (Archer, 1960; Speirs, 1958). The function of two other cell types frequently found in parasitic infection is also unknown. These are the mast cell and the globule leucocyte. It has been suggested (Dobson, 1966a,b; Whur and Gracie, 1967) that the globule leucocyte originates from immunoglobulin producing cells and that its function is to carry antibody into the intestinal lumen. This hypothesis has been refuted (Jarrett et al, 1967a; Jarrett et al, 1967b; Murray et al, 1968). The latter authors have demonstrated that the globule leucocyte is derived from the mast cell, and postulate a role in a local anaphylactic response for both the cell types.

The transfer of immunity using lymphoid cells has led some workers to imply that delayed hypersensitivity may play a significant role in helminth infections. It has been suggested on this basis, for instance, that immunity to T. spiralis in mice (Larsh et al, 1964) and T. colubriformis in the guinea pig (Wagland and Dineen, 1965) is mediated by a delayed hypersensitivity reaction. However, it is possible that the observed protection was due to antibody production by the transferred cells. The latter authors reported failure to transfer protection against T. colubriformis with serum, but it was subsequently shown by Connan (1965) using larger quantities of serum that this was possible.

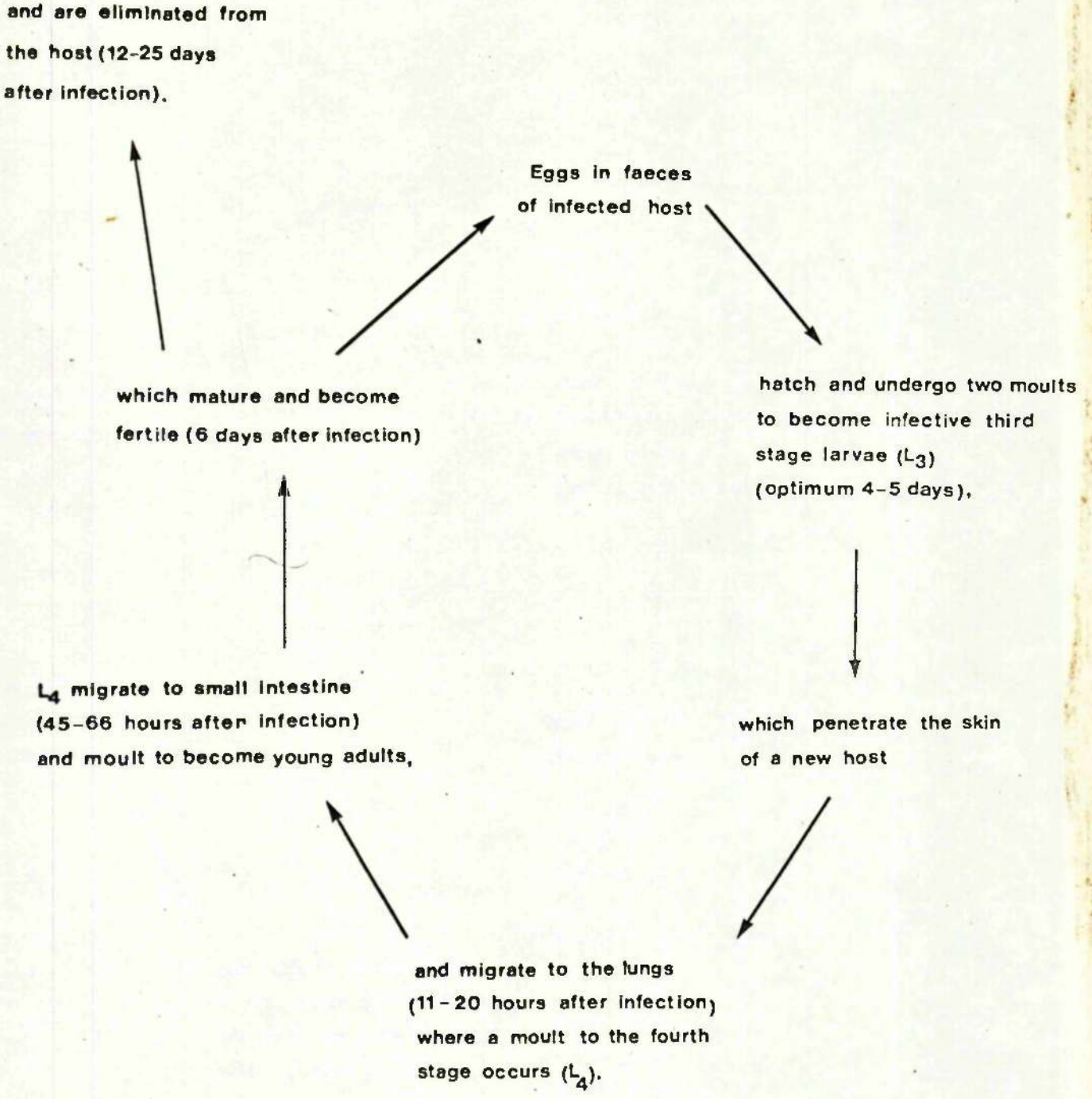
In gastrointestinal parasitic infections there is the particular problem of the means of access of antibody into the gut lumen. It has been suggested (Pierce, 1959) that the means of access of humoral antibody might be afforded by local alterations in tissue permeability; such alterations might possibly be induced by a local anaphylactic reaction (Mulligan et al, 1965). The occurrence of a local antibody producing cell response following infection has already been mentioned, and it is possible that locally produced antibody plays an important role in gut helminthiasis. The demonstration of helminth specific antibodies in intestinal mucous exudates in higher titre than present in serum (Dobson, 1966c) and in intestinal mucosal extracts (Crandall et al, 1967) of infected animals is of particular interest in this context.

LIFE CYCLE

OF

N. BRASILIENSIS

THE LIFE CYCLE OF N. BRASILIENSIS IN A PRIMARY INFECTION



The nematode Nippostrongylus brasiliensis is a natural parasite of wild rats from which it has been isolated in many parts of the world. It was first described in 1922 by Yokogawa who called it Heligmosomum muris Yokogawa but its name has since been changed twice, to Nippostrongylus muris and finally to Nippostrongylus brasiliensis. A pictorial summary of the life cycle is shown opposite and a review of the literature relating to this will be dealt with under four sections entitled 'skin stage', 'lung stage', 'intestinal stage' and 'termination of infection'.

Skin stage

Yokogawa (1922) demonstrated that the natural mode of infection was by skin penetration. The minimum time for penetration of larvae after contact is five minutes (Gharib, 1955) but Twohy (1956) noted that after one hour approximately only 50% of larvae had penetrated and that maximal penetration occurred in rats exposed for four hours.

According to Taliaferro and Sarles (1939) larvae begin to feed soon after penetration of the skin showing an increase in diameter and enlargement of internal organs. Twohy (1955a,b, 1956) however reported that the larvae first underwent a lag phase in growth as evidenced by a decrease in total length, increasing in size only towards the end of the skin sojourn. The lag phase was thought by Twohy to be an inherent characteristic of larval development since it occurred even if larvae were shunted straight to the lungs by injection into the femoral vein.

Yokogawa (1922) stated that after penetration of the host's skin the larvae travelled via the blood stream to the lungs, and this view was supported on the basis of histological evidence by Taliaferro and Sarles (1939). On the other hand, Gharib (1961) failed to recover larvae from the blood but did so from most of the body lymph nodes and so concluded that the migration route is via the lymphatic system. The migration from skin to lungs starts 11-12 hours and is complete 15-20 hours post infection (Yokogawa, 1922; Twohy, 1956).

Lung stage

The larvae remain in the lungs for approximately 35-50 hours (Yokogawa, 1922), undergoing at first a period of logarithmic growth (Twohy, 1955a, 1956). The process of the third moult begins at about 32 hours after arrival in the lungs and subsequently no further growth occurs. Whether the occurrence of a lung specific phase is essential to the development of the parasite has not been definitely established. Schwartz and Alicata (1934) noticed that after oral administration of larvae most were killed in the intestine with very few reaching the lungs. They assumed therefore that a lung stage is essential. But Twohy (1955b) claimed that larvae injected into the portal vein and carried to the liver develop normally and reach maturity if fed to clean rats. Twohy concludes that the lungs cannot be considered the only tissues that provide the physiological requirement for the early growth of the parasite.

Weinstein and Jones (1956) stated that the chief food of the larvae in the lungs was whole blood.

Migration from the lungs to the intestine via trachea, oesophagus and stomach occurs on completion or towards the end of the third moult from 45 hours to 60 hours after infection (Yokogawa, 1922; Twohy, 1956; Hayley, 1958).

The intestinal stage

The final moult occurs in the intestine between 90 and 123 hours after infection (Yokogawa, 1922) and by 137 hours nearly all female worms contain sperm and fertile eggs (Weinstein and Jones, 1959). The first appearance of eggs in the faeces has been most frequently reported to occur on day 6 after infection.

The adult worms live in the duodenum and jejunum where they are found coiled among the villi. Brambell (1965) in his studies on the distribution of a primary infection in the small intestine noted that after day 12 large numbers of worms moved from a region lower down in the duodenum where they had been until that time, up to the pyloric area. At the same time worms could be found in the jejunum and ileum en route to the exterior.

The precise mode of feeding of the parasite remains obscure; according to Porter (1935) and Taliaferro and Sarles (1939) on the basis of histological evidence, the parasites feed on tissue cells and blood. Rogers and Lazarus (1949) after studies with ^{32}P concluded that the parasite fed on host tissues,

but the presence of host intestinal flagellates in the gut of the parasite has been regarded as evidence by Weinstein and Jones (1956) that ingestion of intestinal contents also occurs.

Termination of the infection

The egg count of infected rats becomes positive about day 6 and rises rapidly to the 8th-10th day after infection, remains static for a few days and then rapidly falls to low or zero level 15-20 days after infection (Africa, 1931; Schwartz and Alicata, 1934; Graham, 1934). Shortly after the decline in egg count the host loses the majority of its worms. This phenomenon of worm loss in N. brasiliensis infection has been reported by numerous authors (Africa, 1931; Chandler, 1932; Graham, 1934; Porter, 1935; Hurley, 1959; Hayley, 1961; Mulligan et al, 1965), and forms the subject matter of the first section of this thesis. A more detailed review of the literature relating to this topic will be presented in the introduction to Sections 1 and 2.

MATERIALS AND METHODS

1. Experimental animals

a) Strain and source

All rats used were of the hooded Lister variety. The majority of these were obtained regularly from Animal Suppliers Ltd., London. A small breeding unit of inbred rats (originally obtained from M.F.A. Woodruff, Edinburgh) was also maintained to provide a supply of pre-weaning litters.

b) Age, weight and sex

Animals of two age groups were commonly obtained from Animal Suppliers. For experiments on adult rats, animals of approximately 8 weeks of age, weighing between 150-200 g. were used. For experiments on young rats, animals were sent immediately post weaning, were 19-22 days old on arrival and weighed on average ♂ 56 g. and ♀ 48 g. In any experiment on young animals, rats from one source only were always used, but at no time have any significant differences in reaction to the parasite *N. brasiliensis* been noticed between rats from the two sources. For the purposes of comparison on this point in very young rats, occasional groups of pregnant females were obtained from the commercial source.

Both male and female rats were used in all experiments.

c) Maintenance

The rats were kept in wire cages with mesh floors suspended above sawdust-containing trays. Infected faeces were thus removed from contact

with rats. Cages of infected animals were washed at such intervals as to preclude the reinfection of the animals with larvae developed from their own faeces. Accidental infection in the animal house did not occur on any occasion. Animal house temperature was maintained at approximately 23°C. The rats were fed on a pelleted diet (Diet 41 supplied by Shearers Ltd., Glasgow), this and water being available ad libitum.

2. Parasite

a) Strain

The strain of N. brasiliensis used (originally obtained from Dr. C. Hopkins, Dept. of Zoology, Glasgow University) has been maintained for several years in this laboratory by repeated subinoculation in hooded Lister and occasionally albino Wistar rats.

b) Culture

The culture of N. brasiliensis larvae was after the modified method of Bakarat (1951) adapted by Jennings et al (1963). Faeces were collected from rats with a 7-10 day old infection by placing paper beneath the cages. The faecal pellets were mixed to a paste with a little water in a mortar and a portion of the paste was spread onto the centre of circles of Whatman's No. 1 filter paper, diameter 7 cm., the outer edges of the filter papers being kept clear of faeces. The filter papers were then moistened and

placed on circles of plastic foam of lesser diameter, saturated with water. These were placed in disposable plastic petri dishes (Oxoid Ltd.) which were stacked with lids on, in a humid incubator at 27°C.

After 4-5 days larvae had migrated outwards and could be seen collecting in a fringe around the edges of the filter paper. The larvae were harvested between 5 and 10 days after setting up the culture. This was done by flooding the petri dish with water at 37°C and waiting for approximately 1 minute while the larvae, thus stimulated, swam away from the filter paper leaving a fringe of empty sheaths still attached. The filter paper and plastic foam were then lifted out and discarded. The water containing the larvae was filtered under suction in a large Buchner funnel through strong filter paper (Greens Hydro 904, 18.5 cms. diameter). This paper was then placed inverted on an Endecott sieve (mesh 400) in a Baerman apparatus filled with water at 37°C. The larvae swam down through the sieve and collected at the bottom of the funnel from which they were subsequently run off. Larvae prepared and collected in this fashion were thus separated from any faecal residue.

3. Infection of rats

Counting of larvae was carried out as follows. A 1 ml. sample of the larval suspension was diluted with water to 10 or 100 ml. depending on concentration; the larvae present in 0.025 ml. aliquots of this were counted under a dissecting microscope until a total of at least 400 larvae had been

reached. The number of larvae present in the original suspension was then calculated, and this was diluted so that the required number of larvae for infection purposes was contained in 1 ml. or 0.1 ml. of the suspension.

The above counting procedure was then repeated but usually without dilution of the sample. Again a total of 400 larvae was counted in order to have a dosing error of less than 10%. Final adjustments in dilution were then made if necessary.

The rats were infected by subcutaneous inoculation of the larvae in the groin region. Where baby rats were involved, the dose was contained in 0.1 ml. of water in which case adult control groups were also injected with the same volume. In all other experiments the dose was made up in 1.0 ml. of fluid. Although the natural mode of infection of the parasite is by skin penetration, this method of subcutaneous injection was preferred because of greater accuracy of dosing, higher percentage recovery of larvae (Twohy, 1956) and general practicability.

4. Faecal Egg Counts

A 3 g. sample of faeces from infected rats was homogenized in 42 ml. water to give a total volume of 45 ml. This was passed through a sieve (mesh 50) and 15 ml. of the filtrate centrifuged at 2000 rpm for 2 minutes. The supernatant was discarded while the pellet containing the eggs was re-suspended in 15 ml. of saturated sodium chloride solution in which the

eggs float. With continuous agitation of this suspension, small samples were withdrawn with a pasteur pipette and transferred into the two counting chambers of a McMaster slide. Both chambers, each representing 0.15 ml., were examined under the microscope for parasite eggs which float to the surface. The mean value from both chambers when multiplied by 100 gave the number of eggs present in 15 ml. of homogenate and thus 1 gram of faeces.

5. Recovery of worms from infected rats

a) From the lungs

It had been established in the process of making larval antigens that larvae were extremely difficult to break up by homogenization, many remaining intact even after several minutes at high speed with the Silverson homogenizer. This fact was made use of in the preparation of infected lungs for larval counts. The whole lung of each infected rat was finely chopped with scissors in a universal bottle. The volume was made up to 25 ml. with water and the mixture homogenized in a 100 ml. beaker for 10-15 seconds with the Silverson run at minimum speed. This process broke the lung tissues into single or small clumps of cells but did not damage larvae. A few drops of iodine were added to the homogenate, this staining the larvae deep brown after approximately 5 minutes. One ml. samples of the homogenate were transferred to petri dishes and

the larvae counted, each sample being cleared with a few drops of sodium thiosulphate solution in water. A minimum of 3 samples was counted and the approximate number contained in the total lung homogenate calculated.

b) From the intestine

Rats were killed by a blow on the head, or by Trilene anaesthesia followed by a blow on the head. The entire small intestine was immediately removed and opened longitudinally with blunt scissors. The opened intestine was placed in a gauze bag which was then suspended with a glass rod in a 250 ml. beaker filled with warm saline. This was incubated at 37°C for approximately 1 hour during which time virtually all the worms had migrated through the gauze and collected at the bottom of the beaker. After decantation of the fluid, these worms were then counted in petri dishes under a dissecting microscope. Less than 600 worms were counted in toto but if large numbers of worms were present these were made up to 50 or 100 ml. with water and several 5 ml. aliquots were counted. The total number of worms for each rat was then calculated. Where a quantity of adult worms was being collected merely for some other experiment, the guts, after being opened longitudinally, were put in an Endecott sieve (mesh 50) which was placed on a Baerman apparatus filled with saline at 37°C. This was kept warm by means of lamps. Within 30 minutes the majority of adult worms free from intestinal debris were recoverable at the bottom of the filter funnel. These were run off and counted by a dilution technique as described above.

6. Transplantation of adult worm populations

The method was as described by Neilson (1965). Adult N. brasiliensis were isolated in the manner just described from donor rats, infected with larvae ten days previously and the concentration of the worm suspension was adjusted with warm saline, until 3-5 ml. contained the number of worms to be transferred to a single rat.

While the suspension was kept constantly mixed to ensure even distribution of worms, the appropriate volume was withdrawn with a broad mouthed pipette and delivered into a tapered 15 ml. centrifuge tube. These tubes were kept in a 37°C water bath until the worms were to be transferred and throughout these operations care was taken to expose the worms to a minimum of mechanical damage. Just before transfer to the recipient rat, the worm sample in 1-2 ml. of the suspending fluid was cautiously withdrawn by suction into a length of polythene catheter tubing (2 mm. diameter) fixed to a 5 ml. syringe. A number 1 needle (BSWG) was then placed on the free end of the tubing. The worms tended to swim out along the catheter tubing forming a suspension free from clumps which was easily injectable.

The recipient rat was anaesthetized with Trilene (trichloroethylene B.P., I.C.I. Pharmaceuticals Division, Wilmslow, Cheshire) and the abdomen shaved and washed.

A left paramedian incision of 1-1.5 cm. was made through the skin and musculature approximately 1 cm. caudal to the xiphisternum. The pylorus

was located, usually just beneath this incision and a small length of duodenum was lifted through the incision. The worms were then slowly injected directly through the walls into the lumen of the duodenum with the apparatus described. The intestinal puncture wound was reasonably small and did not require suturing. A drop or two of Terramycin Suspension was dropped into the wound and the incision was closed in two layers with nylon sutures. Care was taken to ensure that the surgical procedure was moderately asceptic. Very few deaths occurred and treated rats usually showed full recovery within 1-2 days.

7. Preparation of immune sera

Serum production rats were infected with a succession of increasing doses of larvae at approximately fortnightly intervals. The first, second, third and fourth doses respectively were 3,000, 5,000, 7,000 and 10,000 larvae. The rats were bled generally 6 days after the last infection by cardiac puncture under Trilene anaesthesia. Serum was removed after clotting had occurred and was centrifuged to remove any suspended red cells. This serum will be referred to as hyperimmune serum.

8. Preparation of whole worm antigen

Adult worms were obtained from rats 10 days after larval infection. The worms were washed with saline, and finally suspended in saline to give

1,000 worms per ml. The worms were then homogenized in the cold using the Silverson homogenizer at high speed. Samples of the homogenate were withdrawn at approximately 60 second intervals and examined under the microscope for disintegration of the worms. When this was judged to be sufficient (usually at least 4 minutes homogenization) the homogenate was centrifuged at 3,000 r.p.m. at 4°C for 30 minutes to remove the particulate matter. The supernatant was used without further preparation for a variety of procedures and was stored at -70°C or -20°C without apparent loss of activity for periods of several months.

9. Passive cutaneous anaphylaxis

Sera to be tested were serially diluted with saline and 0.1 ml. of each dilution was injected intradermally in a shaved area on the back of a normal rat. Each rat could provide 6 injection sites, three on each side, parallel to the vertebral column. Three days after injection of serum 0.5 ml. of whole worm antigen and 0.5 ml. of 1% Evans Blue was injected intravenously into the tail of each rat. A positive response was indicated by a discrete blue patch which appeared 10-20 minutes after injection. The rats were usually killed 30 minutes after injection and the skins pinned onto polystyrene tiles and stored. The titre of the serum being tested was expressed as the highest dilution which gave a positive response. All PCA tests were carried out in duplicate on different rats.

SECTION 1

QUANTITATIVE STUDIES ON THE *N. BRASILIENSIS* INFECTION IN ADULT RATS

INTRODUCTION

It is generally recognised that in many experimental infections with helminths, only a proportion of the dose of larvae becomes established in the definitive site; this number persists for a varying period and then a further phase ensues during which the burden diminishes. A degree of immunity is usually acquired to reinfection and is expressed in the establishment of a smaller proportion of the challenge dose. The development of such worms as do establish may be affected. They may remain inhibited as larval stages; alternatively those which mature may be stunted, the females producing relatively small numbers of eggs.

Although the time scale of these phases varies in different host parasite systems it is possible that the underlying mechanisms may be common to many.

In an attempt to throw light on the mechanisms involved Nippostrongylus brasiliensis in the rat was used as a model system. Many aspects of this system have already been described and it is well established that primary infections are terminated abruptly by an immunological reaction called the "self-cure", and that subsequently rats are relatively immune to reinfection. This was first noted by Africa (1931) who reported a rapid and progressive reduction of eggs in the faeces of rats infected with 200 larvae, between the 16th and 24th day after infection. A loss of worms also occurred over this period with a greater loss of female worms.

Findings of this kind were repeatedly confirmed by subsequent workers (Schwartz et al, 1931; Chandler, 1932; Graham, 1934; Porter, 1935) but a great deal of confusion arose as to the timing of the event.

More recent experiments have revealed a number of factors which may influence the course of infection and may account for the apparent discrepancies of the earlier workers' results. Prominent among these modifying factors, which have been reviewed by Hayley (1962), are age of the host and size of the dose of infective larvae. More will be said about the effect of age in a subsequent section; the effect of size of dose of larvae on the longevity of adult N. brasiliensis in a primary infection was elucidated by Hayley and Parker (1961) who found that the loss from a population of less than 100 worms extended over a 30 day period after infection. Greater worm burdens were more rapidly eliminated so that only approximately 3% remained by the 20th day of infection. This fact was confirmed by Mulligan et al, 1965.

The smallest number of worms which produce a peak in egg count and subsequent decline is 40-80 (Hurley, 1959); infections of 10-20 larvae result in infections with low egg count curves with no obvious crisis and no worm expulsion up to day 28, although the rats are subsequently as immune to reinfection as those given 100 larvae.

Studies on the reactions to challenge infections of N. brasiliensis have been largely preoccupied with the effects of acquired resistance on the migration, development and establishment of the challenge infection.

Schwartz et al (1931) and Graham (1934) found that a prominent feature of reinfection was the retention of up to 50% of larvae in the lungs. Although endorsing this finding, Sarles and Taliaferro (1936) and Taliaferro and Sarles (1939) concluded that after light reinfections immunity acted mainly in the small intestine to cause delayed maturation, stunting of growth and depressed egg count of worms. The successive involvement of lung and skin barriers was noticed only as the numbers of reinfections increased, with the skin barrier becoming effective as infections were continued over several months with final total of up to 50,000 larvae.

Information, however, regarding the timing or rate of worm expulsion in a challenge infection is virtually non-existant in the literature and the published observations of self-cure even in a primary infection have not been made in sufficient detail to adequately quantitate the changes in worm population associated with the various phases.

The aim of the present study was to define in quantitative terms the process of worm expulsion as a function of time and to determine the effect of progressing immunity on the process. The population kinetics of primary, secondary and tertiary infections will be described.

The course of a first, second and third infection using a constant dose

Experimental Plan

The purpose of this series of experiments was to define for one dose rate the parameters of the N. brasiliensis infection. This was done for two reasons. First, to compile quantitative data on the proportion of worms surviving at each site at any given time in rats of defined immune status. Such information is essential to the planning of future experimental studies. Secondly, it was felt that a study of the kinetics of the elimination of the parasite from the intestine was essential to the understanding of the mechanism involved.

Worm survival was studied in adult rats infected for the first, second and third times with 3,000 N. brasiliensis larvae. The re-infections were given one month after the previous infection. The design of the experiments is shown in Table 1. Worms were isolated and counted from the small intestine of each individual animal. The ratio of male to female worms was determined from the bulked sample of each daily autopsy group. Lung counts were carried out for the first four days in First Infection A, in Second Infection B and both third infections. On day 4 after infection, very few larvae could be recovered from the lungs and counts were therefore discontinued until the last or penultimate autopsy day. An analysis of regression (Snedecor, 1966) was carried out using the logarithms of the daily worm counts.

TABLE 1

DETAILS OF EXPERIMENTS COMPRISING SECTION 1

Experiment		Total animals in group	Days of autopsy	No. of rats autopsied/day
First infection	A	60	1 to 10, 20, 30	5
First infection	B	100	9 to 18	10
Second infection	A	50	4 to 12, 14, 17, 19	4
Second infection	B	55	1 to 10, 20	5
Third infection	A	40	1 to 7, 10	5
Third infection	B	40	1 to 8	5

During the periods of zero order kinetics (i.e., when worm expulsion is not occurring) group means are expressed arithmetically, as individual worm counts are normally distributed about the mean; during the period of first order kinetics (i.e., when worm expulsion is occurring) group means are expressed geometrically as the individual worm counts are distributed logarithmically.

The individual worm counts and other details relating to each table will be found in the appendices. Faecal egg counts were made daily over the relevant periods and are shown in the tables.

RESULTS

1. The Course of a First Infection

The course of a primary infection over the first 10 days can be followed from the egg counts and autopsy results of First Infection A (Table 2). In this experiment 5 rats were killed daily from days 1-10 after infection to study the pattern of larval migration from skin to lungs and lung to intestine and also the establishment of worms in the intestine.

The highest count in the lung occurred on day 1, when a mean of 817 larvae was found. This was followed by a sharp decline over days 2 and 3, until on days 4 and 10 a mean of only 10 and 2 larvae respectively remained in the lungs.

TABLE 2

FIRST INFECTION (A) WITH 3000 LARVAE

WORMS RECOVERED OVER THE LOSS PHASE 1 AND PLATEAU PERIODS

Days after infection	Eggs/g. of faeces	Worms Recovered		
		Lungs Mean* ± S.D.	Small intestine Mean* ± S.D.	Lungs and Small intestine $\log_{10}$ Mean *
1		817 ± 419	0	2.91
2		286 ± 200	664 ± 301	2.98
3		20 ± 12	1270 ± 465	3.11
4		10 ± 12	1177 ± 465	3.07
5	0		936 ± 207	2.97
6	16,150		1228 ± 339	3.09
7	43,200		1150 ± 179	3.06
8	30,600		1434 ± 269	3.15
9	43,700		1362 ± 407	3.13
10	31,000		1008 ± 291	3.00

* Arithmetic means and standard deviations are given throughout.

During the first four days of the infection the sum of the number of worms recovered from the lungs and that from the small intestine was virtually a constant, the greater number being first in the lungs and then in the intestine. From then onwards until day 10 no significant change in the number of worms in the intestine occurred. The total mean intestinal worm count over the period of day 4-10 was 1185, this figure representing 40% of the injected dose. It therefore appears that a large number of larvae, i.e., 60% of those injected, have failed to reach the intestine, the major part of this loss occurring during the first day, either at the site of injection or during the course of migration to the lungs.

Autopsy of the remaining rats on days 20 and 30 after infection gave mean intestinal worm numbers of 40 and 48 respectively. The majority of worms had therefore been lost from the intestine between days 10-20.

The moult from fourth larval stage to young adult occurred between days 3 and 5 after infection. Eggs appeared in the faeces on day 6 and the counts rose to remain at a level of 30-40 x 10^3 eggs per gram between days 7-10. The ratio of male:female worms remained at 1:1 until day 10; on days 20 and 30 the male:female ratio was 4:1.

The kinetics of worm loss from the intestine in a primary infection were derived from the autopsy results of First Infection B, during which groups of 10 rats were killed daily from days 9-18 after infection. On this occasion the mean number of worms present on day 9 was 1724, i.e., 57% of the injected dose. The geometric means of worm survival (Table 3) and

TABLE 3

FIRST INFECTION (B) WITH 3000 LARVAE

WORMS RECOVERED FROM THE INTESTINE OVER THE LOSS PHASE 2 PERIOD

Days after infection	Eggs/g. of faeces	Worms Recovered	
		Small intestine Mean* ± S.D.	Small intestine $\log_{10}$ Mean* ± S.D.
6	53,200		
7	108,400		
8	111,900		
9	127,400	1724 ± 151	3.24
10	122,400	1939	3.29 ± 0.32
11	43,300	1403	2.15 ± 0.08
12	21,750	782	2.89 ± 0.36
13	9,900	947	2.98 ± 0.28
14	1,000	566	2.75 ± 0.40
15	250	263	2.42 ± 0.61
16	200	135	2.13 ± 0.62
17	0	73	1.86 ± 0.75
18	0	37	1.57 ± 0.49

* On day 9 the arithmetic mean and standard deviation is given; the other figures are treated geometrically.

the log 10 plot of these figures against time (Fig. 1) show that a rapid and exponential expulsion of worms from the intestine occurred between days 11-18 after infection. The value for the regression coefficient is $b = -0.21 \pm 0.02$, the worm numbers thus falling at a rate of 1 $\log_{10}$ unit in 4.6 days.

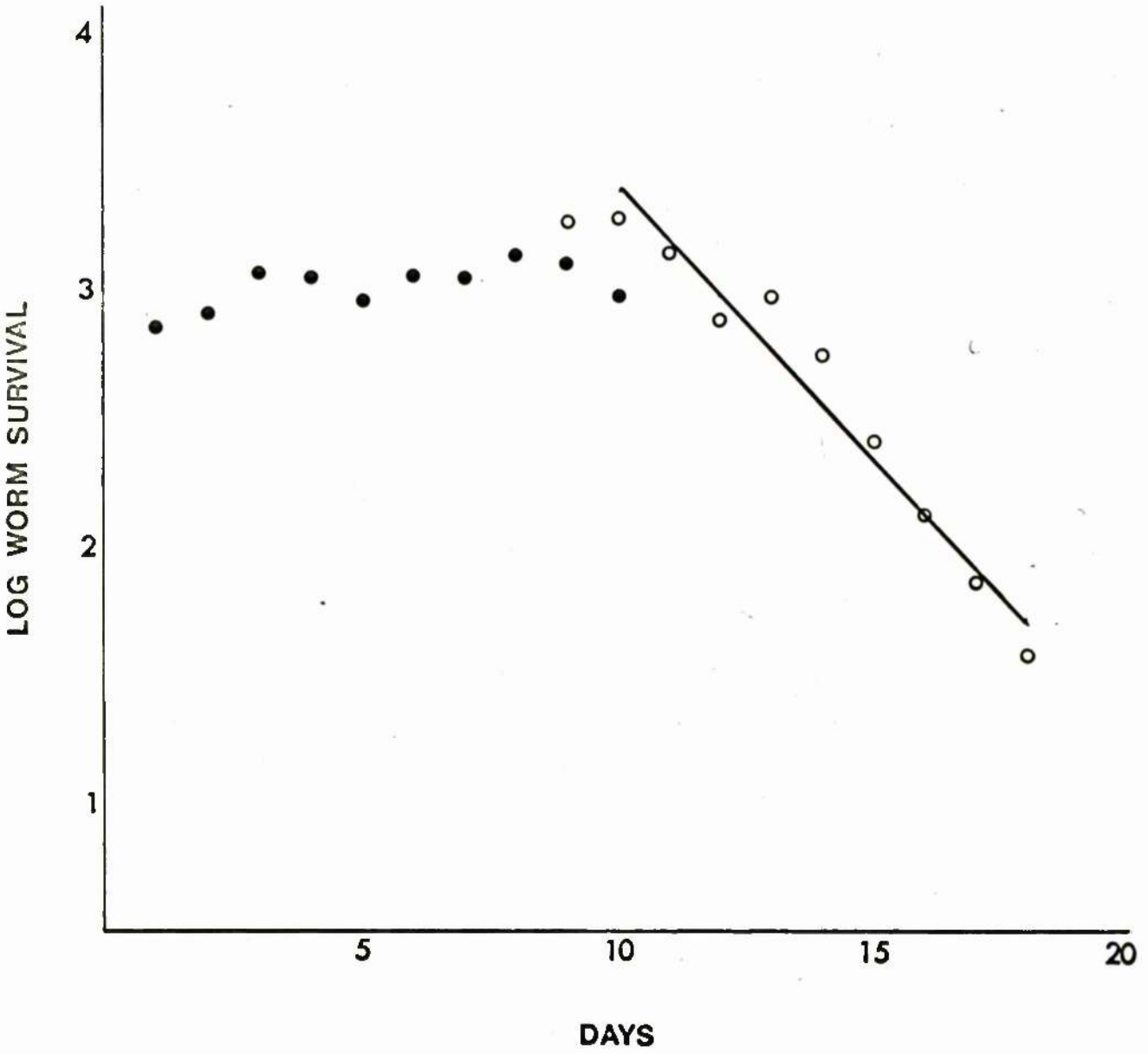
The faecal egg count of this infection (Table 3) rose rapidly to reach a maximum on days 8-10 of 122×10^3 eggs per gram; it then fell steeply over days 11-14 to reach zero on day 17 after infection. Over the period during which worms were expelled from the intestine the male: female ratio changed progressively from approximately 1:1 to 4:1 by day 18.

A comparison of the results of the experiments A and B indicate that the larvae used in the latter were of greater infectivity. The mean number of adults established on day 9 in experiments A and B were 1362 and 1724 respectively and the maximum faecal egg count was 3 times greater in Experiment B. The precise reasons for these variations in infectivity of the larvae on different occasions remain obscure.

To facilitate the presentation and discussion of the subsequent experiments, the main course of events during a first infection of 3,000 larvae can now be summarised. On the basis of the appearance of the curve of $\log_{10}$ worms surviving against time (Fig. 1), it is proposed to divide the infection into 4 phases.

The first is Loss Phase 1, which is a result of the failure of a certain proportion of larvae to reach the small intestine (see next

Fig. 1. The kinetics of worm survival in first infections A and B. Mean worm recovery from the intestine at daily intervals over the first ten days (first infection A ●) and over days 9-18 (first infection B o); regression coefficient (b) = -0.21 ± 0.02. Regression calculated on individual worm counts.



experiment). It is considered that this loss occurs primarily before the larvae reach the lungs since the number of worms arriving in the lungs approximates to that of the final intestinal population when larvae are no longer present in the lungs.

When all the parasites have arrived in the intestine this marks the onset of the Plateau Phase during which worms survive in the intestine with no change in their numbers. This phase in a primary infection lasts from day 3-4 until day 11. During the early part of this period the parasite undergoes the final moult (day 3-5), becomes sexually mature and lays large numbers of eggs.

The Plateau Phase is followed by a phase of rapid exponential expulsion of worms (Loss Phase 2) which is remarkable for its sudden onset.

Finally the last or Threshold Phase represents the continued presence of a small number of worms which have survived the expulsion phase and remain in the intestines of the host for relatively long periods. The majority of these are male worms and the female worms present are largely sterile.

Loss Phase 1 in First Infections of Different Sizes

In First Infections A and B, a mean of 40% and 57% respectively of the larvae injected developed to maturity in the small intestine, i.e., the remainder were lost during Loss Phase 1. Each rat had received 3000 larvae.

More information on the relationship between numbers of larvae injected and numbers of worms developing in the small intestine was obtained by infecting 4 groups of 10 rats with individual doses of 1000, 2000, 4000 and 8000 larvae respectively. The rats were autopsied 8 days later. The results (Table 4) show that means of 51, 58, 61 and 43% respectively of the different doses were recovered as adult worms and indicate that, within limits, Loss Phase 1 is a relatively fixed proportion of the infecting dose.

2. The Course of a Second Infection

The purpose of the first of the reinfection experiments was to determine the time and rate of intestinal worm expulsion in rats which had recovered from a previous infection. Groups of 4 rats were therefore autopsied daily from days 4-12 and thereafter on days 14, 17 and 19.

Due to a shortage of larvae the primary or immunising infection dose was 2400. Faecal egg counts of the immunising infection followed the pattern described previously and worm counts of groups of 3 rats autopsied on days 10 and 20 gave means and standard deviations of 1022 ± 123 and 58 ± 39 respectively. Thirty days after the first infection all but 5 of the rats were reinfected with 3000 larvae. The 5 animals which were not reinfected were autopsied on the last day of the experiment to determine the residual number of worms from the immunising infection.

The results of the daily intestinal worm counts and faecal egg counts are shown in Table 5. The phase of worm expulsion, i.e., Loss Phase 2,

TABLE 4

LOSS PHASE 1 IN FIRST INFECTIONS OF DIFFERENT SIZES

	Larval dose x 10^3			
	1	2	4	8
Worms Recovered Day 8	499	1420	2800	4140
	692	1440	2500	4220
	432	960	2610	2200
	363	1300	1050	2730
	482	1100	3060	5230
	576	640	2520	3600
	474	1240	1240	1980
	398	1580	1840	3700
	620	820	3820	died
	531	1350	2000	"
Mean	508	1155	2444	3450
S.D.	101	353	882	1106
% worms surviving Loss Phase 1	51	58	61	43

TABLE 5

SECOND INFECTION (A) WITH 3000 LARVAE

WORMS RECOVERED FROM THE SMALL INTESTINE OVER THE LOSS PHASE 2 PERIOD

Days after infection	Eggs/g. of faeces	Worms Recovered	
		Small intestine Mean* ± S.D.	Small intestine $\log_{10}$ Mean* ± S.D.
4	0	639 ± 211	2.80
5	0	593	2.77 ± 0.18
6	0	322	2.51 ± 0.33
7	0	133	2.12 ± 0.37
8	0	83	1.92 ± 0.70
9	0	49	1.69 ± 0.56
10	100	27	1.42 ± 0.57

* On day 4 the arithmetic mean and standard deviation is given; the other figures are treated geometrically.

has occurred between days 5 and 10 of the second infection. The regression coefficient for the expulsion period (Fig. 2) is b = -0.27 ± 0.05 and the expulsion rate is 1 $\log_{10}$ unit in 3.7 days. The difference between this slope and that of the first infection was calculated (Goldstein, 1964) and is highly significant ($P < 0.0005$). The expulsion of worms occurring during Loss Phase 2 of the second infection therefore differs from that of the first infection in both time of onset and rate.

Since no change in worm numbers occurred in rats autopsied after day 10 the number of worms on day 10 and the following days represent the Threshold Phase. The last group was killed on day 19 together with the 5 rats kept as residual infection controls for the immunising infection. The mean worm counts and standard deviations for these groups respectively were 44 ± 57 and 43 ± 95.

The results of second infection A have been confirmed in second infection B. In this experiment lung counts were carried out as an additional feature since earlier workers have reported the trapping and death of larvae in the lungs of immune animals (Taliaferro and Sarles, 1939). On this occasion both immunising and second infections consisted of 3000 larvae. The results of the experiment are shown in Table 6.

For an unknown but presumably technical reason the numbers of larvae counted in the lungs were considerably lower than the number of worms which appeared in the intestine. However there was no indication of trapping of larvae in the lungs since after day 3 the majority of lung counts were negative.

Fig. 2. The kinetics of worm survival in second infection A; regression coefficient (b) = -0.27 ± 0.05.

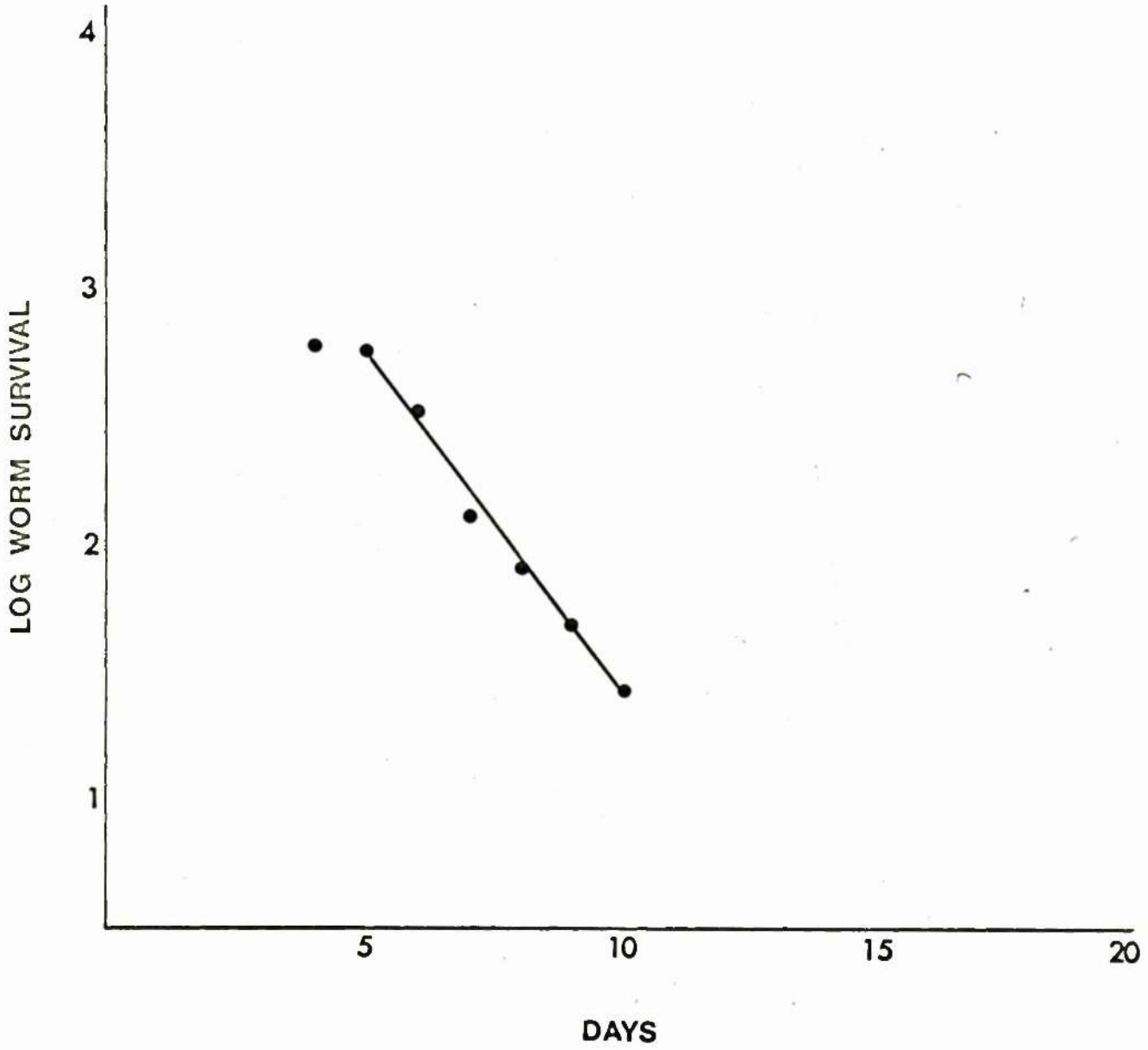


TABLE 6

SECOND INFECTION (B) WITH 3000 LARVAE

WORMS RECOVERED OVER THE LOSS PHASE 1, PLATEAU AND LOSS PHASE 2 PERIODS

Days after infection	Eggs/g. of faeces	Worms Recovered: Lungs Mean* ± S.D.	Worms Recovered: Small intestine Mean* ± S.D.	Worms Recovered: Lungs and Small intestine $\log_{10}$ Mean* ± S.D.
1	0	438 ± 129	54 ± 62	2.68
2		379 ± 132	210 ± 87	2.77
3		56 ± 26	1120 ± 686	3.07
4		2 ± 3	1324 ± 352	3.12
5	0		877	2.94 ± 0.19
6	100		357	2.55 ± 0.28
7	200		182	2.25 ± 0.33
8			169	2.23 ± 0.57
9			9	0.94 ± 0.93
10	150		16	1.21 ± 0.71

* On days 1-4 arithmetic means and standard deviations are given; the other figures are treated geometrically.

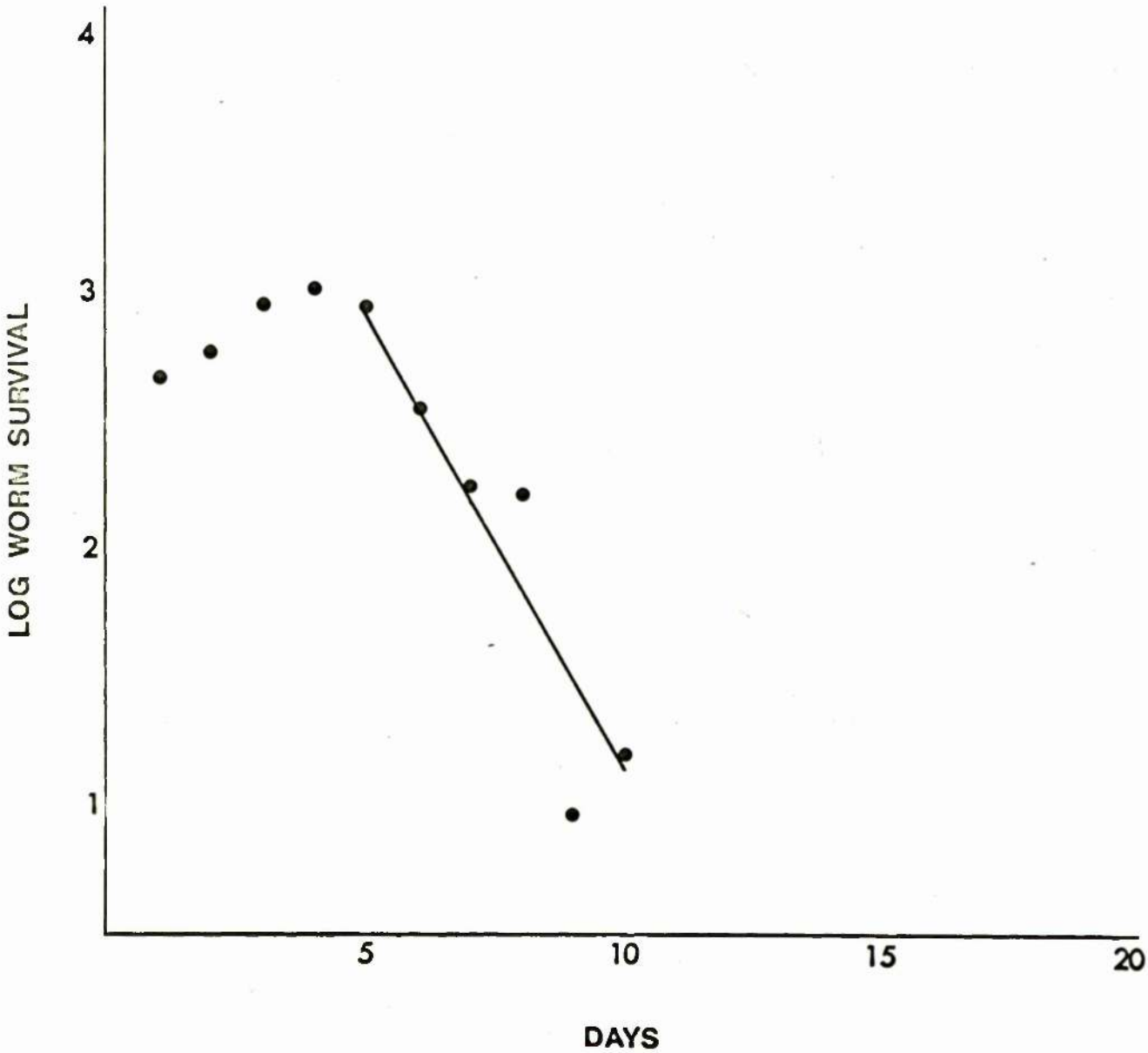
Worm expulsion occurred between days 4 and 10; the slope of the regression line is b = -0.35 ± 0.04 and is an expulsion rate of 1 $\log_{10}$ unit in 2.9 days (Fig. 3). The difference between this slope and that of the first infection is also highly significant ($p < 0.0005$) whereas the slope difference between the two second infections is not.

In both the second infections 10-20% of the parasites did not moult, the remainder doing so on days 5 and 6. Adult worms remained smaller and paler in colour than those of the first infection and egg production was almost totally suppressed.

From these results the main differences between a first and second infection can be summarised as follows. The expulsion of worms from the intestine during a second infection occurs 5 or 6 days earlier than in a first infection, so that by day 10 the Threshold Phase has been reached. The expulsion also proceeds at a significantly faster rate. It is similar to that in a first infection in the sudden onset and exponential kinetics. Other effects on the worms in the intestine are the delay in final moult by 1-2 days with up to 20% of larvae remaining at the fourth stage, stunting and pallor of the adult worms and a severely depressed egg output.

A further point is the possibility that an immune component might become superimposed on the Loss Phase 1 of a reinfection thus making it greater than in a primary infection. The mean numbers of worms in the intestine on day 4 were 639 and 1324 in second infections A and B respectively; the former figure at least suggests the possibility of an increased Loss Phase 1.

Fig. 3. The kinetics of worm survival in second infection B; regression coefficient (b) = -0.35 ± 0.04.



To check on this a dose of 3000 larvae from a common suspension was given to each rat in two groups of rats. The first group had been infected with 3000 larvae 20 days earlier and the second group had not been previously infected.

The results of the autopsies are shown in Table 7. Those on day 4 indicate that, following reinfection, Loss Phase 1 is approximately 30% greater than that of a primary infection. The results on day 9 confirm that the behaviour of both first and second infection was similar to that reported above.

3. The Course of a Third Infection

Animals remaining from second infection B formed the third infection A group of rats. These were infected 30 days after the previous infection and at the same time as first infection A using the same larval suspension.

The results of this experiment (Table 8) confirm that Loss Phase 1 is increased in an immune animal. The highest intestinal worm count occurred on day 4 and represents only 18% of the injected dose. By comparison, in first infection A 40% of the same injected dose became established in the intestine. There has thus been a difference in 'take' of 22% of the infecting dose, between rats infected for the first and third times.

The worms seen in the intestine on day 1 of the third infection are the residual populations of the two previous infections. If the numbers of worms in the lungs and intestines on day 2 are added, this sum approximates

TABLE 7

A COMPARISON OF LOSS PHASE 1 IN A FIRST AND SECOND INFECTION

	4 days after infection		9 days after infection	
	First infection	Second infection	First infection	Second infection
	1,900	670	900	39
	480	293	1,700	31
	1,840	518	1,520	117
	1,660	322	1,500	15
	1,640	887	1,340	121
	1,400	863	385	64
	1,420	248	1,880	63
	1,460	611	1,060	22
	1,760	225	1,460	84
	1,780	497	1,800	
Mean	1,434	513	1,354	62
S.D.	423	237	433	37

TABLE 8

THIRD INFECTION (A) WITH 3000 LARVAE

WORMS RECOVERED OVER THE LOSS PHASE 1, PLATEAU AND LOSS PHASE 2 PERIODS

Days after infection	Eggs/g. of faeces	Worms Recovered: Lungs Mean* ± S.D.	Small intestine Mean* ± S.D.	Lungs and Small intestine $\log_{10}$ Mean* ± S.D.
1	0	322 ± 175	67 ± 73	2.50
2		189 ± 68	366 ± 238	2.74
3		17 ± 10	374 ± 187	2.57
4		32 ± 22	514	2.71 ± 0.17
5	50		210	2.32 ± 0.54
6	50		31	1.49 ± 0.37
7	250		28	1.44 ± 0.46
10	150		20	1.29 ± 0.35

* On days 1-3 arithmetic means and standard deviations are given; the other figures are treated geometrically.

closely to the highest worm count in the intestine later in the infection. After day 2 the number of worms in the lungs falls rapidly and so even in the third infection there is neither real nor circumstantial evidence of worm retention in the lungs.

The Plateau Phase in this infection has become shortened almost to extinction lasting at most 1 or 2 days. The worm expulsion phase occurred very rapidly, starting on day 4 and being complete by day 6-7 (Fig. 4). The slope value of the regression line is $b = -0.43 \pm 0.09$, and so expulsion occurred at the rate of 1 $\log_{10}$ unit in 2.3 days. This expulsion rate was significantly faster than that of both second infections A and B ($p < 0.0005$ and $p < 0.01$ respectively).

The moult from the fourth larval stage in the intestine was delayed as in the second infection. This meant that in the third infection the majority of worms were expelled before moulting. Again as in the second infections the egg count remained extremely low.

Third infection B (Table 9) was done to confirm the rate of expulsion seen in third infection A. The results show a pattern of worm elimination similar to that of the third infection A, though apparently one day later in onset. The value of $b = -0.40 \pm 0.07$ (Fig. 5) and the expulsion rate is 1 $\log_{10}$ unit in 2.5 days. This rate is again significantly different from those of both second infections ($p < 0.0025$ and $p < 0.05$ respectively) but not significantly different from that of the third infection A.

Fig. 4. The kinetics of worm survival in third infection A (●). For comparison first infection A (o) is shown over the same period. Regression coefficient (b) = -0.43 ± 0.09.

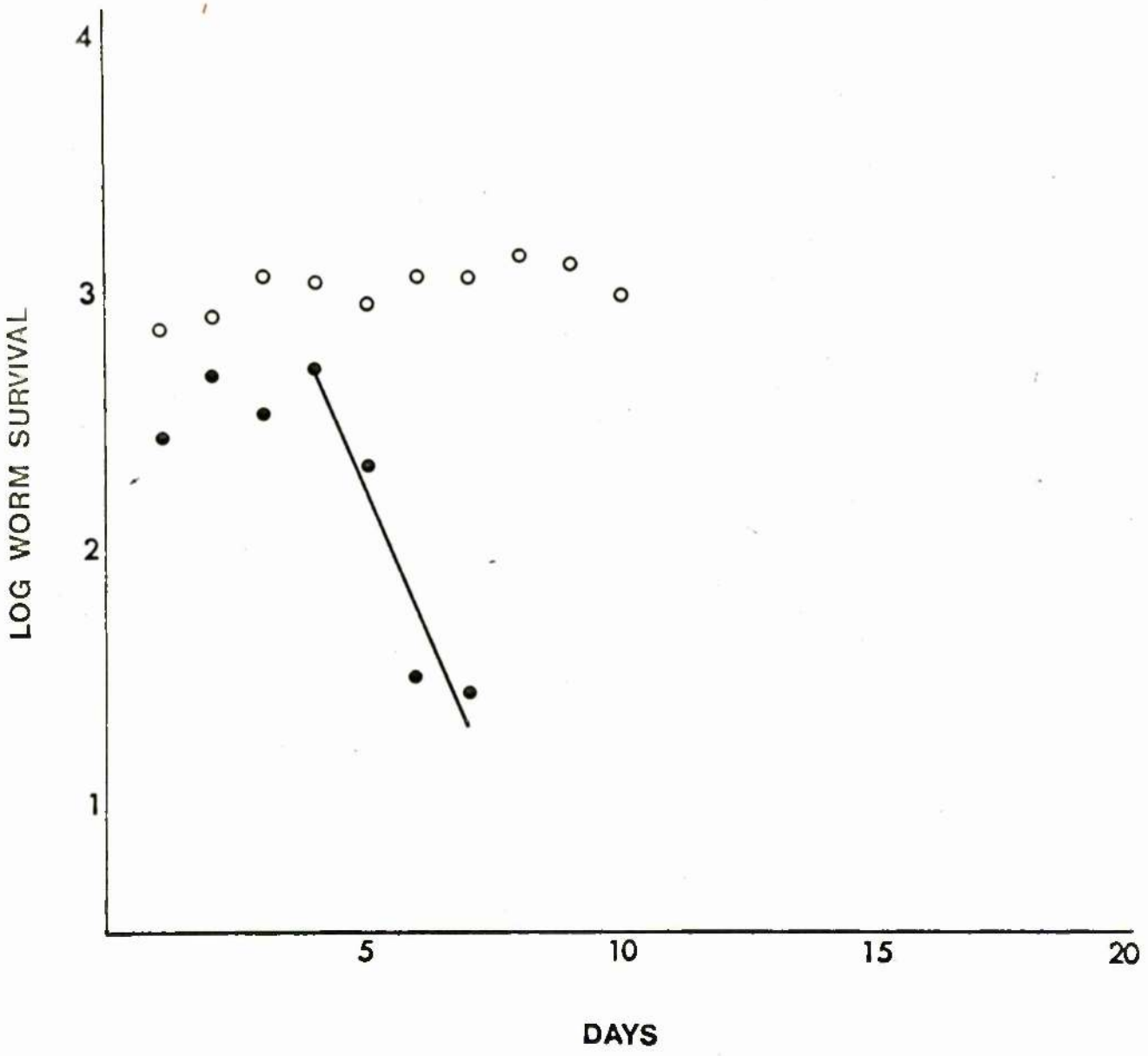


TABLE 9

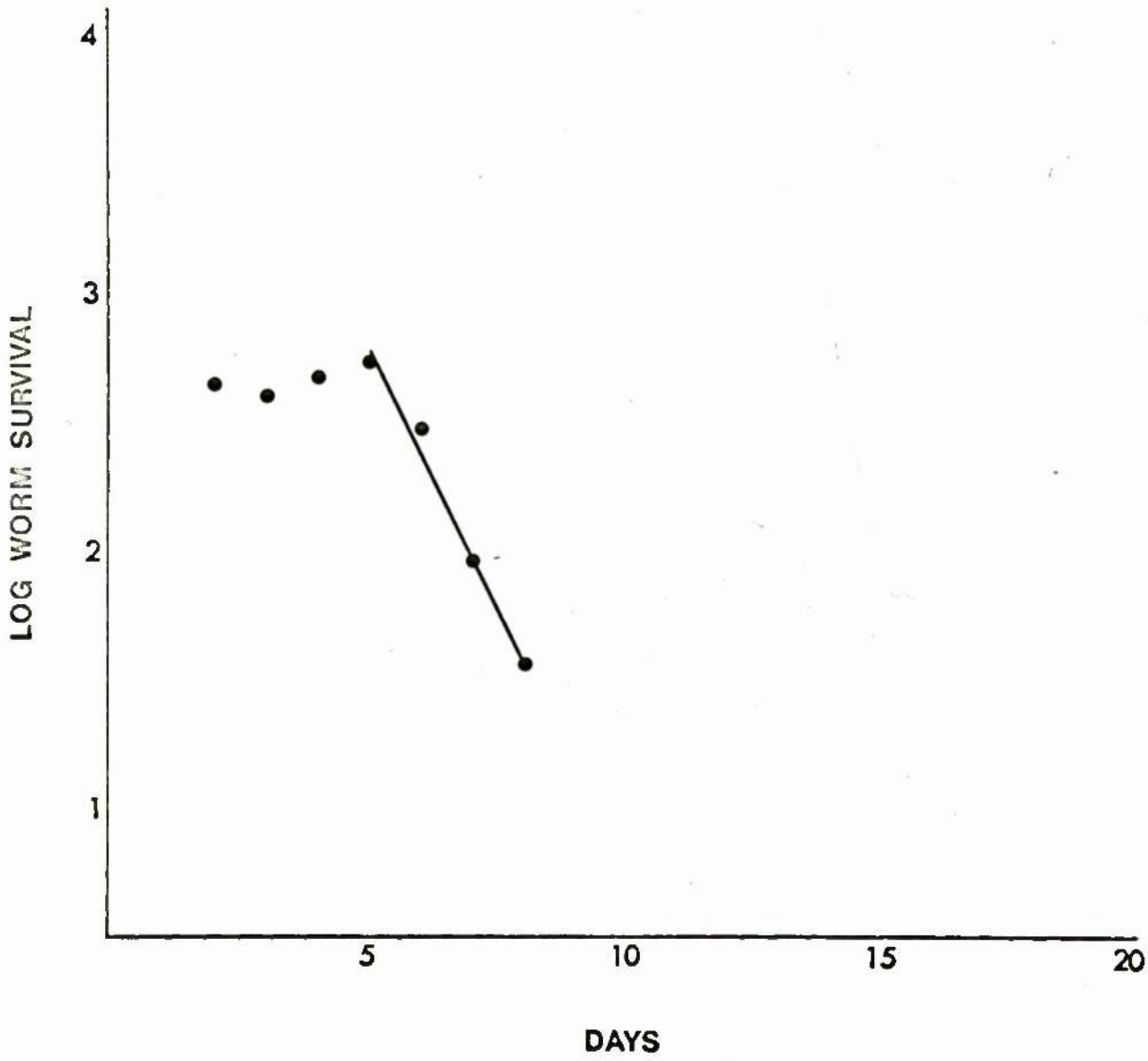
THIRD INFECTION (B) WITH 3000 LARVAE

WORMS RECOVERED OVER THE LOSS PHASE 1, PLATEAU AND LOSS PHASE 2 PERIODS

Days after infection	Eggs/g. of faeces	Worms Recovered: Lungs Mean* ± S.D.	Worms Recovered: Small intestine Mean* ± S.D.	Worms Recovered: Lungs and Small intestine $\log_{10}$ Mean* ± S.D.
1	0	171 ± 102	98 ± 100	2.43
2		282 ± 235	228 ± 73	2.70
3		95 ± 72	514 ± 372	2.78
4		41 ± 18	476	2.68 ± 0.43
5	0		558	2.75 ± 0.29
6	150		301	2.48 ± 0.12
7	0		91	1.96 ± 0.60
8	0		36	1.56 ± 0.29

* On days 1-3 arithmetic means and standard deviations are given; the other figures are treated geometrically.

Fig. 5. Kinetics of worm survival in third infection B; regression coefficient (b) = -0.40 ± 0.07.



DISCUSSION

The results of these experiments provide a comprehensive view of the kinetics of establishment and expulsion of N. brasiliensis in normal and immune rats. Essentially the course of each infection is characterised by four phases. These are: Loss Phase 1 - Following infection a proportion of larvae are immobilized or destroyed at the site of injection or during the course of migration via the lungs to the intestine. The Plateau Phase - The surviving larvae develop to maturity in the small intestine. Their numbers remain static and they lay eggs for a number of days. Loss Phase 2 - After a certain period in the intestine the worms are rapidly expelled in an exponential fashion. Threshold Phase - A small residual population of worms survives.

Loss Phase 1 (LP1)

In a primary infection Loss Phase 1 was almost certainly not an acquired immune response since it occurred within a day or so after infection; indeed as noted previously the bulk of the loss probably occurs before the larvae reach the lungs. Over a wide range of primary infections from 1000-8000 larvae, LP1 ranged between 40%-60% of the injected dose. This result is in accordance with that of Twohy (1956) who found that up to 40% of subcutaneously injected larvae remained at the site of inoculation. Twohy concluded from the small size and unhealthy appearance of these larvae that they were unlikely ever to migrate. Within this range

the different results encountered as LP1 were probably due to minor variables in handling techniques which affected larval viability.

Age of the culture *per se* seems to be of relatively minor importance since Hayley and Clifford (1958, 1960) found cultures to be of constant infectivity until at least four weeks old. The treatment of larvae after harvesting may be more critical; Thorson (1954b) has shown for instance that holding of larvae for prolonged periods in distilled water results in lower adult worm recoveries. It seems important therefore that a minimum of time be involved in isolation in the Baerman apparatus before inoculation into the animal. Hayley (1962) obtained an increase of 24% in adult worm recovery by keeping infective larvae in 0.9% saline instead of distilled water.

Kassai *et al* (1966) have described an increase in infectivity in *N. brasiliensis* larvae following low doses of X-irradiation. In one experiment means of 329 and 901 worms were recovered from doses of 1000 normal and X-irradiated (10 kr) larvae respectively. A subsequent experiment indicated that the infectivity of female worms in these circumstances was of the order of 100%. These observations corroborating those of Ciordia and Bizzell (1960) are of particular interest in view of the fact that the failure of a proportion of parasites to reach the definitive site, has usually been attributed to a series of larval accidents occurring during the course of migration. These results, however, lead to the conclusion that a specific helminth factor is involved in the chain of events that result in LP1 and that this factor is radiosensitive.

First and second reinfections were characterised by an increase in the magnitude of LP1 which was of the order of 80% of the injected dose, i.e., 30% greater than in a primary infection. This was presumably due to the addition of a specific immune component to the non-immunological reaction involved in the LP1 of a primary infection.

Plateau Phase

In a primary infection worms which survived LP1 were found in the intestine where their numbers remained constant between the 4th and 10th days after infection. The last moult was completed by the 5th day after infection and eggs appeared in the faeces on day 6 to rise and remain at a high constant level until day 10. Within the next day or so, but usually on day 11, a sharp decline in faecal egg count occurred and continued over the next few days to reach zero by days 15-17.

In a second and third infection the Plateau Phase was drastically reduced both in numbers of worms and in duration, lasting at most only 1-3 days. It becomes obvious that in dealing with rats of increasing immune status a point would very soon be reached where a Plateau Phase would not be apparent. In such cases, since worms would not accumulate in the intestine, the expelled rather than the surviving proportion would have to be counted to get an estimate of the total number of worms reaching the intestine. Other manifestations of immunity on worms which survived the first day or two of the expulsion phase were a delay in the occurrence of

the last moult and an inability to realise their full adult size and reproductive potential.

Loss Phase 2 (LP2)

The Plateau Phase in both primary and subsequent infections was terminated by a phase of worm elimination from the small intestine. In primary infections this occurred between days 11-18 and if the logarithms of the numbers of worms in the intestine on these days are plotted against time a straight line is obtained. The loss of worms was thus exponential with a decrease of 1 $\log_{10}$ unit in 4.6 days. This phase therefore shows a form compatible with first order kinetics and is consistent with an antigen-antibody reaction. It is not of course proof of this since other processes might result in the same form.

The rate of LP2 is increased with each succeeding reinfection. The expulsion rates in the two second infection experiments were 1 $\log_{10}$ unit in 3.7 and 2.9 days and in the two third infection experiments were 1 $\log_{10}$ unit in 2.3 and 2.5 days. The expulsion coefficients of both the second infections were significantly greater than that of the first infection, and those of the third infections also differed significantly from those of both second infections. The slope value may therefore be regarded as a measure of the immune status of the host and may be a direct reflection of local antibody titre in the small intestine.

It is an interesting feature that LP2 began abruptly, i.e., there was a sharp point of inflexion between the two phases, this appeared to occur in all cases within a 24 hour period. If it is assumed that worm expulsion is the result of antigen-antibody interaction and since the process once initiated proceeds at a constant rate, the implication is that sufficient antibody is present at the point of inflexion to effect this rate of change. It is to be assumed, however, that antibody levels were rising before this time and if antibody was freely available to neutralize the worms then one would have expected the expulsion curve to begin with a shoulder lasting over several days. That this has not occurred implies that antibody is suddenly released or made available for interaction with antigen. Whether this is due to the operation of an effector mechanism which is activated on a threshold basis or some other mechanism inhibiting the action of antibody is not yet clearly established (Jarrett et al, 1967b).

Threshold Phase

Loss Phase 2 did not result in complete expulsion of the worms; a mean of approximately 40 worms remained in the small intestine; and was observed to persist for at least 2 months. This phenomenon did not seem to be affected by changes in the immune status as similar numbers of worms were found following second and third infections. This burden possibly represents a true threshold below which the immunity of the host cannot be expressed.

These experiments have shown that the kinetics of N. brasiliensis infection can be used to express the immune status with a much greater degree of precision than the usual method of killing animals at a single fixed time after challenge. For example, autopsy of rats on day 20 only would not have revealed any differences in the immunity of the groups of rats discussed in these experiments. Likewise a comparison on any single occasion between days 5 and 10, of the numbers of worms in rats from the second and third infections would be most unlikely to reveal significant differences in immune status. It may be that the slope of the line, i.e., the coefficient of expulsion,provides the most sensitive indicator of the level of immunity as it does for example in in vitro virus neutralization and in vivo antigen elimination determinations.

The basic pattern of infection and expulsion described above may be common to many helminth infections. The data presented by Wilson (1966) show a similar sequence in D. viviparus in the guinea pig, admittedly an abnormal host and a truncated infection. It is clear that in a primary infection he obtained a Plateau Phase followed by an exponential expulsion phase; in second infections the immune animals responded to challenge by expelling the worms earlier, exponentially and at a faster rate than in a first infection. Armour et al (1966) and Armour (1967) have suggested that a similar pattern may be involved in Ostertagia infections in sheep and cattle.

In most instances in the literature of the self-cure reaction insufficient animals have been used during the necessary period of time to allow of quantitative analysis of the kinetics of the expulsive phase; this field requires considerable exploration in order to determine if the phenomena described above are a common feature of many helminth infections.

SUMMARY

This section describes the quantitative aspects of establishment and expulsion of Nippostrongylus brasiliensis in the normal and immune rat. It is proposed that this helminth infection may be described in terms of 4 phases. These are: 1) Loss Phase 1 (LP1). A proportion of the infecting dose of larvae is immobilised or destroyed before or during the course of migration via the lungs to the intestine. 2) The Plateau Phase. The worm number remains static in the predilection site. 3) Loss Phase 2 (LP2). This is analagous to the self-cure reaction and during this, worms are expelled from the intestine of the host by an immunological mechanism. The onset of the expulsion is sudden and the process is exponential. It continues at a steady rate until a threshold is reached. 4) Threshold Phase. A small residual population of worms is not expelled and survives for a fairly prolonged period. The pattern is maintained in immune animals undergoing second or third infections but there are quantitative differences. With increasing number of infections LP1 becomes greater, the Plateau Phase is shortened and the expulsion rate of LP2 increases. It is suggested that the rate coefficient of this phase is an accurate measure of the immune status of the animal. The Threshold Phase is of a similar order in first, second and third infections. These results are discussed with regard to their significance as a measure of the immune status of the host, their implication regarding the mechanism of helminth expulsion and their relevance to other helminth infections.

SECTION 2

STUDIES ON THE MECHANISM OF WORM EXPULSION

INTRODUCTION

Helminth parasites may be expelled from the stomach or intestines of infected animals under two apparently quite different circumstances both of which have come to be called self-cure.

In one of these situations which is exemplified by Nippostrongylus infection of rats, worm expulsion begins at a specific time after infection. The period of worm survival before the onset of expulsion corresponds to the time required for the development of the immune response and becomes reduced with each subsequent reinfection. Other infections which are apparently terminated in a similar fashion are those of A. galli in the fowl (Sadun, 1948) and T. retortaeformis in the rabbit (Michel, 1952, 1954).

In the other situation worm expulsion of a pre-existing infection is induced by the intake of a further dose of infective larvae. This phenomenon was first called self-cure by Stoll (1929) who observed a sudden drop in faecal egg count of two H. contortus infected sheep which were grazing pasture contaminated with this parasite. It was subsequently shown by Stewart (1950b, 1953, 1955) that the drop in faecal egg count is usually due to the expulsion of a proportion, or all of the adult population, although occasionally merely due to a temporary suppression of egg laying. The reaction could be brought about not only by natural oral infection, but also by the injection of exsheathed 3rd stage H. contortus larvae into the abomasa of laparotomized sheep. It was also found (Gordon, 1948;

Stewart, 1950b) that the parasites which provoke the reaction may themselves develop to maturity.

A number of theories have been advanced to explain the mechanism of the self-cure reaction. Stewart showed that Haemonchus self-cure was accompanied by a rise in whole blood histamine and that the drop in faecal egg count could often be prevented by the administration of antihistamine. These facts led to the development of a theory of local hypersensitivity and this was supported by the occurrence of a local reaction when larvae were directly implanted into the abomasa of infected sheep. This reaction which was manifested by increased peristalsis and segmentation and the occurrence of abomasal oedema persisted for approximately two hours after implantation. The abomasa of worm free sheep remained flaccid and normal when subjected to the same treatment.

The fact that the histamine response occurred between the 2nd and 4th day after reinfection was taken by Soulsby (1959) as an indication that the antigen provoking self-cure was associated with the larval stages which had undergone some degree of development. Soulsby and Stewart (1960) demonstrated by killing sheep serially after a challenge dose of larvae that an adult population of worms co-existed in the abomasum with the population of the developing larvae until such time as the larvae moulted to the fourth stage. After this time the adults were found disintegrating in the small intestine. Since moulting of H. contortus larvae is initiated 48 hours after infection (Veglia, 1915) the association of the self-cure reaction with the moulting process was strongly suspected. When serum taken after self-cure was tested

by agar diffusion a marked precipitin response was found to occur against exsheathing fluid produced at the time of ecdysis of infective larvae. This antigen is normally released into the rumen (Rogers and Sommerville, 1957), and is unlikely to be the one involved in self-cure for a number of reasons including its dilution, degradation and the time of its release. The marked serologic reaction with exsheathing fluid, however, suggested that an antigenically similar material was also released during the moult from 3rd to 4th larval stages and it was postulated that this antigen initiated a local anaphylactic reaction which resulted in self-cure.

In so far as the reaction could remove not only H. contortus but also a number of other abomasal and small intestinal parasites such as O. circumcincta, T. axei and T. colubriformis, it seemed that the physical effects of the anaphylactic reaction resulted in worm expulsion, rather than any specific antigen-antibody reaction with the adult worm. Although self-cure of the abomasal parasites caused elimination of the small intestinal parasites the reverse was not true.

A number of theories have been advanced to explain the mechanism of the self-cure reaction in Nippostrongylus infection. One of these (Sarles and Taliaferro, 1936) postulated a primary role for serum antibodies acting at specific local sites, i.e., skin, lungs and small intestine. In support of this theory the authors were able to passively protect rats with serum taken from rats hyperimmune to the parasite. It was subsequently shown (Mulligan et al, 1965) that the passive transfer of immune serum to rats

would confer a significant degree of protection not only to larval infection but also against a transplanted population of adult N. brasiliensis. It was suggested that this action of the immune serum need not necessarily have a direct anti-worm effect, but might depend on passive sensitization of the intestine. Subsequent anaphylaxis stimulated by antigen from the transplanted adults might produce an unsuitable environment for the worms survival.

The operation of such a mechanism would bring self-cure in N. brasiliensis infection into line with the theory suggested by Stewart (1953) to explain self-cure in Haemonchus contortus infection. This theory was supported by the fact that rats which have undergone self-cure could be shocked with an intravenous injection of N. brasiliensis antigen (Urquhart et al, 1965), the reaction being principally expressed in gross alterations in the small intestine, i.e., increased capillary permeability, hyperaemia and increased mucous production.

In a study of the pathogenesis of N. brasiliensis infection it has been shown by Jarrett and Sharp (personal communication) that a villar lesion develops in which the epithelium becomes separated from the underlying capillaries and that the space so formed is filled with protein containing fluid. The presence of the large volume of fluid causes marked stretching of the epithelial sheet. If the dye Evans blue is injected intravenously into infected rats, areas of intense blue staining of the small intestinal mucosa are found at autopsy and frequently blue stained fluid is present in the lumen (Urquhart et al, 1965).

This evidence of leakage and the presence of a microscopical extravascular 'leak lesion' suggest a third possible mechanism for Nippostrongylus self-cure; namely that the increased capillary permeability associated with a local anaphylactic reaction might be important as a means of allowing a significant amount of antibody into the gut lumen, where it may then act directly on the worms. Such a possibility was first suggested by Pierce (1959).

The present section is a description of the development of the 'leak lesion' under different circumstances of infection. An experiment is described in which the lesion was reproduced in uninfected rats by a heterologous system. This system was then used to study the effect of the lesion in rats infected with transplanted adult worms, and passively immunized with serum.

1. The influence of the size of infection and of the effect of cortisone treatment on the development of the intestinal 'leak lesion'

Introduction

The ability of cortisone and related drugs to inhibit the immune response is well documented and it has been shown that the immune response to nematodes may also be inhibited. One effect of this type of drug is the prolongation of many parasitic infections beyond the time when worm elimination has occurred in control animals. Such an effect has been described for T. spiralis and Trichuris muris infections of mice (Coker, 1955; Campbell, 1963) and N. brasiliensis infections of rats (Ogilvie, 1965, Urquhart et al, 1965). In the latter infection worm elimination can be delayed for as long as cortisone treatment is carried out but is re-established some days after the administration is discontinued. The mechanism whereby cortisone has this action remains obscure.

The purpose of the present experiment was twofold; first to make a comparative study of the pathogenesis of N. brasiliensis infections of normal and cortisone treated rats with particular reference to the development of the leak lesion. If cortisone could be shown to diminish or abolish the leak lesion some useful evidence would be obtained to support the theory that this lesion played a significant role in the self-cure reaction. The second purpose was to estimate the effect of the size of the worm burden on the extent of the leak lesion.

Materials and Methods

Four groups of 25 rats were infected as follows:

Group 1 a single infection of 2000 larvae

Group 2 a single infection of 5000 larvae

Group 3 a single infection of 5000 larvae and cortisone treatment from one day before infection onwards

Group 4 a single infection of 5000 larvae followed by cortisone treatment from the 8th day of infection onwards.

The cortisone treatment consisted of 0.1 mg. of Betsolan (Betamethazone, Glaxo Laboratories) subcutaneously on alternate days over the periods designated. Terramycin 2.5 mg. was given simultaneously with each injection of the cortisone.

Two rats from each of the first 3 groups were autopsied on every second day from days 4-20 inclusive after infection. Autopsy of group 4 was started on day 10.

The characteristic lesion in nippostrongylosis was located by the following method. Each rat was injected intravenously with 0.5 ml. of a 1% solution of Evans blue. Thirty minutes later the animals were killed and the entire small intestine opened longitudinally and examined macroscopically to assess the extent and intensity of blue staining of the mucosa and the amount of leakage of blue stained fluid into the lumen. Two uninfected control rats were similarly injected with Evans blue on the first day of the experiment and were autopsied to determine the degree of blue staining of the mucosa in uninfected animals.

Blocks for histopathological examination were cut from Evans blue stained (EB) areas and from adjacent non-stained (NEB) areas, were fixed in corrosive formol (Carleton and Drury, 1957), dehydrated, cleared in an alcohol-amyl acetate-chloroform series and embedded in paraffin wax. The sections were stained routinely with haemalum and eosin.

Egg counts were carried out on all four groups throughout the experiment and are shown in Table 10.

Results

A. Uninfected rats: The intestines of the control rats were thin-walled and of regular diameter. The entire mucosa was a diffuse and even pale blue colour; no blue stained fluid was found in the lumen. On microscopical examination the villi appeared slender, elongate and evenly spaced.

B. A 2000 and a 5000 larval infection (Groups 1 and 2)

Day 4: On day 4 the worms were still very small, were lying quite deeply in the mucosa and appeared as dark speckles on a diffuse blue background (Fig. 6). Many more worms were present in the higher dose group. In both groups the ileum was distended with viscid yellow mucous which was blood-stained in the case of one of the group 2 rats.

Since there were no areas of intense blue staining on this day, blocks for histopathology were taken from areas of greatest worm density. A notable histological feature was the presence of numerous parasites lying between

TABLE 10

EGG COUNTS OF RATS INFECTED WITH 2000 OR 5000 N. BRASILIENSIS LARVAE (GROUPS 1 & 2) AND RATS TREATED WITH TWO REGIMENS OF CORTISONE AND INFECTED WITH 5000 LARVAE (GROUPS 3 & 4)

Day	GROUP			
	1 2000 larvae	2 5000 larvae	3 5000 larvae + cortisone from D-1 onwards	4 5000 larvae + cortisone from D8 onwards
7	29,400	85,300	185,200	250,200
8	44,200	80,000	114,100	125,700
9	35,000	74,600	73,500	150,000
10	15,000	64,900	176,000	170,000
11	4,600	5,400	57,000	103,500
12	7,600	1,500	91,900	184,600
14	100	200	71,100	83,800
15	0	0	89,600	189,000
16			42,600	75,000
17			37,900	56,500
19			58,800	47,900

Fig. 6. Gross appearance of duodenal mucosa on day 4 showing immature adult worms.

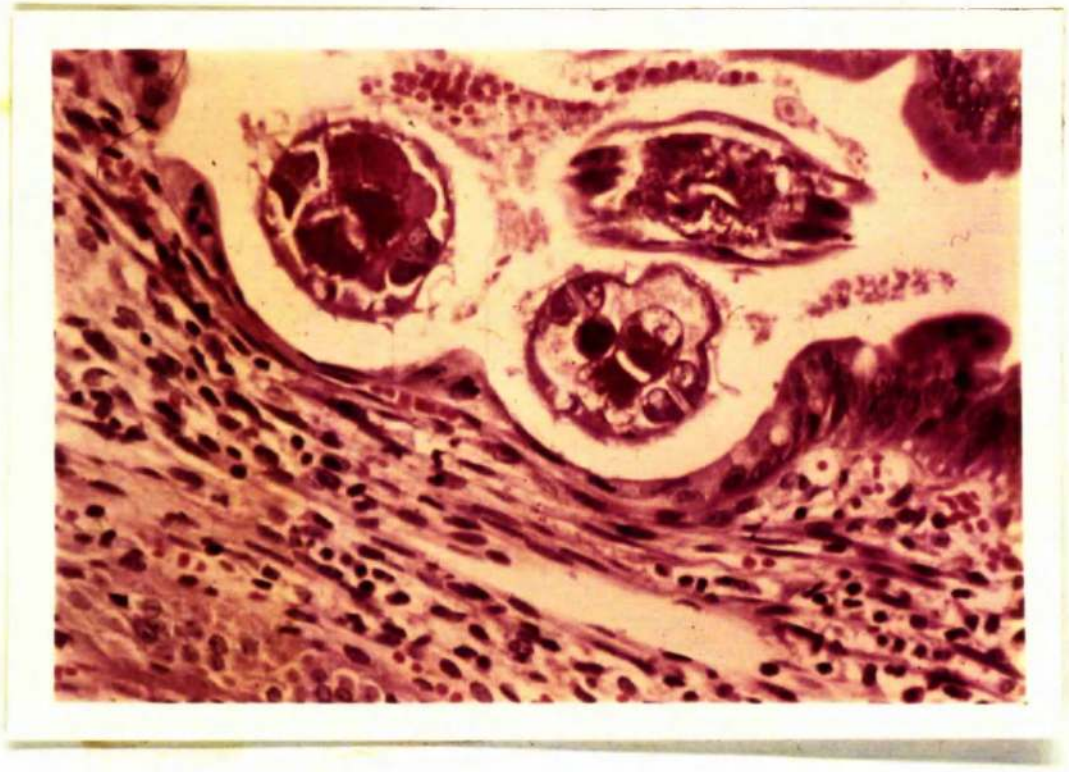


Fig. 7. Parasites lying between villi on day 4; the villi are frequently indented, and the epithelial cells adjacent to the worms are flattened. (x 350)

villi which had become indented to 'fit' round them (Fig. 7). The epithelial cells adjacent to the worms were frequently flattened to a layer of extremely thin cells with dark strap-like nuclei. In group 2, slight villary oedema was already apparent on day 4, especially at the tips of the villi. In this group also, the villar capillaries were congested and some quite extensive haemorrhage had occurred, usually at the bases of the villi.

Day 6: The intestines of both groups before being opened appeared distended and dark blue-black in the area corresponding to the highest concentration of worms (Fig. 8). This distention was more marked in the higher dose group. It was found on opening the intestines that the worms, which now appeared red in colour, were almost exclusively confined to an area lying between the 3rd and 8th inches of the duodenum. The mucosa in this area was stained deeply blue in a mottled fashion (Fig. 9), the degree of staining being greater in group 2 and being proportional to the density of worms present at a particular site. Outside the limits of the 'worm patch' the intensity of blue staining faded abruptly. The walls of the intestine were swollen and oedematous. In the higher dose group this oedema was more extreme and much thin yellow fluid was present in the lumen.

Sections cut from the Evans blue stained (EB) areas showed that villary oedema occurred on day 6 in the 2000 dose group but was much more

Fig. 8. Appearance of the intact duodenum following Evans blue injection on day 6 (Group 2), showing marked distension and discolouration of the area where the worms are located.

Fig. 9. The opened intestine on day 6, showing blue staining of the mucosa and the presence of mature adult worms.

severe in the 5000 dose group. Almost all villi in EB sections were very distorted (Fig. 10). Over the tips of the villi and extending down the later faces the epithelium had been 'lifted' off the underlying lamina propria and the space so formed was filled with oedema fluid (Fig. 11 and 12). The villar capillaries were congested, especially at the periphery of the villi (Fig. 13) and widespread haemorrhage into the lamina propria had occurred. A general increase in the number of epithelial cells as was described by Jarrett and Murray (personal communication) had become obvious. This was manifest by convolution and thickening of the lumenal epithelium, an increase in the number of crypt cross sections and by an increased number of mitotic figures in the epithelial cells of the crypt area.

Sections cut from non-Evans-blue-stained (NEB) areas adjacent to the EB areas gave in both groups a histological appearance differing little from that of uninfected control intestines (Fig. 14). Very few worms were seen in these sections, villary oedema was absent and the mucosa was also much thinner than in the EB areas.

Day 8: The distention of the intestine commented on in the day 6 infection still occurred but was less marked in the lower dose group. In this group also, blueing of the mucosa was less intense and more diffuse than on day 6. Very many worms were present in the 5000 dose group but were found in an area slightly lower down than on day 6. Blue staining in this group was still intense with large dark blue mottled areas in the region of the worms.

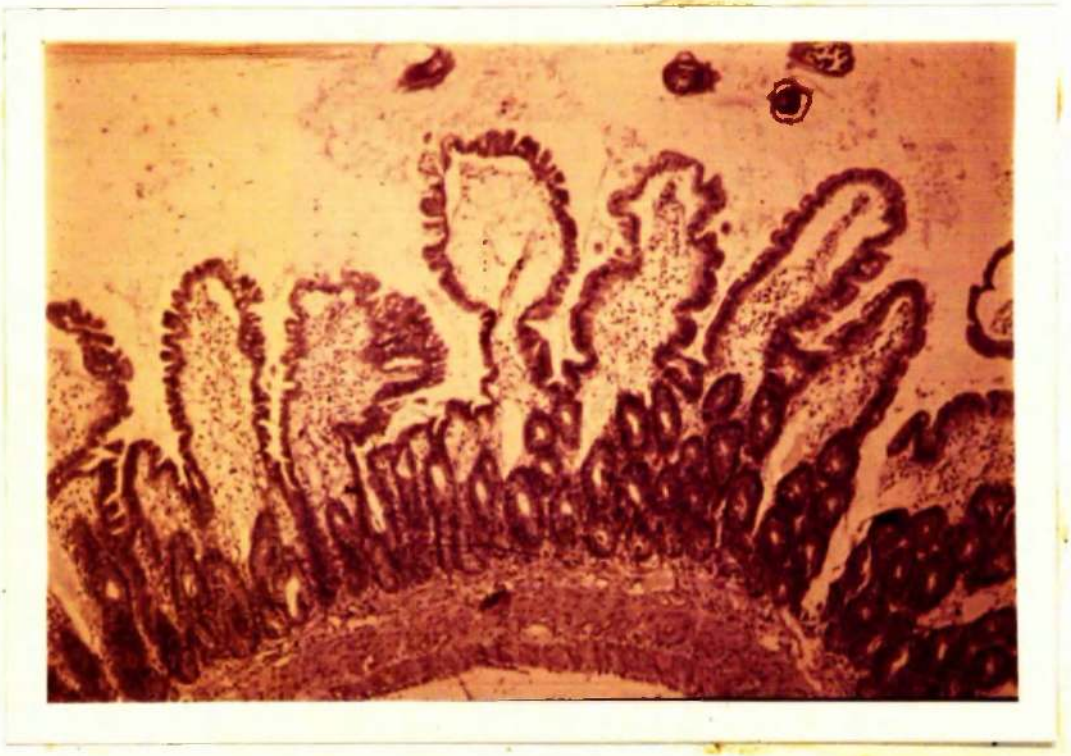


Fig. 10. Histologic appearance of Evans blue stained area of the mucosa showing oedematous and distorted villi on day 6. (x 50)

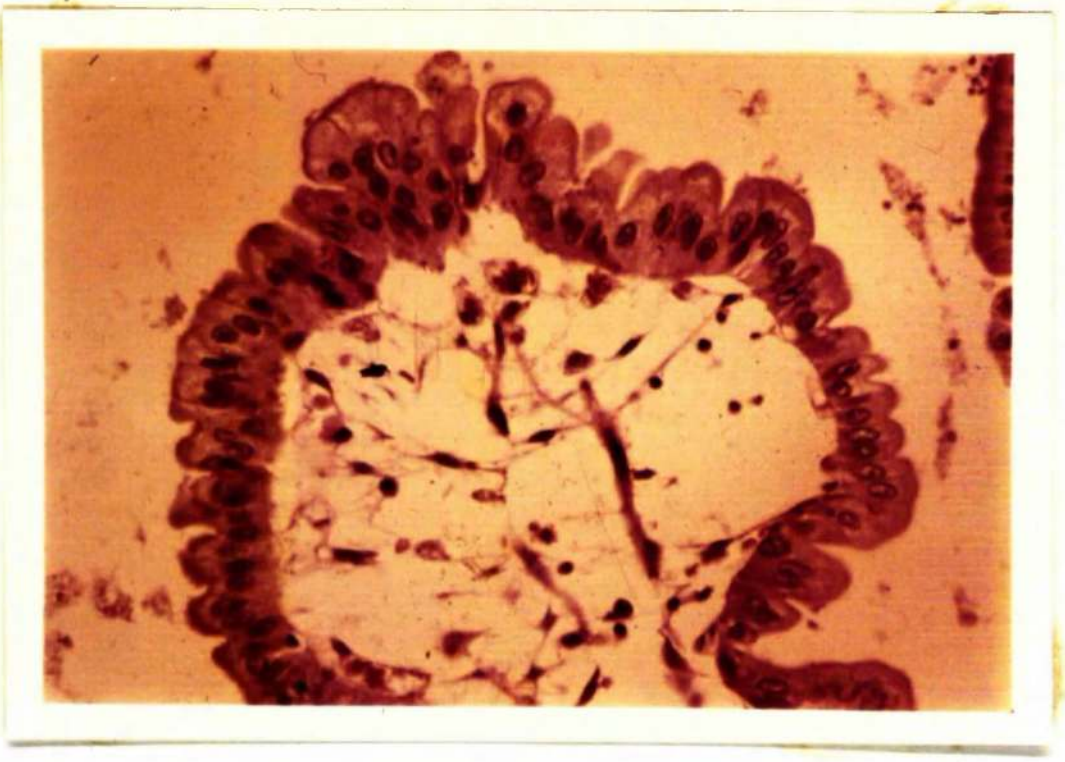


Fig. 11. Detail of tip of villus showing the sub-epithelial 'leak' lesion. Day 6. (x 350)

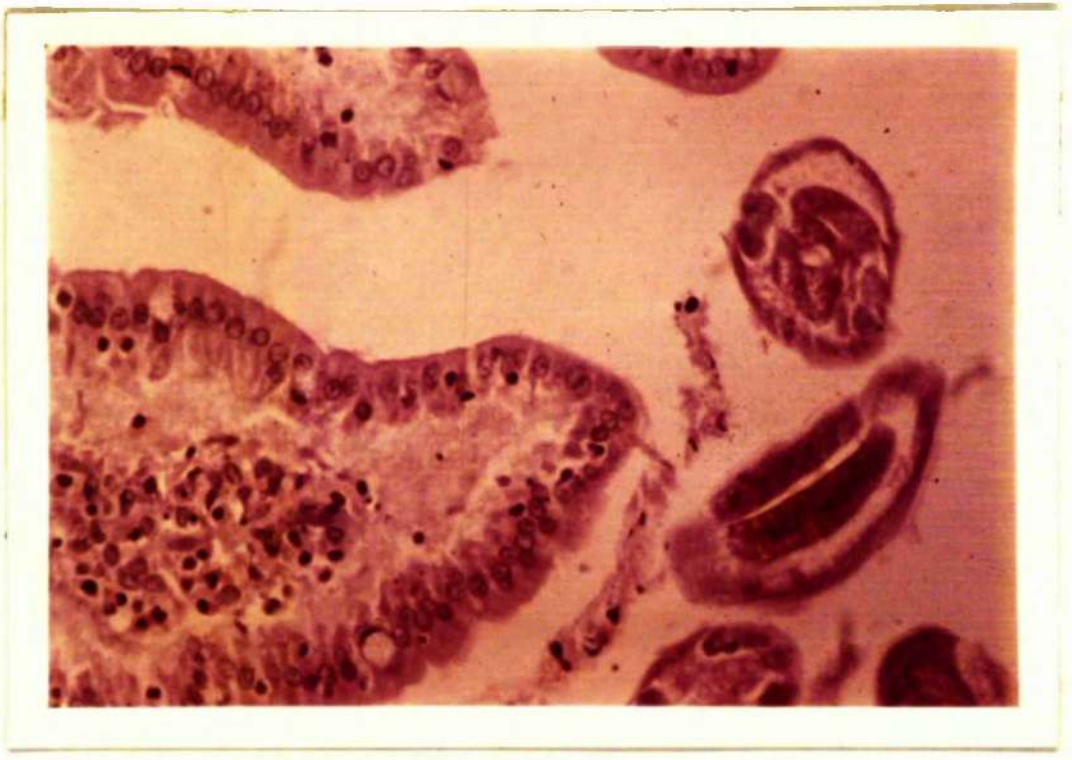


Fig. 12. Detail of villus to demonstrate the extension of sub-epithelial oedema down the lateral faces. Day 6. (x 245)

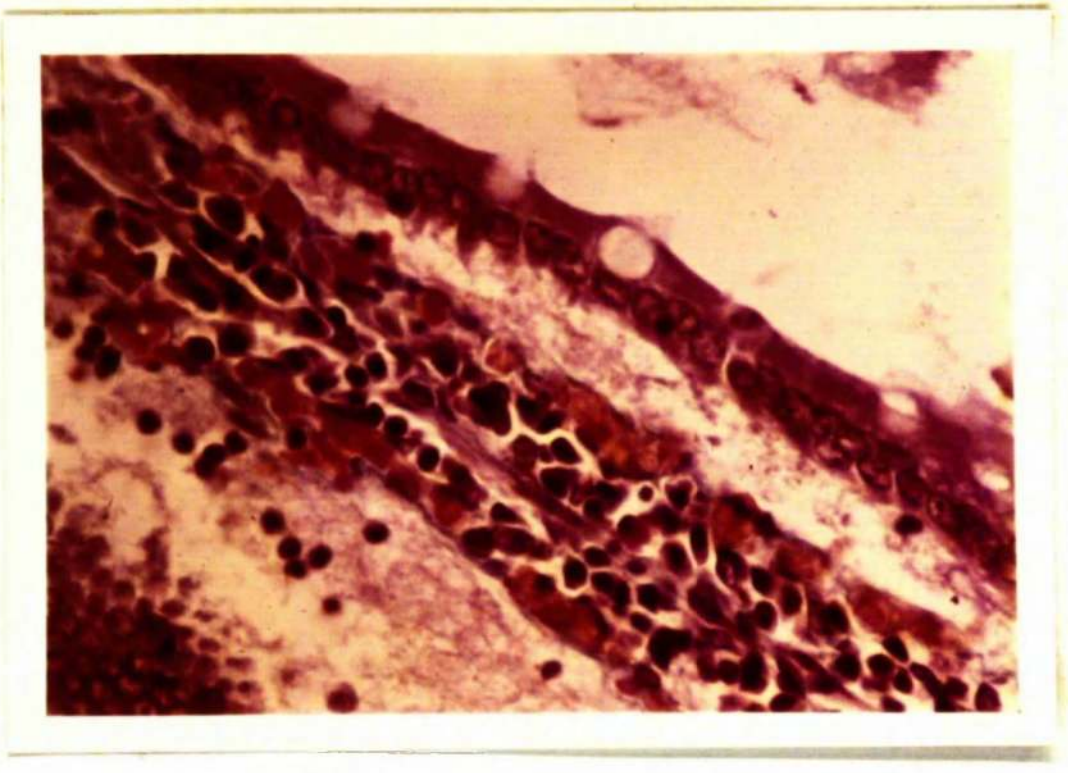


Fig. 13. Congestion of the peripheral villar capillaries on day 6. (x 350)

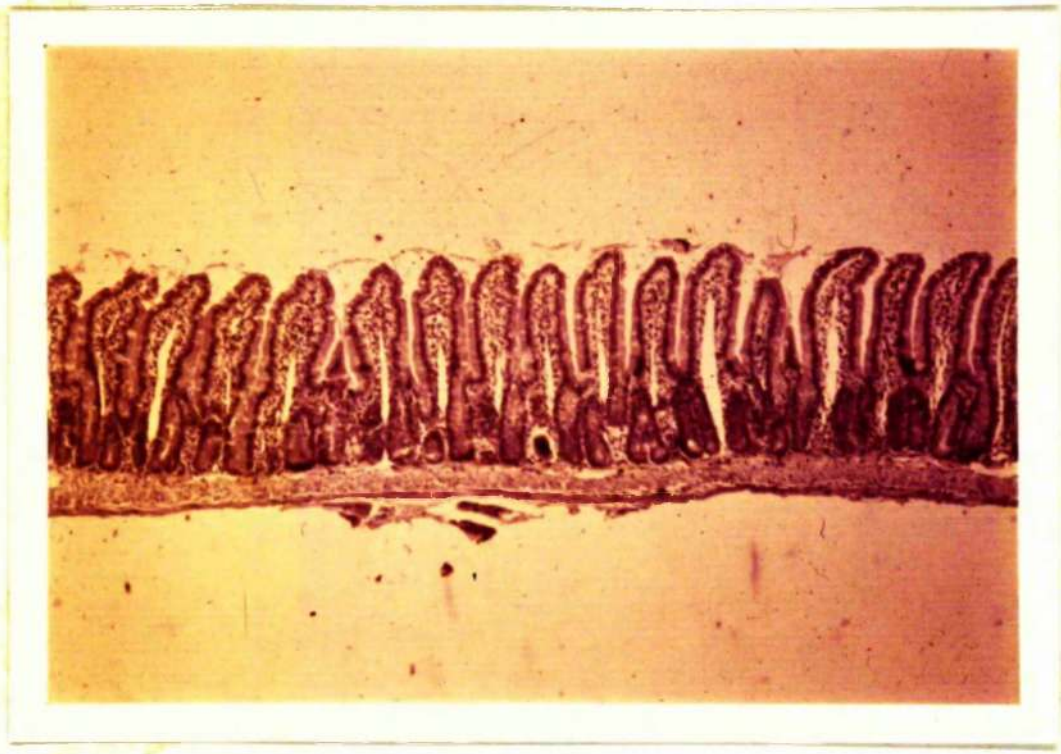


Fig. 14. Appearance of the unstained mucosa on day 6; the leak lesion is absent and the villi are regular. (x 50)

The histological picture of villary oedema appeared much the same as on day 6 but was if anything less marked. A small degree of oedema was found on day 8 in some of the NEB sections. A cellular reaction consisting of eosinophils, lymphocytes and plasma cells which had been building up since day 4 was approaching maximum at this time. This reaction and especially the increase in the number of eosinophils was more marked in group 2. An apparent increase in the number of Paneth cells was noticed to be occurring during the infection especially in group 2. On day 8, 4-5 Paneth cells could be recognised in many crypt cross sections in this group. Most of these cells were well granulated but, as the time after infection increased, many came to have a reticulated appearance. Some appeared to be discharging granules into the crypt lumina while others were vacuolated and appeared to be empty.

Day 10: On day 10 the worm population was more evenly distributed over the anterior half of the small intestine though still of highest density in the previously defined worm area. The worms were no longer adherent to the mucosa but were suspended in much fluid. The blue mottling had become less marked in both groups and was fading into the general diffuse background blue of the worm area.

Histologically villary oedema in both groups appeared to be reduced with villi assuming a more normal and elongate pattern. Congestion of villus tip capillaries still occurred in the high dose group but there was

less haemorrhage than seen previously. Epithelial desquamation especially over the tips of the villi was noticed in this group. The macroscopical reduction in contrast between EB and NEB areas was reflected also in the histology; most of the NEB areas at this stage in the experiment showed lesions of similar intensity to those seen in the EB areas although capillary congestion was probably less.

Day 12: The two rats of group 1 killed on this day both still harboured many worms and the mucosa was patchy blue in the worm area in which a large proportion of the worms were found. In both group 2 rats there were apparently less worms and a few small isolated blue areas corresponding to worm clumps were found in the jejunum and ileum. Thick blue mucus was present in the lumen at the worm area and the mucosa was thickened here but there was no evidence of the watery oedema seen on days 6 and 8.

Histologically the villary oedema was more marked than on day 10; whether this reflects a real increase or a chance variation in 'takes' between different rats is not known. At this stage of the infection however the oedema was complicated by a cellular reaction.

Day 14: On day 14 the rats of both groups harboured noticeably less worms and these were spread out over a large area of the duodenum. The mucosa was only slightly more stained in the worm area than elsewhere but deeper

coloured mucous fluid was still present over this site. Thinner blue-green fluid was found further down in the intestine.

Villary oedema was markedly reduced, in many areas to a barely perceptible villus tip oedema; there was no visible distinction between EB and NEB areas.

After day 14 the appearance of the small intestine reverted rapidly to that of normal uninfected animals. A few worms were still occasionally seen but were not apparently sufficient to result in the persistence of the leak lesion.

C. Cortisone treated rats: Examination of group 3 rats which had been cortisone treated since the day before infection showed until the 10th day after infection only relatively minor differences, of degree rather than kind, in the lesions described for group 2 rats. The worms were distributed in a similar fashion but Evans blue staining of the mucosa did not appear to be quite as intense as in the non-cortisone treated animals. Much fluid was often present in the lumen but the intestinal distension seen in group 2 rats on days 6-8 was less marked in the cortisone treated group.

Histologically villary oedema occurred in group 3 as in group 2 but was marginally less severe. Although epithelial proliferation appeared to have occurred to a similar extent to that in non-cortisone treated rats it was felt that the eosinophil and plasma cell reaction was reduced.

On day 12 after infection the pattern of villary oedema in both groups of cortisone treated rats resembled that of the milder day 6 infection of

untreated rats. This appearance continued until day 20 when the experiment was terminated. At this time the intestines of the untreated rats resembled closely those of the uninfected control animals.

It therefore appears that between days 10 to 20 after infection most of the differences in appearance between the intestines of the cortisone treated and untreated rats could be attributed to the continued presence of the worms in the former groups. Self-cure did not occur in either of the cortisone treated groups and no differences were noticed in the macroscopical or histopathological picture of group 3 and 4 despite the different dosing regimens. From day 12 onwards the worms in these groups were no longer confined to the worm area but became scattered throughout the small intestine. Similarly oedema and Evans blue staining spread in the wake of the worm movement.

Discussion

On examination of the small intestine of infected rats which have been intravenously injected with the dye Evans blue, patches of intense blue staining of the mucosa are seen. These have been found on microscopical examination to correspond to a vascular and oedematous lesion of the villi and the extent and intensity of the blueing was shown to provide a gross estimate of the severity of this lesion.

The lesion basically consists of the development of a large fluid-filled space between the blood vessels and the epithelium of the villus.

Since the Evans blue molecule combines with albumin its presence in the sub-epithelial spaces and in the intestinal lumen indicates that the lesion is related to a specific site of macromolecular leak.

The severity of the leak lesion has been found to be proportional to the dose of larvae given; the blue mottled areas were invariably found in the region of clumps of worms and were more intense and covered a larger area in the group of rats which were infected with 5000 as opposed to 2000 larvae.

The time of onset of the leak lesion was dependent on the size of the infection. In the high dose group it was detectable microscopically on the 4th day after infection; in both groups it was grossly and microscopically apparent and at a maximum on the 6th day post infection. Subsequently the lesion remained relatively static in intensity until about day 10 when its severity regressed, although blue stained fluid was still visible in the lumen of the intestine. Around this time the worms were also found to be spread over a much larger area than that to which they had been confined for the first 8-10 days.

In the cortisone treated rats (groups 3 and 4) all the components of the leak lesion also occurred although not to the same degree. The most striking difference was the persistence of the lesion in both cortisone treated groups until day 20 when the experiment was terminated. During this period when self-cure was operating in the untreated groups it was very apparent in the cortisone treated groups that the persisting worm

populations were spread throughout the whole small intestine. Possibly because of this, i.e., the lower density of worms per unit area of intestine, no dark patches of Evans blue occurred, the whole intestine showing moderate staining and leakage.

One of the primary objects of this experiment was to find if cortisone abolished the leak lesion. Since on the evidence presented here it does not appear to do so, one must consider alternative ways in which cortisone might interfere with the expression of the self-cure reaction. One possible explanation recently reported elsewhere (Jarrett et al, 1967b) depends on the observation that cortisone treatment using the same regimen as described here markedly reduces the total number of mast cells in the mucosa of the small intestine. When cortisone treatment is stopped the mast cells reappear in quantity; shortly after self-cure occurs. The possible association between the mast cell and self-cure is discussed elsewhere.

2. The development of the intestinal leak lesion following transplantation of adult worms

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Introduction

The results of the previous experiment have shown that when a sufficiently large dose is used, the intestinal leak lesion may develop as soon as four days after larval infection, i.e., only 48 hours or so after the first worms have arrived in the small intestine. This period is a suspiciously short one for the development of an anaphylactic lesion.

The experiment to be described here was carried out to determine the time of onset of Evans blue leakage and the oedema lesion in rats infected by laparotomy with a transplanted adult worm population.

Both this and the following experiment were carried out in preparation for another experiment, designed to test the role of the leak lesion in worm expulsion, and described later in this section.

Materials and Methods

Two groups of 12 rats were infected as follows:

Group 1 Transplant of 700 adult worms

Group 2 Transplant of 3000 adult worms.

The adult worms for transplant were removed on the 10th day of infection from a group of donor rats. The method of transplantation is described in the Materials and Methods section.

Two rats from each group were killed on days 2, 4, 6 and 9 following the injection of Evans blue as described in the previous experiment.

Results

On the second day after infection the rats which had received 3000 larvae showed typical Evans blue mottling of the small intestinal mucosa in the region where the worms were found. Blue fluid was present in the lumen and surrounding the worms. The rats of the lower dose group showed no Evans blue staining or leak on day 2.

Histologically the leak lesion was present in both groups on day 2. In group 1, capillary congestion and villar tip oedema was discernable but was not marked or extensive. The group 2 lesion was already severe and the villi had already become blunted and appeared fused over large areas.

On day 4 and after both groups showed similar lesions varying only in degree and these were identical to those described in the previous experiment.

Discussion

The results have shown that starting at 2 days and prominent at 4 days after receiving a transplant of adult worms, the small intestine of rats showed patches of deep blue staining which were related to the presence of large clumps of worms. These areas were associated with the characteristic leak lesion located immediately below the epithelium of the villi.

Because of the shortness of the time which elapses between the presence of the worms in the intestine and the onset of this lesion, i.e., 2-4 days, it is unlikely that the mediators are released by an immunologic reaction in this instance.

3. The leak lesion produced by ovalbumin induced anaphylaxis

Introduction

Intestinal lesions similar to those which have been described for nippostrongylosis occur in several disease entities of different etiology; these include tropical sprue (Butterworth and Perez-Santiago, 1958) and Asiatic cholera in man and experimental animals (Sprinz, 1961). Similar intestinal lesions are also associated with experimental shock induced by various methods. They are seen in rats following the injection of the histamine liberator 48/80 and of histamine and 5.hydroxytryptamine (Fell, Boyne and Cuthbertson, 1961).

In order to test the possible role of the leak lesion it was found desirable to produce the lesion by a system not associated with Nippostrongylus. Following irregular results with the injection of 48/80, histamine or 5 HT a satisfactory leak producing system was obtained by ovalbumin induced anaphylaxis.

Materials and Methods

Rats were sensitized to ovalbumin after the method of Sanyal and West (1958). Each rat was injected intraperitoneally with 1 ml. of 4 per cent (w/v) ovalbumin (Sigma Chemical Co. Ltd., St. Louis, Missouri) together with 1 ml. of Pertussis adjuvant 20,000 x 10^6 organisms per ml. (kindly supplied by Burroughs Wellcome, Park Langley). Shock was induced 12 days after

sensitization by the intravenous injection of 1 ml. of the ovalbumin solution. Evans blue was used as a marker of protein leak as described previously and was injected (0.5 cc. of a 1 per cent solution) together with the shocking dose of antigen.

Rats were autopsied at intervals of 15 minutes to 2 hours after injection of the shocking dose.

Results

Approximately 2 minutes after the injection of the shocking dose of antigen the rats became prostrate and dyspnoeaic. Gross examination of the small intestine at 15 minutes and 30 minutes after infection showed extreme hyperaemia of the swollen mucosa and intense Evans blue staining along the entire length of the small intestine. There was much blue mucus in the lumen.

Histologically villar capillary congestion and oedema was seen. A sub-epithelial fluid filled space as described for nippostrongylosis had formed by 15 minutes and was even more extreme at 30 minutes after injection (Fig. 15). Also at this time the tips of many villi gave the appearance of having exploded under the fluid pressure and epithelial cells were lying free in the lumen.

At 2 hours after injection the above lesions although still present appeared to be regressing.

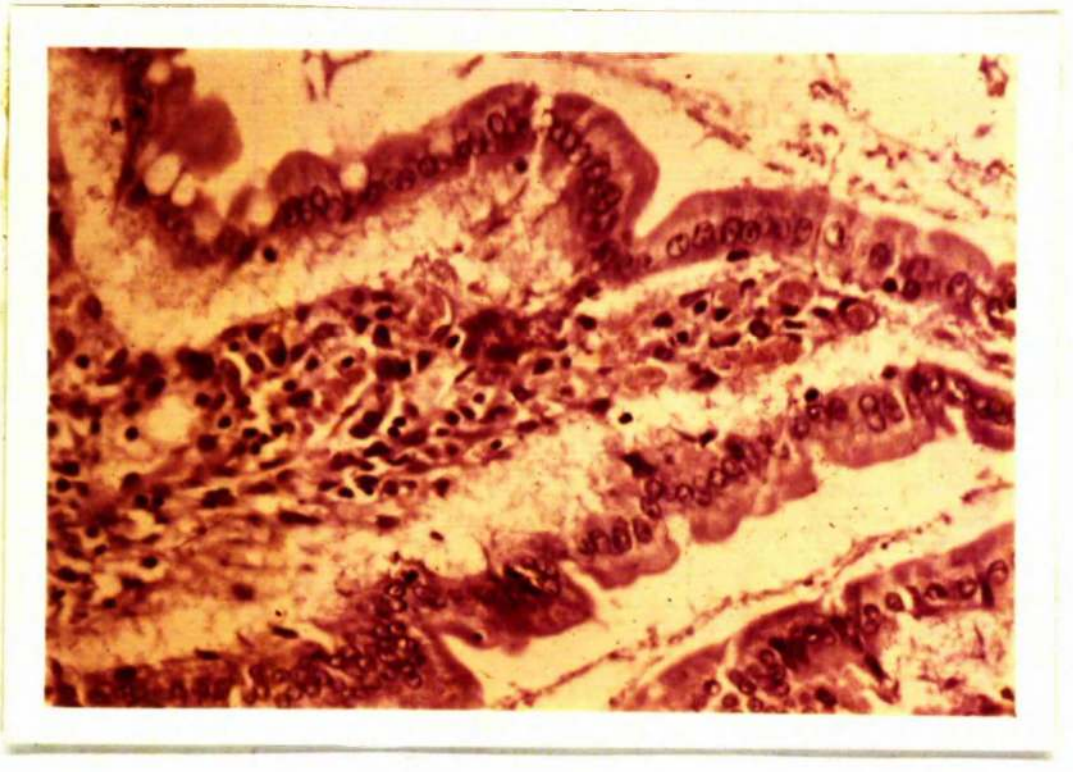

Fig. 15. Sub-epithelial 'leak' lesion in villus of small intestine produced by ovalbumin-pertussis anaphylaxis. (x 245)

Discussion

The intestinal lesions produced in rats by ovalbumin-induced anaphylaxis resemble these present in infected rats before and at the time of self-cure, although the distribution and duration of the lesions is different. In the ovalbumin shocked rats the whole small intestine shows diffuse blue staining of the swollen mucosa and this is associated with the diffuse distribution of the villar lesion, but this persists for only a few hours.

4. The role of the leak lesion in worm expulsion from the intestine

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Introduction

This part describes an experiment which was designed to test the role of the leak lesion in the expulsion of worms from the small intestines. Two alternative functions have been postulated for the anaphylactic-like state of which the leak lesion is a symptom (see introduction to this section); first, that the associated structural and functional changes provided an environment which is unsuitable for worm survival, or secondly that the leakiness associated with the altered state provides the means of access of antiworm-antibody into the intestinal lumen; the antibody may then act directly on the worms.

In an effort to obtain more information on the specific mechanisms involved a comparison was made between two methods of attempting to produce premature expulsion of worms in rats infected with a transplanted adult population. Both groups were subjected to heterologous intestinal anaphylaxis; the second group was also passively immunized.

The results indicate a possible role for the leak lesion in worm expulsion.

Experimental Plan

All materials and methods are as described in the Materials and Methods section, or as previously described in this section.

The experiment outlined in Table 11, was designed to determine if the production of heterologous anaphylactic shock would induce premature self-cure in passively protected (Group A) or normal rats (Group C) harbouring a transferred adult infection.

Whether or not a degree of worm expulsion had occurred was determined by counting the number of worms in the rats at autopsy 36 hours after shock, and comparing these with the numbers of worms present in unshocked rats (Groups B and D) which had received hyperimmune serum and normal serum respectively.

Since it is known that transferred adult worms are not expelled from passively protected rats for at least 3 days (Mulligan et al, 1965; Neilson, 1965) the numbers of worms in the group B rats (hyperimmune serum alone) were expected to be similar to those of group D rats (normal serum alone) at the time of autopsy. If the numbers of worms in group C rats (anaphylaxis alone) were significantly reduced, it could be concluded that the physical changes associated with anaphylaxis per se were responsible for the expulsion of the worms. If on the other hand the mean number of worms in this group was similar to groups B and D and that of group A alone was significantly reduced, it would indicate that the role of anaphylaxis had been confined to facilitating the passage of antiworm-antibody into the intestinal lumen.

Results

Results are shown in Table 12.

TABLE 11

DETAILS OF TIMES OF TREATMENT OF FOUR GROUPS OF RATS INFECTED WITH SURGICALLY TRANSPLANTED ADULT WORMS

Time	A	B	C	D
Day 1	Sensitized*	-	Sensitized*	-
Day 10	Adult worms transferred surgically to rats in all groups			
Day 12	Hyperimmune serum	Hyperimmune serum	-	Normal serum
18 hours after serum	Shocked*	-	Shocked*	-
36 hours after shock	Rats in all groups autopsied and residual worms counted			

* Sensitized and shocked with ovalbumin

TABLE 12

THE RESULTS OF ATTEMPTS TO PRODUCE ACCELERATED EXPULSION IN RATS INFECTED WITH TRANSFERRED ADULT WORMS

	A Hyperimmune serum and shock	B Hyperimmune serum	C Shock	D Normal serum
No. worms recovered	216	462	419	360
	181	396	256	324
	169	256	253	319
	163	256	251	317
	165	213	221	292
	164	187	218	253
	131	173	207	232
	104	172	159	199
	74	170	152	173
	37	132	151	5
			69	
Mean	140	241	214	247
S.D.	51	101	85	99
Test of significance (t test)	P = 0.01	NS	NS	

The rats which received hyperimmune serum and were later subjected to anaphylaxis had a mean burden and standard deviation of 140 ± 51 worms. This was significantly lower than the burden of the rats which received hyperimmune serum alone (241 ± 101), or those which were shocked without serum (214 ± 85). The burdens of these two later groups were not significantly different from the group of rats which received normal serum only (247 ± 99).

All of the shocked rats showed clinical signs of anaphylaxis (i.e., prostration, dyspnoea) of medium severity lasting not less than one and not more than 10 hours. Two rats from groups A and C were killed 30 minutes after injection of the shocking dose of antigen and the presence of the characteristic intestinal lesion was confirmed grossly and microscopically.

Discussion

In the experiment described above in which rats infected with an adult population of Nippostrongylus brasiliensis were subjected to intestinal anaphylaxis induced by ovalbumin and Haemophilus pertussis it was found that the consequent physical alteration of the mucosa did not result in the expulsion of worms.

On the other hand the production of intestinal anaphylaxis in passively immunized rats produced a significant expulsion of worms compared to rats which were only passively immunized. These results suggest that the physical changes associated with anaphylaxis had facilitated the extravascular passage

of antibody where its effect was specifically directed against the worms. This supports the idea that the lesion found in the intestines of infected rats may play a similar role in the self-cure reaction.

5. Failure of attempts to accelerate self-cure in primary infections

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Introduction

In part 4 of this section an experiment was described in which adult worms were transplanted into the small intestine of uninfected rats by laparotomy. This experiment, undertaken to elucidate the mechanism of self-cure, indicated that the reaction might depend on the availability of antiworm-antibody which had gained access to the worms via an intestinal leak lesion.

If this hypothesis is correct it might be reasonably inferred that a primary infection could be prematurely terminated by the injection of hyperimmune serum, since the lesion component is present from the 5th day onwards.

Some evidence which superficially supports this hypothesis is presented in Section 3 in which it is shown that the size of a primary infection is reduced by the administration of hyperimmune serum before and during the early stages of a larval infection; on reflection, this is rather equivocal since it is possible that the early migrating stages are affected by the treatment.

In the first experiment described below, serum was administered on the 5th and 7th day after infection in an attempt to advance the onset of Loss Phase 2.

Experiment 1 - The use of hyperimmune serum alone

Plan

Two groups of 8 rats were infected with 1,000 larvae. The rats of one group (group 1) received 6 ml./100 g. of hyperimmune serum intraperitoneally on days 5 and 7 after infection. The other control group (group 2) received no serum. Both groups of rats were autopsied on the 10th day after infection, this being before the normal onset of LP2.

Results

The results shown in Table 13 indicate that expulsion had not been advanced in group 1 rats by treatment with hyperimmune serum since means of 283 and 331 worms were recovered from the treated and control groups respectively.

Discussion

One possible explanation of the failure of hyperimmune serum to produce premature self-cure is that the leak lesion in primary infections although established by the 5th day as measured by gross and histological criteria may lack some essential component until the 12th day. A second experiment was therefore carried out to investigate this hypothesis.

Experiment 2 - The use of hyperimmune serum and heterologous anaphylaxis

Plan

Two groups of 10 rats (groups A and D) were sensitized to ovalbumin

TABLE 13

ATTEMPTS TO ADVANCE THE ONSET OF LOSS PHASE 2 IN A PRIMARY INFECTION

1. Administration of hyperimmune serum on day 5 and 7 after infection

Intestinal worm count on day 10

	Group 1	Group 2
	325	540
	361	433
	292	439
	290	405
	281	368
	263	324
	261	362
	189	223
		237
		173
		234
Mean ± S.D.	283 ± 50	331 ± 115

as described in part 3 of this section. Eight days later both these groups and 2 other groups (groups B and C) were infected with 1,000 larvae. On the 5th day of infection, the rats of group A (sensitized) and group B received 6 ml. of hyperimmune serum per 100 g. intraperitoneally. On the 6th day after infection (14 days after sensitization) the rats of both of the ovalbumin sensitized groups (A and D) were shocked by intravenous ovalbumin injections. As in the previous experiment all groups were autopsied on the 10th day after infection.

Results

It is again apparent (Table 14) that this combined system failed to advance the onset of worm expulsion.

Discussion

From the results of the 2 experiments it seems likely that the inability of the immune serum to induce self-cure in a primary infection is due to a factor other than the failure to develop a competent leak lesion. This subject is further discussed in the General Discussion of this section.

TABLE 14

ATTEMPTS TO ADVANCE THE ONSET OF LOSS PHASE 2 IN A PRIMARY INFECTION

2. Administration of hyperimmune serum and shock

Intestinal worm count on day 10

	Groups			
	A Hyperimmune serum	B Hyperimmune serum + shock	C No serum No shock	D No serum + shock
	69	128	6	188
	184	328	112	209
	238	91	279	395
	235	168	201	363
	294	120	153	322
	119	121	90	197
	136	251	243	302
	311	19	289	223
	269	179	266	78
	227	229	361	
	251	180	421	
	398	157	87	
	511	278	98	
	58	173	212	
	492	144	245	
	246	187	409	
	118	384	540	
		66	181	
		240	107	
		238	93	
		356		
Mean ± S.D.	244 ± 131	191 ± 93	220 ± 137	253 ± 101

GENERAL DISCUSSION

In Section 1 it was shown that the onset of worm expulsion in a Nippostrongylus infection was sudden, but once initiated, proceeded in an exponential fashion. This observation suggested that the mechanism whereby adults are expelled might be a two-stage reaction depending first on the presence of antiworm-antibody and secondly on a specific factor which permitted the sudden expression of this antibody.

It was considered that the specific factor might be the development of a lesion which facilitated the passage of antibody from the blood or connective tissue of the villi onto the mucosal surface. Such a lesion was shown to exist. Basically, it consists of the development of a large fluid-filled space between the blood vessels and epithelium of the villus; it is a specific site of macromolecular leak as shown by the presence of Evans blue in the sub-epithelial spaces and in the intestinal lumen.

Subsequent work, however, showed two reasons why it is unlikely that the leak lesion is solely responsible for the sudden onset of worm expulsion. First, if sufficiently large infections are used the lesion may develop as soon as 4 days after larval infection and 2 days after adult worm transplant. It then persists until the time of self-cure. Secondly, Mulligan (personal communication) in experiments using isotopically labelled polyvinyl pyrrolidone and ^{51}Cr-labelled human albumin has shown that there is a progressive increase in the passage of these substances from the circulation into the intestinal lumen during the infection. It is therefore unlikely that intestinal

permeability arising from the leak lesion *per se* (as defined by the technique used) is the sudden onset event responsible for antibody release.

Detailed studies of the etiology and development of the leak lesion have indicated a particular aspect which may be significant in the sudden release of antibodies.

It has been shown using the dye Evans blue that the magnitude of the lesion is dose-dependent and that the sites of leakage from the blood vessels are correlated exactly with the position of the worms. This also occurs in cortisone treated animals which do not self-cure. It has also been recently demonstrated (Jarrett *et al*, 1967b) that degranulation of the intestinal mast cell population occurs at approximately the same time as the onset of the leak lesion. Since this time is thought to be too early to attribute the onset of the leak lesion to the immunologically released mediators, it is highly probable that both the mast cell degranulation and the associated oedema of the villi are mediated by a permeability inducing factor produced by the worm. In support of this theory, a small molecular weight and non-antigenic mast cell degranulator has recently been isolated from the parasite *Ascaris suus* by Uvnas and Wolde (1967). It has also recently been found (unpublished results) that antigen prepared from *N. brasiliensis* contains a substance which degranulates rat skin mast cells. It may be in the parasite's interest to invoke this reaction in the intestine, possibly as part of the feeding mechanism.

It is also evident that hypersensitivity results from *N. brasiliensis* infections. This is shown by the ability to induce anaphylaxis expressed primarily in intestinal changes in immune rats with *N. brasiliensis* antigen

(Mulligan et al, 1965) and by the appearance of reaginic antibodies in the serum of rats 1-2 weeks after self-cure (Ogilvie, 1964), stimulated by the secretion by the parasite of a specific allergen. We have also recently found (Jarrett et al, 1967b) that following the original degranulation referred to above the mast cell population reappears and increases by the time of self-cure to 2-3 times its original number. This is followed by a second degranulation or amine discharge around the time of self-cure. This subject is to be fully discussed in a Ph.D. thesis to be submitted by Miller. It is possible that the action of allergens and reaginic antibodies result in the rise and subsequent second degranulation of the mast cells and that this in turn contributes quantitatively or qualitatively to the permeability initiated by the worms. Incidentally, this theory, if correct, might explain our failure reported earlier to accelerate the onset of self-cure in a primary infection by the administration on the 5th and 7th day post-infection of hyperimmune serum alone or followed by albumin induced shock. As mentioned above, granulated mast cells are almost absent at this time.

Locally available antibodies have been shown to be of primary importance in defence against a variety of pathogenic bacteria, protozoa and viruses (see review by Pierce, 1959). Pathogens affected by such antibodies are usually characterised by their non-invasiveness being confined to mucous surfaces or in the superficial mucosa. A rise in local antibody titre may be a reflection of either local antibody production or the extravasation of

humoral antibody arising from local alterations in tissue permeability. It has been suggested that the second mechanism may be the function of the intestinal leak lesion seen in nippostrongylosis and it has been demonstrated that a similar lesion produced artificially will facilitate the passage of antibody into the gut lumen and thereby expedite worm expulsion. It seems reasonable to attribute a similar role to the natural lesion.

Current work in this field is directed towards the elucidation of the possible role of locally produced antibody in Nippostrongylus infection. It is possible that passive protection induced by the injection of hyper-immune serum produces its effect by a method which is not the most significant in the natural infection; in the latter antibody may be produced locally in the intestinal mucosa and may come into contact with worms in concentration far greater than occurs in the serum.

With regard to the class of immunoglobulin involved, it is known that IgA is the predominant type of γ-globulin found in many secretions (Tomasi et al, 1965) and that the production of IgA is the primary role of the plasma cells in the intestinal tract (Crabbe et al, 1965; Crabbe and Heremans, 1966). Local antibodies to polio virus in duodenal fluid have been demonstrated to occur in this type of immunoglobulin (Berger et al, 1967).

In nippostrongylosis there is both a tissue migrating and a local intestinal phase; it is likely that several types of antibody of different classes are produced both locally and systemically and that their relative concentrations may alter not only during a primary infection but also in

successive reinfections. Thus the interaction of the different antibody classes to produce the spectrum of immunological effects may be more complex than was hitherto thought.

The present approach includes fluorescent labelling experiments using antisera specific for each of the rat immunoglobulin classes, to determine which types of antibody are being produced or fixed locally, during each stage of the Nippostrongylus infection. Attempts to isolate antibody from the intestinal mucosa are also in progress.

SUMMARY

Experiments undertaken to elucidate the mechanism of worm expulsion from the intestine are described. Observation of the sudden onset of LP2, and the subsequent exponential kinetics suggests that the mechanism whereby adults are expelled might be a two stage reaction, depending first on the presence of antiworm-antibody and secondly on a specific factor which permits the sudden expression of this antibody. It was considered that the specific factor might be the development of a state of permeability of the vascular and/or epithelial tissues which facilitates the passage of antibody from the blood or connective tissue of the villi onto the intestinal mucosal surface. A lesion which gives rise to such a state was shown to exist. It consists basically of the development of a large fluid-filled space between the blood vessels and the epithelium of the villus. It is a specific site of macromolecular leak as shown by the presence of Evans blue in the sub-epithelial spaces and in the intestinal lumen. The lesion develops as soon as four days after infection with N. brasiliensis larvae and two days after a transplant infection of adult worms; it is considered that its mediators are produced by the worm itself at least during the early part of the infection.

In an experiment in which the lesion was produced by a heterologous system, its presence appeared to facilitate the passage of passively transferred antibody into the intestinal lumen, causing the premature expulsion

of a transplanted adult worm population. By itself the lesion had no effect on the worms. The results are discussed in relation to recent findings concerning the intestinal mast cell and reaginic antibodies.

SECTION 3

N. BRASILIENSIS INFECTION OF YOUNG RATS

INTRODUCTION

It is well established that the immune response of very young animals may be defective both in quality and quantity. It is also possible that antigenic stimulation during this period may produce a state of specific immunological unresponsiveness which can persist into adult life (see review by Good and Papermaster, 1964).

Since both animals and man are frequently exposed to helminth infection in very early life the possibility exists that immunological immaturity and unresponsiveness may play significant roles in the epidemiology and pathogenesis of particular helminth diseases. The subject is as yet largely unexplored. Immunological immaturity to infection with Haemonchus contortus has been described in several breeds of British sheep aged 6 months or less (Manton et al, 1962; Urquhart et al, 1966); in the East African Merino the period is apparently even longer (Lopez and Urquhart, 1968). Immunological unresponsiveness has been reported to occur in calves infected, when under 5 days of age, with Cysticercus bovis or Mesistocerrus digitatus (Soulsby, 1963b). In all of these instances the failure of the immune response was demonstrated by the relative inability of the host to resist successfully a second infection.

An experimental approach to the problem in large animals presents all the usual difficulties encountered in such investigations, the cost of the experiments and accommodation required being but two. A laboratory model of immunological unresponsiveness to a helminth parasite would overcome these problems and provide a convenient system for obtaining a background of

experimental fact. This was the object of the experiments described in this section.

In helminth immunity part of the antibody response is associated with functional immunity which, in N. brasiliensis infection, is responsible for self-cure and resistance to reinfection. But as in other helminth infections the immune response is accompanied by a diverse antibody response which may be measured by a variety of serological techniques. There is no evidence that these antibodies play any important part in the immune mechanism or that their titre is an indication of the immune status of the animal; the presence of these antibodies may be quite incidental to functional immunity and may merely reflect the large number of antigens to which the host is exposed as a result of helminth infection. It is relevant in this context that attempts to produce comprehensive immunological tolerance to foreign complex antigens such as bacteria or a mixture of protein antigens have been largely unsuccessful (Smith, 1961; Smith et al, 1963; Hyde et al, 1965; Ben Shaul, 1962, 1963), although in these cases tolerance may be induced to some determinants while antibody response occurs to others.

The question then arises as to how unresponsiveness should be best identified and measured. Obviously unresponsiveness to the incidental, i.e., non-protection inducing antigens, is of little practical importance. Protective antibody on the other hand at present can only be detected by a direct in vivo effect on the parasite.

It was the intention at the outset of this work to explore in baby rats

some aspects of the Nippostrongylus infection which pertained especially to problems of unresponsiveness.

These were:-

1. The behaviour of an infection in young rats; would normal self-cure occur? If not, how would the pattern of infection differ from that of adult rats?

2. The reaction to later reinfection; would there be any evidence of the induction of tolerance or alternatively would an infection when young produce as good an immune response as when adult?

As it transpired a form of unresponsiveness apparently unrelated to immunological tolerance occurred and the mechanism of this will be discussed in the light of current information of unresponsiveness and the self-cure reaction.

Part 1 The Pattern of Infection in Young Rats

Introduction

The course of Nippostrongylus infection in young as compared to adult rats has not been previously investigated. However, several authors during studies on the development of age resistance to the parasite compared some isolated features of the infection between young and older rats. Using the course of egg production, Africa (1931) found this to be much higher in 2 month old than in 8 month old rats. A similar result was reported by Graham (1933). Chandler (1932) using the number of worms recovered at autopsy as the criterion found that young, i.e., 4-5 week old, rats harboured about one third more worms at approximately 10 days after infection than did older animals.

The experiments to be described in part 1 of this section were designed to show the pattern of infection and self-cure in baby rats and to determine if this differed from the characteristic sequence seen in adults (see Section 1). The experiments fall into two groups; first exploratory infections of baby rats and secondly more quantitative experiments designed on the basis of the information obtained from the preliminary work.

Materials and Methods

The method of infection of baby rats and all other techniques were as described in the Materials and Methods section. The dose of larvae and age

of rats infected in the individual experiments of this part are included in the text where appropriate.

1. Preliminary experiments

Results

Experiment 1

In the first experiment two groups of baby rats were infected with 500 larvae each. We have shown in the course of other experiments that this dose of larvae will cause a normal self-cure reaction in adult rats. The groups infected were aged 3-4 weeks and 5-6 weeks respectively. Half of each group was killed on days 10 and 26 for examination of the intestinal worm burden. On day 26, 5 of the young rats, 2 from the first and 3 from the second group, were tested for susceptibility to specific anaphylaxis by the intravenous injection of 0.5 ml. Nippostrongylus antigen with 0.5 ml. 1% Evans blue. All the rats killed on day 26 were bled and the serum from each group was pooled and titrated for PCA activity.

The results, listed in Table 15, show that the infection had not been eliminated by day 26, by which time in adult rats self-cure is complete.

On the other hand, all the young rats injected with Nippostrongylus antigen showed signs of severe shock, i.e., prostration, dyspnoea, etc., and one of these rats died 10 minutes after injection. The small intestines of these rats were oedematous and the mucosa was deeply blue stained with

TABLE 15

500 LARVAL INFECTION IN TWO AGE GROUPS OF YOUNG RATS;
WORMS RECOVERED FROM SMALL INTESTINE

	Days after infection			
	10		26	
	Group 1*	Group 2x	Group 1	Group 2
	225	148	198	370
	223	133	144	271
	94	145	226	201
	153	173	181	250
	175	318	206	167
	143	204	177	230
	97	264	374	179
		177		17
Mean	177	195	215	210
S.D.	82	65	75	101

* Group 1 - rats aged 3-4 weeks at infection

x Group 2 - rats aged 5-6 weeks at infection

Evans blue. Histological examination showed the typical shock lesion (see Section 2) in the small intestine of all of these animals. In this feature their reaction is similar to that of adult rats. The PCA titre of the pooled serum was in the region of 1/600 for both groups. This titre is similar to that of adult rats receiving the same infection.

This experiment showed that self-cure of a 500 larval infection did not occur before day 26 in rats aged up to 6 weeks. However, despite the lack of this immune reaction the young rats had developed the susceptibility to shock and a high serum titre of reagins.

Experiment 2

The second experiment was designed to ascertain the duration of a primary infection in baby rats. Five litters aged between 2-3 weeks received 500 larvae/rat. Egg counts were made at weekly intervals on each litter. Since the results from the individual litters were very similar, mean values only are shown in Table 16. Although an appreciable fall occurred in the egg output between days 12-25 after infection, the level then remained between $5\frac{1}{2}$ and $3\frac{1}{2}$ thousand e.p.g. for over five weeks. In view of the continuing egg count, groups of rats were first autopsied on day 52 and subsequently on day 67 and 87 after infection. The results of the worm counts on these days are also shown in Table 16.

It is apparent that these rats had not undergone the self-cure reaction and still harboured the major part of their adult worm burden as late as day 67 and a large part still on day 87 of the infection.

TABLE 16

500 LARVAL INFECTION IN 2-3 WEEK OLD RATS; WORMS RECOVERED FROM THE SMALL INTESTINE AND EGGS/GRAM OF FAECES

	Days after infection		
	52	67	87
	191	135	158
	132	182	121
	141	148	55
	128	90	92
	124	148	34
			95
			25
Mean ± S.D.	143 ± 27	140 ± 33	83 ± 48

Days after infection	E.P.G.
7	14,700
12	27,250
19	13,600
25	4,500
33	5,500
39	4,500
48	3,600
55	2,500
61	2,100
67	3,600
74	1,600
81	1,200

On day 52 of the infection a group of 10 of these rats at this time 9-10 weeks old were challenged with an infection of 3,000 larvae together with a control group of 6 uninfected rats of the same age. The egg counts of this infection, and worm counts, following autopsy on days 10 and 16 are shown in Table 17.

While the egg count results show some depression of egg output in the reinfected rats, the worm counts show no manifestation of immune elimination either due to an increased LP1 or an earlier LP2, i.e., on day 10 the mean worm burden of the reinfected and control rats was the same. By day 16 both the challenge and control group are undergoing LP2, this occurring possibly rather more rapidly in the previously unresponsive than the control rats. However this point, due to the small group sizes, cannot be taken as confirmed.

Discussion

The preliminary experiments indicated that if rats were infected when under 6 weeks of age with 500 larvae they did not undergo during the second week after infection the characteristic self-cure reaction that is a feature of the response of adult rats. Instead these animals underwent a prolonged infection that extended well into adult life and which cannot therefore be attributed solely to immunological immaturity.

In this aspect therefore the rats were unresponsive; however an antibody response to at least one worm antigen, probably the allergen described by Jones and Ogilvie (1967), was demonstrated by the susceptibility of the rats to specific anaphylaxis and by the production of reaginic antibody.

TABLE 17

CHALLENGE INFECTION OF UNRESPONSIVE AND CONTROL RATS: WORMS RECOVERED FROM THE SMALL INTESTINE AND EGGS PER GRAM OF FAECES

	Days after infection			
	10		16	
	Unresponsive rats	Control rats	Unresponsive rats	Control rats
	1500	1450	195	283
	1495	1220	385	231
	1330	46	5	261
	324		29	
	92		4	
Mean	927	905	123	258
S.D.	684	719	166	26

E.P.G.

Days after infection	Unresponsive rats	Control rats
7	46,700	41,200
8	30,050	53,400
9	31,700	87,300
10	4,200	25,600
11	2,000	18,000
12	1,200	6,400
14	100	400
15	0	100
16	0	0

The evidence at this stage might lead one to speculate that classical immunological tolerance (Billingham et al, 1956) had been induced to a worm parasite infection. In common with tissue transplant tolerance, the continuous presence of antigen might predispose to the upkeep of such a state. In this context the demonstration of one antigen-antibody reaction need not be at odds, since split tolerance, i.e., the simultaneous existence of tolerance to one antigen and antibody response to another, has been demonstrated not only in the homograft system (Brent and Courtney, 1962) but also with bacteria (Smith et al, 1963) and mixtures of protein antigens (Ben Shaul, 1962, 1963).

Less auspicious for the furtherance of the tolerance concept, however, is the age up to which this unresponsive state is inducable, i.e., approximately 6 weeks. This is well beyond the reported limits of the postnatal period in rats during which the induction of tolerance has been possible. With the homograft system this period of tolerance responsiveness in rats has been found to extend only into the first postnatal week (Billingham et al, 1962) although Woodruff and Simpson (1955) found that a small proportion of rats could still be made tolerant by inoculations as late as 2 weeks after birth.

While it is now recognised that tolerance can be produced even in the adult animal, it must be pointed out that the rats in this case had been exposed to a relatively small amount of antigen.

In addition, the results of the preliminary challenge infection make

it unlikely that tolerance is involved. Thus the first infection given when young and still persisting at the time of challenge, while not having stimulated the high degree of resistance to reinfection characteristic of adult rats did not on the other hand interfere with the development of an immune response to the challenge infection. The evidence suggests that this occurred in a manner similar to that of rats which are experiencing a primary infection.

2. The response of young rats to different doses

The unresponsiveness to Nippostrongylus demonstrated in the preliminary experiments followed infection with a dose of 500 larvae which led to an established infection of 140-200 worms. The object of the next experiment was to enlarge on this result and find out if the unresponsive state induced in these rats would be altered either quantitatively or in duration by increasing the size of the first larval infection.

Results

Experiment 1

One hundred rats aged between 19-22 days were divided into 3 groups and infected as follows:

Group 1 - 33 rats infected with 500 larvae per rat

Group 2 - 33 rats infected with 1,000 larvae per rat

Group 3 - 34 rats infected with 2,000 larvae per rat.

Egg counts in each group were made at frequent intervals. The rats were sacrificed in groups of 10 to 12 from each group on days 12, 24 and 48 after infection.

The results of the faecal egg counts are shown in Table 18. The highest counts were found in the group infected with 2,000 larvae, but in this as in the other groups the counts fell until by day 20 the counts of all 3 groups were at the 2,000-3,000 e.p.g. level. From then onwards until day 48 the situation remained unchanged with the egg count in all 3 groups fluctuating between 1,000-4,000 e.p.g.

The means of the worm burdens of the rats on days 12, 24 and 48 after infection are shown in Table 19 and graphically in Fig. 16. The adult worm burden established from a dose of 500 larvae on day 12 was 102 ± 38 worms and the results of subsequent autopsies on days 24 and 48 showed that this had not changed significantly. Similarly the group of rats receiving 1,000 larvae in which 269 ± 175 worms were established on day 12 showed no significant difference in worm burden as late as day 48. In both these groups it is apparent that expulsion of the adult worms had not occurred. On the other hand in the third group infected with 2,000 larvae the worm burden fell from 696 ± 369 on day 12 to 409 ± 323 on day 24 and finally to 253 ± 80 on day 48 after infection.

TABLE 18

DOSE RESPONSE EXPERIMENT 1. THE EGG COUNT (E.P.G.) OF RATS INFECTED WHEN 19-22 DAYS OLD WITH A SINGLE DOSE OF LARVAE

Days after infection	Dose of larvae		
	500	1000	2000
7	21,900	53,400	172,000
8	18,300	50,000	43,950
9	11,250	28,600	63,900
12	7,000	15,800	19,700
13	5,750	18,000	26,300
14	4,400	10,800	17,100
15	8,300	16,800	13,400
16	5,850	7,350	8,900
20	2,000	3,200	1,900
21	1,450	3,700	1,700
22	2,900	7,500	2,200
24	2,050	2,200	1,600
27	2,350	4,500	3,150
28	3,750	3,950	2,500
29	4,100	4,100	2,850
34	3,400	3,900	2,950
35	2,750	4,950	3,350
36	1,250	2,750	4,050
37	2,050	2,600	2,700
41	2,850	2,050	4,000
42	1,850	3,200	3,150
43	2,350	1,750	4,200
48	2,600	2,500	1,350

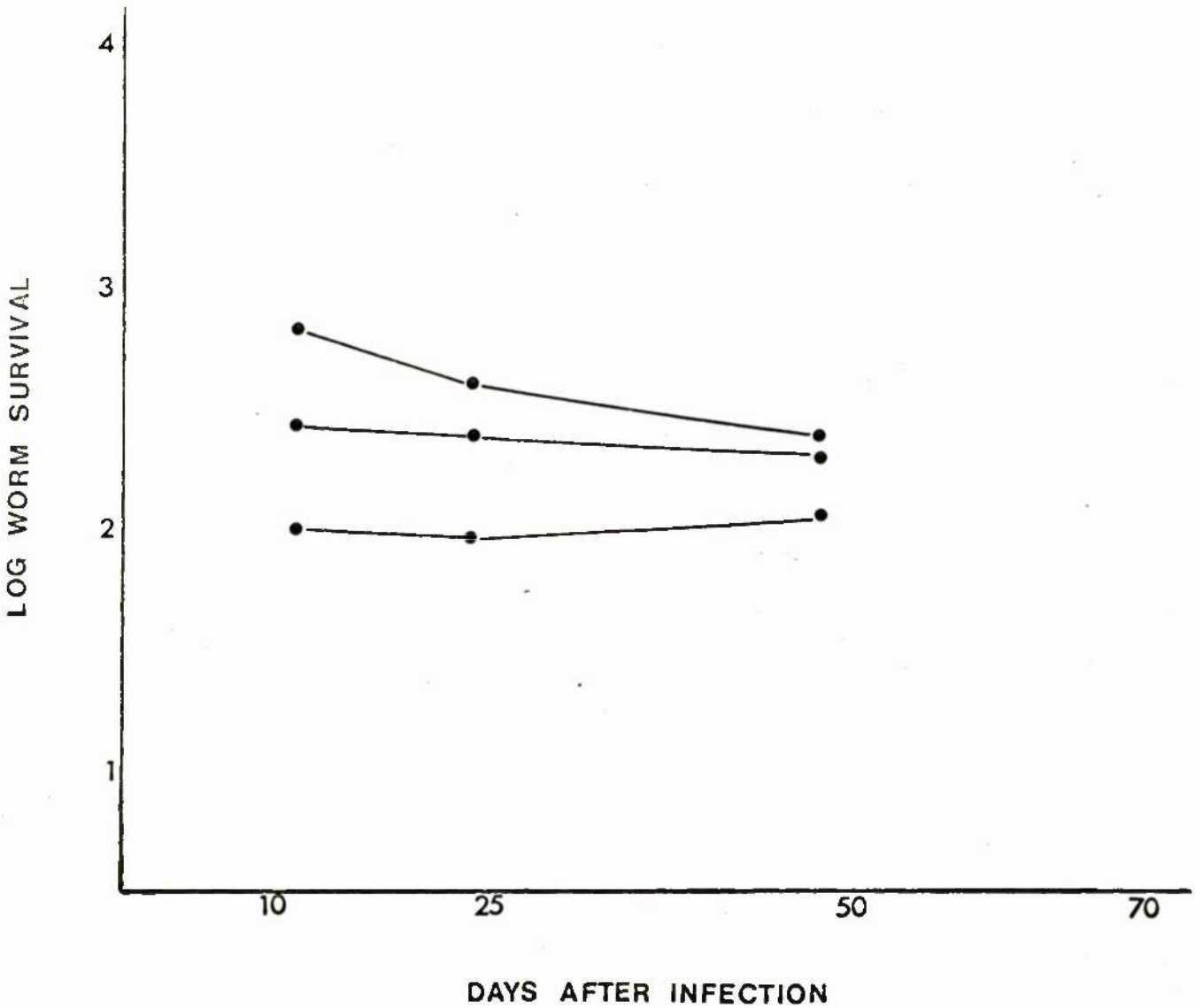
TABLE 19

DOSE RESPONSE EXPERIMENT 1. THE MEAN* NUMBER OF WORMS RECOVERED AT AUTOPSY FROM GROUPS OF RATS KILLED AT INTERVALS AFTER INFECTION WITH A SINGLE DOSE OF LARVAE

Days after infection	Dose of larvae		
	500	1000	2000
12	102 ± 38	269 ± 175	696 ± 369
24	96 ± 57	254 ± 122	409 ± 323
48	121 ± 46	208 ± 111	253 ± 80

* ± standard deviation

Fig. 16. Baby Dose Response Experiment 1. The numbers of worms surviving in 3 groups of rats infected when 3-4 weeks old. The groups were infected with 2,000, 1,000 and 500 larvae/rat, and appear in that order from top to bottom in the figure.



In summary the results of this experiment showed that infection of young rats with a dose of 500 or 1,000 larvae resulted in the establishment of an adult worm population which was not eliminated either at the expected time of self-cure or at any time up to day 48 after infection. An increase in dose to 2,000 larvae resulted in an adult worm infection, two-thirds of which was eliminated by day 48, by which time the mean worm burdens of the groups infected with 1,000 and 2,000 larvae were numerically identical. It would thus appear that by increasing the dose of larvae and hence the size of the adult worm population an immune response of sorts can be evoked in baby rats. This response also differed from the response of adult rats in that worm elimination occurred over a greater period than that which normally spans the self-cure phase. Whether worm elimination would have continued after day 48 in this group was not established.

Experiment 2

The second dose response was designed to answer two questions arising from the first experiment. First, would worm elimination have continued after day 48 in the group infected with 2,000 larvae, or, having reached a level which in the 1,000 group was being continuously tolerated, would it remain static? Secondly, would the immune response be increased by further increasing the size of the larval infection?

Thirteen litters, initially 89 rats, aged between 1-4 weeks, were divided into 3 groups and infected as follows:

Group 1 - 30 rats infected with 800 larvae per rat

Group 2 - 29 rats infected with 1,600 larvae per rat

Group 3 - 30 rats infected with 3,200 larvae per rat.

Egg counts were carried out on the bulked faeces samples from each group at frequent intervals. Five or more rats from each group were autopsied for the purpose of intestinal worm counts on days 10, 25, 50 and 70 after infection. All the rats killed on the last autopsy day were bled and the serum of each rat kept separate. The individual sera of 5 rats in each group were titrated for PCA activity.

The results of the faecal egg counts are shown in Table 20. As in the previous experiment, the egg counts in all 3 groups fell drastically after day 11. The fall in egg count at this time after infection in young rats is similar to that which occurs with adult rats but differs from it in that it does not fall to zero.

Results of worm counts at autopsy are shown in Table 21 and Fig. 17. The mean adult worm burdens which had established in the 3 groups on day 10 from larval doses of 800, 1,600 and 3,200 were 357 ± 178, 776 ± 255 and 1,273 ± 363 respectively. Between days 10 and 25 after infection a variable proportion of the worm burden was eliminated in each group, with the result that on day 25 the means of the three groups ranged between 202-233 worms. Having reached this level further worm expulsion did not occur for at least another 25 days, since on day 50 the worm numbers were still the same. However, by day 70 the mean worm burdens had fallen to between 103 and 113 worms in all groups.

TABLE 20

DOSE RESPONSE EXPERIMENT 2. THE EGG COUNT (E.P.G.) OF RATS INFECTED WHEN 1-4 WEEKS OLD WITH A SINGLE DOSE OF LARVAE

Days after infection	Dose of larvae		
	800	1,600	3,200
7	48,600	44,500	44,100
9	25,100	64,900	85,600
11	15,600	30,400	10,700
13	7,300	7,900	6,100
15	1,100	2,300	300
19	1,650	2,000	650
20	3,000	3,250	650
22	5,350	2,500	1,300
26	1,250	1,750	1,300
28	3,250	2,700	1,600
33	3,950	3,200	1,000
35	1,750	2,700	450
37	3,350	4,500	400
41	1,600	2,000	3,650
42	2,750	5,300	6,250
47	2,250	1,500	3,650
50	500	4,000	1,850
55	2,100	2,400	700
57	350	1,200	800
63	250	450	500
65	1,600	1,150	1,750
69	400	750	2,350
70	200	800	900

TABLE 21

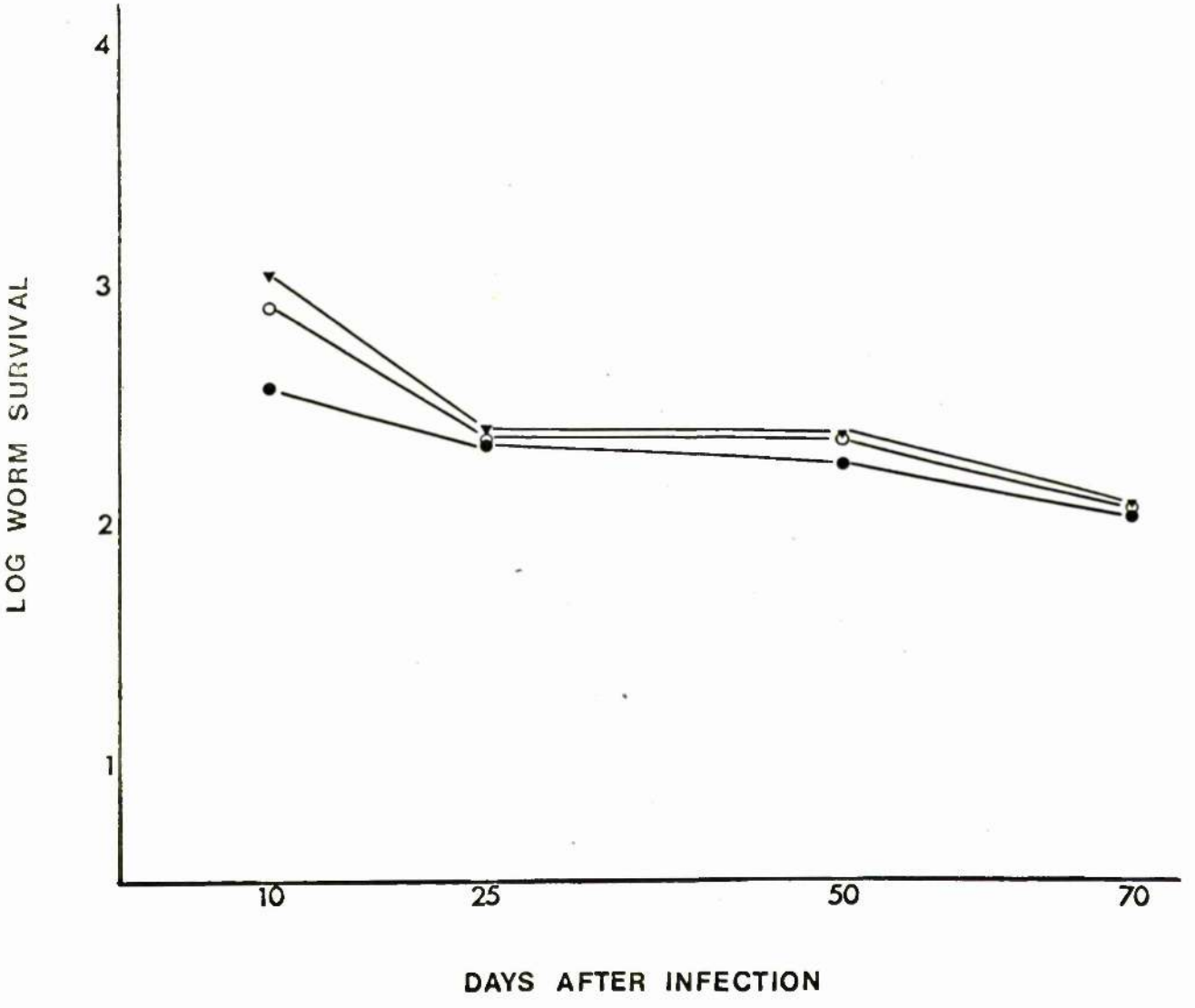
DOSE RESPONSE EXPERIMENT 2. THE MEAN* NUMBERS OF WORMS RECOVERED FROM GROUPS OF RATS KILLED AT INTERVALS AFTER INFECTION WITH A SINGLE DOSE OF LARVAE

Days after infection	Dose of larvae		
	800	1,600	3,200
10	357 ± 178	776 ± 255	1273 ± 363
25	202 ± 110	202 ± 98	233 ± 50
50	171 ± 62	216 ± 81	226 ± 79
70	103 ± 67	113 ± 78	107 ± 89

* ± standard deviation

Fig. 17. Baby Dose Response Experiment 2. The numbers of worms surviving in 3 groups of rats infected when 1-4 weeks old. The groups were infected as follows:

Group	1	800 Larvae/rat	●
Group	2	1,600 Larvae/rat	o
Group	3	3,200 Larvae/rat	▼



Titration of the rat sera for PCA activity on day 70 gave titres of less than 1:8 for all rats tested in each group.

Comparison of the Expulsion Phase in Young and Adult Rats

Since the 2 dose response experiments show that a partial loss of the adult worm burden occurred, usually before day 25, in young rats infected with the higher larval doses, 2 experiments were carried out to compare this incomplete worm expulsion occurring in baby rats with that of similarly infected adults.

Experiment 3

A group of young and a group of adult rats were infected with 2,500 larvae. The young rats aged 19-22 days at infection originally numbered 25 but 5 of these died in the first 2-3 days after infection. The rats were therefore killed in groups of 4 on alternate days from 9 to 17 after infection.

The results of worm counts at autopsy (Table 22) show that the adult group had as expected lost the greater part of their worm burden between days 11-17 (regression coefficient (b) = -0.15 ± 0.02), the expulsion occurring at a rate of 1 $\log_{10}$ unit in 6.6 days. In contrast the worm burdens of the baby rat group remained relatively even over these days and the figure suggests that expulsion started only towards the end of this period.

TABLE 22

COMPARISON OF LP2 IN YOUNG AND ADULT RATS INFECTED WITH 2,500 LARVAE

Days after infection	Mean intestinal worm recovery ± standard deviation	
	Young rats	Adult rats
9	1520 ± 570	1315 ± 462
11	1400 ± 363	1275 ± 163
13	1160 ± 298	800 ± 335
15	1070 ± 256	491 ± 305
17	941 ± 607	223 ± 103

Although worm expulsion had not occurred by day 17 in the young rats, the 2 dose response experiments had shown that it was almost invariably complete by day 25 after infection. Consequently, a second experiment was carried out to obtain more information on the timing and rate of the expulsion phase in young rats.

Experiment 4

A group of 65 young rats infected with 2,500 larvae were autopsied in groups of 5 between days 10 and 22. The results are shown in Table 23.

Between these days the worm burden fell from a mean of 1498 ± 509 to a mean of 305 ± 149. This worm loss occurred very much more gradually than that in adults; because of this and the large standard deviation it is difficult to say precisely when worm expulsion began. This point is illustrated in Fig. 18. Regression analysis using the geometric mean worm burdens from days 15-22 inclusive shows that over this period expulsion occurred at the rate of 1 $\log_{10}$ unit in 12.5 days (regression coefficient (b) = -0.08 ± 0.007). This was almost exactly half the rate of the worm expulsion seen in the adult group infected with the same dose.

Discussion

When an adult rat is subjected to a single infection of 500 or more larvae a relatively fixed proportion, of the order of 50-60%, fails to become established in the intestine. This has been called Loss Phase 1

TABLE 23

INFECTION OF YOUNG RATS WITH 2,500 LARVAE. WORMS RECOVERED OVER THE LOSS PHASE 2 PERIOD

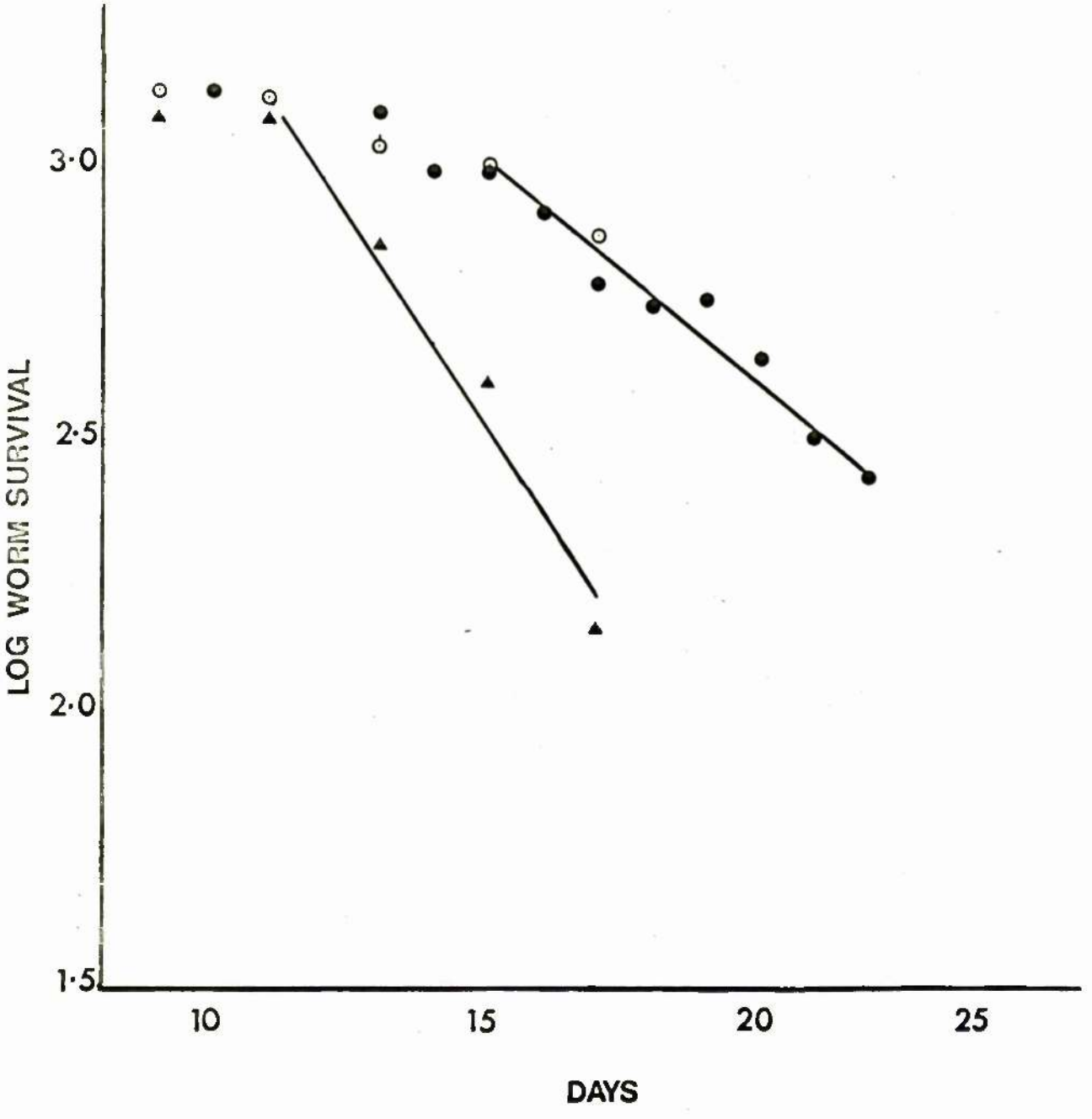
Days after infection	Eggs/gram of faeces	Mean worm recovery ± standard deviation
10	261,000	1498 ± 509
13	151,800	1340 ± 421
14	57,000	1060 ± 278
15	33,000	1064 ± 316
16	14,900	936 ± 317
17	12,000	780 ± 523
18	2,850	668 ± 347
19	3,750	658 ± 277
20	1,800	514 ± 211
21	2,100	358 ± 153
22		305 ± 149

Fig. 18. A comparison of the worm expulsion rates in young and adult rats infected with 2,500 larvae on day 0.

Adult rats ▲ Regression coefficient (b) = -0.15 ± 0.02

Young rats o Exp. 3

● Exp. 4 Regression coefficient (b) = -0.08 ± 0.00



(Section 1). The remainder become adult by day 5 and their number persists unchanged (the Plateau Phase) until about day 11 when the great majority are expelled exponentially at a measurable rate. This immunological reaction has been termed Loss Phase 2 although it is more generally referred to as the self-cure phenomenon. By day 20 only a small residual population in the region of 40 worms remains in the intestine (the Threshold Phase).

The response of young rats was found to be very different in that rats infected with 500 N. brasiliensis when under 6 weeks of age failed to undergo the Loss Phase 2 reaction. Instead the Plateau Phase persisted well into the adult life of the rat. Similar results have been subsequently reported by Ogilvie and Jones (1967) and Kassai and Aitken (1967) although the unresponsiveness seen in their breeds of rats was not as dramatic as that seen in our laboratory with hooded Lister rats. These observations raised 3 points. First the cause of the inability of the young rat to develop a normal immune response; secondly, the reason for its failure to develop even when the rat had become adult; thirdly, the subsequent response of such rats when subjected to reinfection. The experiments reported in part 1 of this section are primarily concerned with the first question.

The results of the dose response experiments confirmed that Loss Phase 2 did not occur in the group of rats which received 500 larvae, the Plateau of 100 worms persisting until the experiment was terminated on day 48. A partial Loss Phase 2 did occur in the rats of the other groups which were dosed with larger numbers of larvae, although in retrospect its intensity appears to be

related to the actual numbers of worms which established in the intestine rather than to the numbers of larvae administered. Thus in the first experiment a smaller number of adult worms and a less marked LP2 developed from a dose of 1,000 larvae than from a dose of 800 larvae in the second experiment.

When the infections from both experiments are arranged in order of size of the adult worm burden at days 10-12 (Table 24), the following pattern emerges. When the number of worms which becomes established from the infecting dose is around 250 or less Loss Phase 2 does not occur. If considerably more than 250 worms are established Loss Phase 2 occurs but this is incomplete compared with that which occurs in adults in that it terminates when the worm population falls to a mean of approximately 200. This has occurred in 4 out of 5 groups by day 25 after infection. As seen previously these adult worm infections then persist until at least day 50, thereafter declining to approximately 100 worms by day 70. This decline is possibly due to a senescence of the worms rather than to any specific immune effect.

It is thus apparent that a worm threshold of 200-250 operated in rats infected when immature in the sense that when less than this number of worms was present in the intestine the expulsion mechanism did not operate. This occurred irrespective of whether this figure had never been exceeded or whether it had dropped down to this threshold level as a result of Loss Phase 2. The situation is analogous to that of adult rats in which more

TABLE 24

SUMMARIZED RESULTS OF DOSE RESPONSE EXPERIMENTS IN YOUNG RATS WITH GROUPS ARRANGED IN ORDER OF SIZE OF INITIAL INTESTINAL INFECTION. MEAN NOS. WORMS IN INTESTINE

Plateau burden day 10-12	Days after infection		
	24-25	48-50	70
102	96	121	-
269	254	208	-
357	202	171	103
696	409	253	-
776	202	216	113
1273	233	226	107

than approximately 40 worms are required for self-cure to occur (Hurley, 1959) and in which a mean of 40 worms remains after LP2 is complete (Section 1). The fact that there are similarities between the thresholds of mature and immature rats is suggestive of a similar but quantitatively different mechanism for their operation.

Loss Phase 2 in rats infected when immature was also characterised by its relatively late onset around day 15 and by its slower rate (Fig. 18).

The explanation for the different sequence of events in rats infected when immature as compared to adult depends ultimately on an adequate explanation of the mechanism of Loss Phase 2.

It has been suggested (see Sections 1 and 3) that Loss Phase 2 could be a two-stage reaction depending, first, on the production of antiworm-antibody and secondly on its liberation into the intestinal lumen by an effector mechanism which may be activated on a threshold basis. It is therefore possible that either defective antibody production, or reduced sensitivity of the effector mechanism, or both, may explain the characteristic features of Loss Phase 2 in young rats.

On general grounds qualitative or quantitative defects in antibody production of the young rat are a possibility (see review by Good and Papermaster, 1964; Binaghi et al, 1966; Deichmuller and Dixon, 1960; Sterzyl and Trnka, 1957) and could explain the apparent requirement for a greater dose of antigen for Loss Phase 2 to occur in the young rat, and its delayed onset and slower rate.

Alternatively or additionally the effector mechanism may be deficient. We have shown that intestinal mast cells are present in large numbers at the onset of Loss Phase 2 in adult rats and have suggested that the action of worm allergen and reaginic antibody on these cells may constitute at least a part of the effector mechanism for the release of antiworm-antibody into the intestinal lumen (Jarrett et al, 1967b). The much smaller number of intestinal mast cells in the young rat may be of significance in this context. An effector mechanism which is quantitatively deficient may result in a reduced rate of worm expulsion and may require a higher number of worms for its activation.

The present results do not permit discrimination between these hypotheses; further experiments are in progress to attempt to clarify the situation.

Since even rats showing no evidence of protective antibody have high titres of reagin a few weeks after infection it is possible that the allergen is antigenically 'stronger' (Good and Papermaster, 1964) than the antigen or antigens involved in the induction of protective antibody or alternatively that the immunoglobulin class of the reagins can be synthesized earlier in the young animal. However, an interesting point is that by the 70th day after infection the reagin titre in these rats with an average worm burden of 100 worms has fallen to less than 1 in 8 in all cases. The implications of this finding will be discussed later in this section.

While the hypotheses discussed above may account for the reduced or absent protective response of immature rats they do not adequately explain the subsequent failure of these rats to develop the full reaction to the tolerated worm burden on reaching maturity. This point will be discussed in a later part.

Part 2 The immune status following infection at an early age

Introduction

Initially the experiments in young rats were designed to answer two questions: first, would the immune response of the young animal to a helminth infection differ from that of the adult, and secondly would such an early infection affect the animal's ability to respond to a subsequent infection at a later age?

Regarding the first question, it has been shown that the protective immune response of young rats is deficient when compared with that of adults. In young rats the extent of LP2, i.e., the self-cure reaction, is directly proportional to the size of the worm burden established during the Plateau Phase: when this is increased above a certain level an immune response does occur, but is diminished and incomplete. Following infection, and irrespective of dose, all young rats harbour a worm burden which in itself is numerically sufficient to stimulate expulsion in rats infected at an older age but this abnormally high burden is not eliminated even when the rats become adult. Since the type of unresponsiveness which has been found is thought to be unrelated to immunological tolerance and only during the initial part of the infection due to immunological immaturity, the mechanism of the prolonged unresponsiveness which allows the persistence of the original parasitic worm burden requires to be investigated.

The second question therefore becomes more complex. Groups of young rats which have shown a partial elimination response, or a nil response, during the primary infection are indistinguishable from each other on the basis of worm burden when adult. The reaction of both of these types of animals may be different.

With regard to the above considerations it seemed relevant to study two aspects of the immune status following baby infections.

First, the problem of the persisting primary infection merits the investigation of the antibody status of rats during this period. In other words, is the phenomenon due to absence or insufficiency of protective antibody, or alternatively is such antibody present but by some mechanism frustrated in its action. To test for the presence of antibody, passive protection tests were carried out using the sera of rats infected when young and bled some time after infection.

Secondly a more direct measurement of immunity to reinfection was obtained by challenge of the rats after they had reached adult size, with a second infection.

The results of these two methods of approach are presented separately and are summarised at the end of this section.

Materials and Methods

Passive protection tests

Two ways of assessing the passive protective effect of serum were used.

The first consisted of transferring by laparotomy a 10 day old adult worm population to adult rats which subsequently received the test serum intraperitoneally at a dose-weight ratio of 4 ml./100 g. Evidence of antibody action was assessed by a significantly decreased number of worms at autopsy 5 days later (Mulligan et al, 1965) in the rats receiving the test serum. Two control groups were used, one group receiving saline intraperitoneally and another receiving hyperimmune serum. This second control was included to ensure the effectiveness of the test system.

The second method was by intraperitoneal injection of the test serum into adult rats at the same dose-weight ratio on every second day, starting one day before a larval infection followed by autopsy and worm counts before day 12. Control groups as above were again included.

Sera

1. Baby infection serum was obtained from rats which had been infected when under 4 weeks of age. The infection of these serum production rats was followed by egg counts and worm counts were done when the rats were bled out. The rats were bled except where otherwise stated on day 50 after infection. At this time they weighed between 100-160 g.

2. Hyperimmune serum was prepared in adult rats subjected to a series of infections of increasing size as described in the Materials and Methods section.

The technique of adult worm transplant and all other methods used in this part are described in the Materials and Methods section.

1. Passive protection tests using serum from rats infected when young

Results

Three experiments of this kind were carried out. In one of these experiments the passively immunized rats were infected with adult worms and in the other two with larvae.

Experiment 1

The test serum was taken from 41 rats which had been infected at the age of 8-12 days with 800 larvae. The rats were bled on day 50 when their mean worm burden was 157 ± 120.

Three groups of 10 rats were infected by laparotomy with 1,000 adult worms per rat. The test serum was injected 24 hours after laparotomy into each rat of group 1, groups 2 and 3 receiving hyperimmune serum and saline respectively.

The results of egg counts during the first four days and worm counts on day 5 after infection are shown in Table 25. It will be seen that the saline control group at this time harboured a mean of 620 worms while in the group injected with hyperimmune serum a mean of only 353 worms survived. The group injected with the baby infection serum harboured a mean burden of 409 worms. A test of significance (Student's t test, Snedecor, 1965) showed that whereas the difference between the saline and hyperimmune serum injected groups was significant ($p < 0.05$) that between the saline group and the baby infection serum group was not ($p = 0.1$).

TABLE 25

AN ATTEMPT TO ASSESS THE ANTIBODY CONTENT OF BABY INFECTION SERUM USING A PASSIVE PROTECTION TEST IN RATS TRANSPLANTED WITH AN ADULT WORM INFECTION. EXPERIMENT 1.

	Days after infection	Group 1 Baby infection serum	Group 2 Hyperimmune serum	Group 3 Saline
Eggs	2	43,900	35,100	56,400
per	3	45,200	32,100	30,900
gram	4	34,800	10,000	21,700
Worms in intestine	5	311	402	224
		172	412	561
		183	455	398
		670	831	28
		505	156	774
		384	597	756
		489	69	879
		552	171	796
			314	1142
			129	648
	Mean	409	353	620
	S.D.	180	238	329
	t test	Group 1 and 3	$p = 0.1$	
		Group 2 and 3	$p < 0.05$	

Since it was felt that the difference between the saline control and the group injected with baby serum was suggestive even if not significant, an attempt was then made to improve on the passive protection routine by using the second of the two techniques outlined in the Materials and Methods.

Experiment 2

The baby infection serum for this experiment was obtained from 52 rats on the 50th day after infection. The rats were infected when under 4 weeks of age with 500 larvae. The mean burden and standard deviation at autopsy on day 50 was 179 ± 106 worms.

Three groups of rats were infected with 1000 N. brasiliensis larvae. One of these groups was injected with the test baby infection serum intraperitoneally at the dose rate of 4 ml./100 g. on the day before infection, the day of infection and subsequently on days 2, 4 and 7 after infection. The second group received hyperimmune serum according to the same schedule and the third group received no serum. Since each rat was given a large total amount of serum (approx. 30 ml.) the group sizes were of necessity limited to 4 rats.

Table 26 shows the results of egg counts after infection, and worm counts on day 8 after infection when all the rats were autopsied. There was no evidence of antibody action on the group injected with baby infection serum, since the mean worm count figures for this group and the uninjected control group were similar. The group injected with the hyperimmune serum

TABLE 26

AN ATTEMPT TO ASSESS THE ANTIBODY CONTENT OF BABY INFECTION SERUM USING A PASSIVE PROTECTION TEST IN RATS INFECTED WITH LARVAE. EXPERIMENT 2.

	Days after infection	Group 1 Baby infection serum	Group 2 Hyperimmune serum	Group 3 No serum
Eggs	6	8,200	0	2,100
per	7	23,600	750	26,100
gram	8	19,700	0	20,600
Worms	8	427	80	342
in		506	305	457
intestine		155	62	301
		156	73	372
	Mean	311	130	368
	S.D.	182	117	66
	t test	Group 2 and 3	$p < 0.02$	

on the other hand showed a significant decrease in worm burden ($p < 0.02$) when compared with the control: other signs of the action of the hyperimmune serum were the very low egg count of this group and marked stunting of the worms recovered at autopsy.

The baby infection sera used so far in these experiments had been taken from rats which received low larval doses, i.e., 500-800 larvae, doses which have been shown to cause little if any worm expulsion in the infected baby rats. Since higher larval doses than these do result in a measure of worm loss implying the presence of antibody, it was decided to repeat the above experiment using serum from rats which had shown some reaction.

Experiment 3

A pool of serum which had been taken from the rats of all groups in the baby dose response experiments on days 24-25 and 48-50 was available; this serum was used although it was not considered ideal for the purpose since it contained serum from several groups some of which had not shown a marked LP2.

The serum was injected into a group of 3 rats at the same dose ratio and on the same days as in the previous experiment but in addition on day 10 after infection. A control group of 3 rats received no serum. The rats were infected with 1000 larvae and autopsied on day 11 after infection.

The results are shown in Table 27. It will be seen that on this occasion the baby infection serum has conferred a significant measure of

TABLE 27

AN ATTEMPT TO ASSESS THE ANTIBODY CONTENT OF BABY INFECTION SERUM USING A PASSIVE PROTECTION TEST IN RATS INFECTED WITH LARVAE. EXPERIMENT 3.

	Days after infection	Group 1 Baby infection serum	Group 2 No serum
	6	350	16,250
Eggs	7	48,000	28,700
per	9	3,500	42,900
gram	10	2,400	31,500
	11	1,700	25,600
Worms	11	180	387
in		91	512
intestine		155	273
	Mean	142	390
	S.D.	46	120
	t test	$0.05 > p > 0.02$	

protection ($p < 0.05$) when compared with the non-infected controls. This result is substantiated by the marked difference in the egg count between the groups.

Discussion

In the three experiments just described, the serum from rats infected when young was tested for the presence of protective antibody by passive protection techniques. The results have shown that protective antibody is present in the serum of groups of rats infected when young with more than 800 larvae, but have failed to reveal antibody activity in rats infected with lesser doses. However, it is considered that a negative result with this technique, because of its relative insensitivity, cannot be interpreted as indicating the complete absence of antibody.

One of the main objectives of the passive protection test approach has therefore not been fully realised. The question of whether or not the 500-800 larvae infection rats do produce antibody is of importance in the consideration of mechanisms of unresponsiveness. The possibilities are several: First, this amount of antigen may be sub-threshold for the production of antibody in the immature rat and young rats infected with such doses of larvae may in fact produce no antibody. Secondly, a small amount of antibody may be produced but be of insufficient titre to exert an effect on the worms. Thirdly, it is possible that sufficient antibody, potentially capable of expelling worms, is synthesized, but cannot exert its action for one of several reasons. For instance, the amount of antibody

synthesized or antigen released may be insufficient to trigger an antibody release mechanism. Although the intestinal mast cell has not as yet been definitely implicated in this release mechanism, it is of interest that less than half the number of mast cells seen in adult rats are present in young animals.

Finally, it has been shown that in young animals in general IgM production precedes IgG production by several weeks (Good and Papermaster, 1964), and Binaghi et al (1966) have demonstrated that in 2-3 week old rats anaphylactic antibody can be produced at a time when the precipitin producing capacity is still deficient. This being so, it is possible that in the young rat infected with Nippostrongylus, antiworm-antibodies of a different class from that which normally operates are preferentially produced. Such antibody might actually block the action of small amounts of protective antibody synthesized. It is impossible on present evidence to discriminate between these various possibilities.

2. The Challenge of Rats Infected When Young

Results

Challenge Experiment 1

Two groups of young rats and 2 groups of adult rats received a primary infection as follows:

Group	No. of rats	Age of Rat	Dose of larvae
1	10	Adult	3,200
2	10	Adult	1,600
3	19	2-4 weeks	3,200
4	16	2-4 weeks	1,600

Egg counts of the infection were carried out at intervals after day 10 during the primary infection and the results (Table 28) show that in the young rats the usual prolonged egg count occurred. This tended to remain uniformly higher in the lower dose group but after day 50 fell in both groups. The egg count of the young rats infected with 3,200 larvae fell to a very low level between days 19 and 25 after infection with a subsequent rise. Attention is drawn to a similar finding in the previous part (see Dose Response Experiment 2) but the significance of this finding is not yet known. The egg count of both adult groups although unusually low followed the expected pattern and had fallen to zero by day 19.

On day 70 after infection, 4 rats were autopsied from each of the groups infected as babies to determine the worm burden remaining from the primary infection. These worm burdens for groups 3 and 4 were 155 ± 58 and 105 ± 77 respectively. At this time the rats weighed between 150 and 210 grams. On the same day the remaining rats in all 4 groups were challenged with 1,000 larvae and another group of 10 adult rats (Group 5) which had not been previously infected, was given the same dose. The rats were killed in 2 groups on days 8 and 23 after infection.

TABLE 28

CHALLENGE EXPERIMENT 1. EGG COUNT OF THE PRIMARY INFECTION OF YOUNG AND ADULT RATS

Day of infection	Eggs/gram			
	1,600 larvae		3,200 larvae	
	Young rats	Adult rats	Young rats	Adult rats
10	16,500	8,200	26,700	7,500
11	20,100	3,600	13,100	8,650
12	12,000	1,350	5,500	3,200
13	7,000	250	2,550	350
14	5,700	100	1,800	100
18	3,000	0	4,800	0
19	4,500	0	0	0
20	5,200	0	0	0
21	3,750	0	200	0
25	13,240	0	100	0
31	4,900		1,800	
35	4,450		8,400	
41	1,500		400	
45	2,550		6,050	
50	200		50	
57	100		50	
68	300		850	
71	1,000		50	

The egg counts after the challenge infection are shown in Table 29. The highest count occurred in the control group 5 which received the 1,000 larvae as a primary infection. In contrast, all the other groups which had been previously infected and were challenged with 1,000 larvae had depressed egg counts. This was least marked in group 4, i.e., the rats previously infected with the lower dose of larvae when under four weeks of age.

The mean worm counts at autopsy are shown in Table 30. On day 8 after infection, the take (i.e., plateau) from a dose of 1,000 larvae in the previously uninfected rats (group 5) was 416 ± 123 worms. In both groups of rats which had been first infected when adult a combination of increased Loss Phase 1 and advanced Loss Phase 2 had resulted in worm loss to the threshold level by day 8. This was also the case for the group of rats which had been initially infected with 3,200 larvae when under 4 weeks of age (group 3). However, the group infected with 1,600 larvae at under 4 weeks (group 4) had a similar number of worms on day 8 after challenge to the group of control rats which had not been previously infected (group 5). It must be remembered however that approximately 100 worms per rat presumably remained in this group from the original infection. Nevertheless it would seem in this group, as in the control group, that Loss Phase 2 had not occurred before day 8. On day 23 after challenge the worm burdens of all the groups had fallen to low threshold values.

TABLE 29

CHALLENGE EXPERIMENT 1. EGG COUNT AFTER THE CHALLENGE INFECTION

Day of challenge infection	Eggs/gram				
	Group 1	Group 2	Group 3	Group 4	Group 5
6	300	0	300	2,400	15,500
7	1,050	200	450	4,000	23,350
8	450	300	500	1,250	34,800
9	150	150	410	300	26,300
12	0	0	0	0	6,800
13	0	0	350	50	1,850

TABLE 30

CHALLENGE EXPERIMENT 1. MEAN NUMBERS OF ADULT WORMS IN DIFFERENT GROUPS OF RATS CHALLENGED WITH 1000 LARVAE AND AUTOPSIED 8 AND 23 DAYS LATER

Group treatment before challenge		Numbers of worms after challenge ± S.D.	
		8 days	23 days
Group 1	3200 larvae when adult	6 ± 4	7 ± 7
Group 2	1600 larvae when adult	58 ± 102	3 ± 3
Group 3	3200 larvae when 2-4 weeks old	23 ± 14	46 ± 34
Group 4	1600 larvae when 2-4 weeks old	478 ± 275	17 ± 27
Group 5	None	416 ± 123	34 ± 36

From these results it seems that rats infected at an early age may respond in adult life to a subsequent challenge infection in one of two ways. They may show a secondary response, as do adult rats which have been previously infected, which is manifest by worm expulsion occurring before day 8 (see Section 1). Alternatively they may respond in the same way as do normal adult rats which have not previously experienced the antigen, i.e., by worm expulsion occurring between days 12-20. The type of response is determined by the size of the initial infection, the secondary response occurring in the group of rats which had previously been infected with a high dose of larvae and the primary response in rats previously infected with a lower dose. The worm count on day 23 shows that the rats have lost not only the challenge but also the residual primary worms. These results are confirmed in the next experiments.

Challenge Experiment 2

In this infection the primary infection consisted of 2,000 larvae. Two groups of rats were set up as follows:

Group 1. 40 rats aged 19-22 days given 2,000 larvae/rat

Group 2. 28 rats aged 19-22 days not infected.

It was considered that the response of adult rats to a second infection is sufficiently well documented so an adult group was not infected at this time. The egg count of the primary infection followed the usual pattern (Table 31).

TABLE 31

CHALLENGE EXPERIMENT 2. EGG COUNT OF THE PRIMARY INFECTION OF YOUNG RATS WITH 2000 LARVAE, AND OF THE CHALLENGE INFECTION WITH 1000 LARVAE

	Eggs/gram	
Day of primary infection	Group 1 2000 L.	Group 2 Challenge control
10	24,900	
12	10,400	
14	9,500	
16	2,000	
18	800	
20	2,350	
24	2,100	
28	3,800	
32	5,500	
36	2,150	
40	2,500	
44	2,950	
49- Thibenzole	1,250	
50	0	
54- Both groups infected with 1000 larvae		
Day of challenge infection		
6	2,000	14,700
7	900	12,600
8	850	17,000
12	100	2,700
13	0	800
15	0	0

On day 49 after infection 3 rats from group 1 were killed to determine the worm burden from the primary infection. The mean burden and standard deviation was 243 ± 163 worms; this confirmed the expected state of unresponsiveness to this infection. The remaining rats of group 1 were treated by stomach tube with thibenzole at a dose rate of 40 mg. per 100 g., it having been previously established that this dose is effective in eliminating virtually the entire intestinal worm burden. Egg counts after thibenzole treatment fell to zero.

On day 54 after the primary infection both groups of rats, now weighing between 120-150 g., were infected with 1,000 larvae. The rats were killed in groups of seven on days 5, 9, 15 and 20 after the challenge infection.

The egg counts of the challenge infection seen in Table 31 show that egg output was depressed in the previously infected rats. The worm counts are shown in Table 32. On day 5 the number of worms in both groups was very similar. This shows that there had not been an increase in Loss Phase 1 in the previously infected rats in comparison with the rats infected for the first time. On day 9 the worm count of group 1 is reduced. This reduction in worm burden is not significant but suggests that Loss Phase 2 has started in the former group and that a proportion of the worm burden has already been expelled. On days 15 and 20 the worm burdens of both groups are decreasing, the rather high mean seen on day 15 in group 1 being contributed to by one high worm count in this group (see Appendix).

TABLE 32

CHALLENGE EXPERIMENT 2. MEAN NUMBERS OF ADULT WORMS IN RATS CHALLENGED WITH 1000 LARVAE

Group treatment before challenge	Number of worms after challenge ± S.D.			
	day 5	day 9	day 15	day 20
Group 1. 2000 larvae when 19-22 days old	370 ± 211	165 ± 81	81 ± 142	15 ± 28
Group 2. None	320 ± 185	288 ± 232	29 ± 38	3 ± 3

This result is in harmony with that of the previous experiment where it was seen that young rats infected with 3,200 larvae underwent a secondary type of response and those infected with 1,600 larvae a primary response. In this experiment the primary dose of 2,000 larvae lay between those used in the previous experiment. As a reflection of this it seems that the rats on challenge gave a response midway between the two seen in the first experiment. Thus worm expulsion started at some time after day 5 but was not complete by day 9. It was complete in the majority of rats by day 15. From the egg output of these rats there was also evidence of enhanced immunity to the challenge infection.

Challenge Experiment 3

The effect of the size of the primary infection of baby rats in determining the response to the challenge infection has been established. In the previous experiments rats were challenged with 1,000 larvae. In this experiment baby rats infected with 500 larvae were subsequently challenged with the same dose after receiving thibenzole treatment to eliminate the primary infection burden. It was thought that this small primary infection, followed by an equally small challenge might allow uninterrupted expression of the unresponsiveness occurring to the primary infection.

Two groups of rats aged 1-3 weeks were set up as follows:

Group 1. 50 rats given 500 larvae each

Group 2. 25 rats not infected.

Following large litter mortalities only 31 rats remained in group 1 and 17 in group 2 at the time of challenge. On day 50 after infection the rats still weighed only on average 80-95 g. so the challenge infection was delayed until day 79 when the rats weighed between 140-200 g. On day 75 the rats of group 1 were treated with thibenzole at the same dose-weight ratio as in the previous experiment. Four rats from this group were killed and had a mean burden and standard deviation of 96 ± 18 worms. Following the challenge infection of 500 larvae the rats of both groups were autopsied in groups of 5 or 7 on days 5, 8 and 15 after infection. The results of the egg count of the primary and the challenge infection are shown in Table 33. It will be seen that after challenge the egg count of the previously infected rats was depressed in comparison with that of the control group. The worm counts (Table 34) show that in both the previously infected group and the controls worm expulsion occurred between days 8 and 15. The previously infected rats have thus undergone a typical primary response as manifest by worm expulsion although the depressed egg count of this group gives evidence of a degree of enhanced immunity. There is no evidence of the continuation of the unresponsive state to the challenge worm burden in that on day 15 after infection a mean of 10 worms only remained in the small intestine.

Challenge Experiment 4

The evidence of the previous experiment strongly suggests an antigenic difference between the old and the new worm populations, although it might be

TABLE 33

CHALLENGE EXPERIMENT 3. EGG COUNT OF THE PRIMARY INFECTION OF YOUNG RATS WITH 500 LARVAE, AND OF THE SUBSEQUENT CHALLENGE INFECTION WITH 500 LARVAE

Day of primary infection	Eggs/gram	
	Group 1 500 larvae	Group 2 Challenge control
10	15,900	
15	6,150	
20	450	
25	1,500	
30	5,100	
35	6,300	
40	2,800	
45	4,800	
50	4,600	
55	4,200	
60	1,300	
65	3,300	
70	600	
75 - Thibenzole		
79 - Both groups infected with 500 larvae		
Day of challenge infection		
7	2,500	14,700
8	1,200	13,900
10	6,300	19,500
11	1,550	12,600
12	400	6,500
13	0	350
15	0	0

TABLE 34

CHALLENGE EXPERIMENT 3. MEAN NUMBERS OF ADULT WORMS IN RATS CHALLENGED WITH 500 LARVAE

Group treatment before challenge	Numbers of worms after challenge ± S.D. day 5	day 8	day 15
Group 1. 500 larvae when under 4 weeks old	203 ± 91	181 ± 61	10 ± 16
Group 2. None	153 ± 101	172 ± 100	31 ± 11

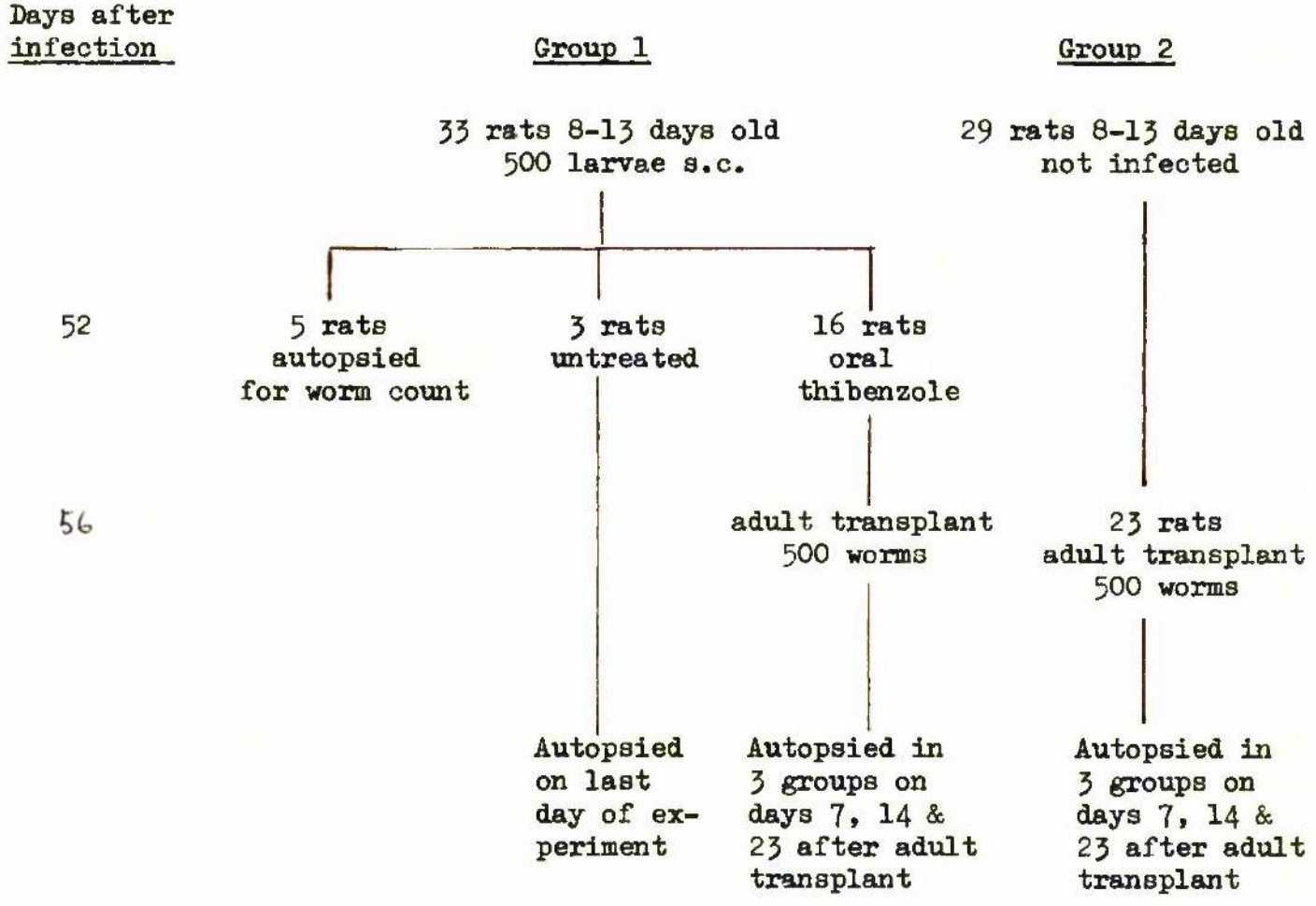
argued that it is the experience of the larval antigens that has provoked worm elimination in the previously unresponsive rats. This experiment was designed to effect the substitution of a new, surgically transplanted, worm burden for the already existing one in unresponsive rats. By this method stimulation with larval antigens would be avoided. A plan of the experiment is shown in Fig. 19.

Eight litters aged between 8-13 days were divided into two groups and the rats of one group were infected with 500 N. brasiliensis larvae each (group 1). The other group remained uninfected (group 2). The egg count of group 1 after infection was typical for baby rat infections. On day 52 after infection when both groups of rats weighed between 110-150 g. 17 of the infected rats were treated with thibenzole at the usual dose rate. Egg counts after thibenzole treatment were negative. Five rats of this group 1 were autopsied on this day to establish the existing worm burden. This was 173 ± 104 worms. A remaining 3 rats were left untreated to serve as residual infection controls on the last autopsy day. Four days after thibenzole treatment the rats of both groups were given an adult transplant of 500 worms. This dose was calculated to give rise to a worm burden numerically similar to that previously present in the thibenzole treated rats. The egg counts of both groups fell to low levels by day 7 after reinfection with that of group 1 rats falling slightly more rapidly.

The rats were autopsied in groups of 5 or more on days 7, 14 and 21 to establish whether self-cure was premature, normal or absent.

FIG. 19

TRANSPLANTATION OF AN ADULT WORM POPULATION TO UNRESPONSIVE RATS. PLAN OF EXPERIMENT



The results shown in Table 35 were somewhat unexpected in that although self-cure occurred in the previously unresponsive rats this was apparently delayed in comparison to the previously uninfected rats, since the figures for the day 14 worm counts are significantly different ($p < 0.05$). In both groups self-cure was complete by day 21 after infection. On this day (i.e., day 79 of the original infection) the 3 remaining residual control rats were autopsied and found to have a burden of 73 ± 25 worms. This was still markedly higher than these rats which received the substitute infection.

Discussion

In these experiments it has been shown that when rats, which have been infected when immature, are challenged when adult several types of response may occur. The response may be of a primary or secondary nature, or a reaction intermediate between the two, and appears to depend on the size of the original infection.

In experiment 1, young rats which had received a primary infection of 3000 larvae underwent Loss Phase 2 in the manner of a secondary response following challenge infection. In contrast in the rats initially infected with 1,600 larvae Loss Phase 2 occurred between days 8-23 during which period the previously uninfected controls also self-cured. In this experiment not only the challenge infection but also the residual unresponsive infection was expelled during the reaction.

In the subsequent experiments the original residual infection was eliminated

TABLE 35

THE FATE OF A TRANSPLANTED ADULT WORM INFECTION IN UNRESPONSIVE RATS AFTER REMOVAL OF THE ORIGINAL POPULATION WITH THIBENZOLE

Days after transplant	Infected and thibenzole treated rats	Previously uninfected rats
7	146 ± 131	119 ± 85
14	111 ± 64	28 ± 25
21	14 ± 12	18 ± 21

with thibenzole to provide an uncomplicated picture of the challenge infection kinetics. In the second experiment a primary infection dose intermediate between the two of the first experiment was used and an intermediate type of response also occurred on challenge. Thus worm expulsion was a little advanced in comparison with the control primary infection but did not occur at the rate of a secondary infection.

It might be speculated from these results that reduction of the dose of the primary infection to minimum size and subsequent challenge with an equally small dose might allow uninterrupted expression of the state of unresponsiveness. In experiment 3, in which rats were both infected and challenged with 500 larvae, this was not found to be the case; Loss Phase 2 again occurred in the manner of a primary infection. However even in this group a low degree of pre-existing immunity is evidenced by the depressed egg output of the experimental group.

In experiment 4 in which a transplanted adult infection was substituted for the original unresponsive infection self-cure again occurred more or less in the manner of a primary response, but possibly was slightly delayed.

These results have given no real evidence of a state of unresponsiveness or immunological tolerance following the challenge infection. Kassai and Aitken (1967) have reported the induction of immunological tolerance to Nippostrongylus in rats of the albino Wistar strain. Several points arising from their work are worthy of comment. First, in one experiment in which young rats were given a primary infection (experiment 1) they report the demonstration of tolerance in one experimental group. The mean difference of this from the control group is however only 15 worms, which in our estimation

is not a significant difference. In any case the worm burden of all the groups was at the expected level for the adult Threshold Phase (i.e., approximately 40 worms). In another experiment (experiment 3) a state of tolerance is claimed following a single or double reinfection in young rats. The time of challenge was always in the region of 6-7 weeks, and while it is difficult to draw comparisons between different strains of rats it is possible that the worm burdens which these authors attribute to induced tolerance are in fact a manifestation of infection during the original unresponsive period. Alternatively it may be found that the time between the original and challenge infections is critical for the continuance of the unresponsive state. This possibility is being investigated. Finally, these authors attribute the failure of previous workers to induce tolerance in parasitic systems to the use of neonatal infections which were too small. From the results shown in Part 2 of this section this assumption at least as far as concerns Nippostrongylus is seen to be incorrect, since larger primary infections evoke an immune response whereas small ones do not.

The results of this part confirm those of the previous part in which it was shown that baby rats infected with a sufficiently large dose of larvae did develop an immune response although this was inferior to that of similarly infected adult rats. In addition, the results of the challenge experiments show that such rats develop immunological memory of the N. brasiliensis antigen as evidenced by their more rapid response to a challenge infection. In contrast a dose of larvae which is subthreshold for the development of the immune

response in young life does not evoke a memory response to subsequent challenge. Conversely there is no evidence that it has led to a continuous state of unresponsiveness to the relevant antigens, since a challenge infection is dealt with not less efficiently than by an animal infected for the first time. A possible exception to this was seen in experiment 4.

It is almost certain that several factors are interacting to produce the complex of phenomena observed after infection or reinfection of young rats with N. brasiliensis. The possible factors involved in the initial immunological immaturity which results in the failure to self-cure after the first infection have already been discussed. It was considered most likely that either qualitative or quantitative defects in antibody production, or a deficient effector mechanism or a combination of both was responsible. This, however, did not explain the failure to eliminate the original 200 worm burden when the rats subsequently became immunologically mature. The demonstration of the effectiveness of substituting the old unresponsive infection for a new but not larger infection leads to the interesting speculation that there is an antigenic difference between the two worm populations. This would mean that the old population of worms, not recognised in early life due to immaturity, is not subsequently recognised as the rat matures due to the loss of an essential antigenic component. This may be introduced with the new infection as it were for the first time.

It is also possible that self-cure after reinfection in experiments 3

and 4 was at least partly due to the recovery of a depleted cellular response during the five days' period between anthelmintic treatment and the reinfection. This latter factor might also explain in experiment 4 the delay in self-cure of the reinfected group when compared with previously uninfected controls.

Research in this field is still continuing in our laboratory at the time of writing, and it is difficult at present to be more precise about the mechanism of action of this interesting manifestation of unresponsiveness.

SUMMARY

Section 3 reports a study of N. brasiliensis infection in young rats. Preliminary experiments indicated that if rats were infected when under 6 weeks of age with 500 larvae they did not undergo during the second week the characteristic self-cure reaction (LP2) that is a feature of the response of adult rats. Instead these animals underwent a prolonged infection that extended well into adult life and which cannot therefore be attributed solely to immunological immaturity.

The pattern of primary N. brasiliensis infections of different sizes was followed in young rats. It is shown in these dose response experiments that when the number of worms which becomes established from the infecting dose is around 250 or less worm expulsion as seen in adult rats does not occur. If considerably more than 250 worms are established, worm expulsion occurs but is incomplete in comparison with that of adult rats in that it starts later, proceeds at a slower rate, and terminates when the worm population falls to a mean of approximately 200. These worm infections then persist well into adult life of the rat.

Using passive protection tests it was shown that the serum of rats infected when young with the higher doses of larvae contains protective antibodies. Such antibodies could not be demonstrated in the serum from rats infected with low doses of larvae.

When rats which have been infected when immature are challenged when

adult, the response may be of a primary or secondary nature or a reaction intermediate between the two and appears to depend on the size of the original infection. During the reaction to the challenge infection the previously tolerated worm burden is also expelled.

Attempts are made to interpret these facts in the light of current information on immunological unresponsiveness and the self-cure reaction.

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APPENDICES

APPENDIX 1 (Table 2)

FIRST INFECTION (A) WITH 3000 LARVAE

WORMS RECOVERED FROM THE LUNGS AND SMALL INTESTINE OVER THE LOSS PHASE 1 AND PLATEAU PERIODS

	Days after infection										
	1	2	3	4	5	6	7	8	9	10	20
Larvae recovered from lungs	578	367	23	10						0	
	761	57	21	0						8	
	910	286	0	0						2	
	364	572	34	11						0	
	1470	147	22	30						0	
Mean	817	286	20	10						2	
S.D.	419	200	12	12							
Worms recovered from intestine	0	886	710	1900	873	1370	1410	1520	1612	870	69
	0	680	1482	1310	616	1590	1030	1260	1720	1450	19
	0	449	1463	726	1100	906	990	1850	1680	1080	36
	0	287	870	1120	1130	1443	1060	1380	640	980	18
	0	1018	1826	830	960	830	1260	1160	1160	660	59
Mean	0	664	1270	1177	936	1228	1150	1434	1362	1008	40
S.D.		301	465	465	207	339	179	269	407	291	

APPENDIX 2 (Table 3)

FIRST INFECTION (B) WITH 3000 LARVAE

WORMS RECOVERED FROM THE SMALL INTESTINE OVER THE LOSS PHASE 2 PERIOD

	Days after infection									
	9	10	11	12	13	14	15	16	17	18
Worms Recovered	2070	2390	1210	1800	1690	810	484	213	97	6
	2650	2120	1070	510	820	1400	59	10	4	141
	1730	1430	1600	920	680	850	74	21	11	78
	2150	1950	1570	770	221	101	1260	109	182	36
	2050	1890	1090	165	1115	288	685	44	61	79
	1490	1880	1850	1390	700	1305	290	45	56	4
	2300	2130	1320	865	640	135	1050	248	21	53
	2000	1980	1460	980	1930	1080	1110	660	98	34
	1150	1760	1420	1000	1590	950	95	1160	980	80
	1700	2020	1640	680	1810	670	27	1090	801	42
Mean	1724	1955	1423	908	1119	758	513	360	231	55
S.D.	151	253	253	450	494	461	482	447	354	41
Geometric Mean ($\log_{10}$)	3.27	3.29	3.15	2.89	2.98	2.75	2.42	2.13	1.86	1.57
S.D.	0.10	0.32	0.08	0.36	0.28	0.40	0.61	0.62	0.75	0.49

APPENDIX 3 (Table 5)

SECOND INFECTION (A) WITH 3000 LARVAE

WORMS RECOVERED FROM THE SMALL INTESTINE OVER THE LOSS PHASE 2 PERIOD

	Days after infection						
	4	5	6	7	8	9	10
Worms Recovered	625	570	164	435	446	171	40
	410	328	191	84	24	98	4
	920	780	853	62	18	47	41
	603	850	404	137	247	7	75
Mean	639	632	403	179	183	81	40
S.D.	211	235	319	173	205	71	29
Geometric Mean ($\log_{10}$)	2.79	2.77	2.51	2.12	1.92	1.69	1.42
S.D.	0.19	0.18	0.33	0.37	0.70	0.56	0.57

APPENDIX 4 (Table 6)

SECOND INFECTION (B) WITH 3000 LARVAE

WORMS RECOVERED FROM THE LUNGS AND SMALL INTESTINE OVER THE LOSS PHASE 1, PLATEAU AND LOSS PHASE 2 PERIODS

	Days after infection										
	1	2	3	4	5	6	7	8	9	10	20
Larvae recovered from lungs	480	300	46	0	0						
	598	297	53	6	0						
	250	544	100	4	6						
	390	256	50	0	0						
	473	498	31	0	0						
Mean	438	379	56	2	1						
S.D.	129	132	26	3							
Worms recovered from guts	16	164	1100	1060	435	288	327	294	77	32	30
	0	212	1140	1620	1325	625	93	140	0	12	65
	29	192	160	1680	934	793	115	86	10	69	26
	73	126	1100	1400	1420	175	504	36	64	41	17
	153	354	2100	860	680	231	113	1092	0	0	46
Mean	54	210	1120	1324	959	422	230	329	30	31	37
S.D.	62	87	686	352	419	271	108	436	38	27	
Geometric Mean (log_{10})	1.34	2.29	2.93	3.11	2.94	2.55	2.25	2.28	0.94	1.20	
S.D.	0.84	0.28	0.42	0.13	0.19	0.28	0.33	0.57	0.93	0.71	

APPENDIX 5 (Table 8)

THIRD INFECTION (A) WITH 3000 LARVAE

WORMS RECOVERED FROM THE LUNGS AND SMALL INTESTINE OVER THE LOSS PHASE 1, PLATEAU AND LOSS PHASE 2 PERIODS

	Days after infection									
	1	2	3	4	5	6	7	8	9	10
Larvae recovered from lungs	437	79	23	35						0
	529	192	25	33						0
	70	244	0	29						13
	280	246	14	62						0
	296	184	21	0						0
Mean	322	189	17	32						3
S.D.	175	68	10	22						5
Worms recovered from guts	3	320	396	453	32	9	5			59
	158	271	312	468	124	69	22			8
	2	318	674	513	473	32	57			10
	129	773	321	945	583	85	33			26
	42	149	169	348	37	17	78			23
Mean	67	366	374	534	250	42	39			21
S.D.	73	238	187	232	259	33	29			21
Geometric Mean ($\log_{10}$)	1.34	2.50	2.53	2.71	2.32	1.49	1.44			1.29
S.D.	0.71	0.26	0.22	0.17	0.54	0.37	0.46			0.35

APPENDIX 6 (Table 9)

THIRD INFECTION (B) WITH 3000 LARVAE

WORMS RECOVERED FROM THE LUNGS AND SMALL INTESTINE OVER THE LOSS PHASE 1, PLATEAU AND LOSS PHASE 2 PERIODS

	Days after infection							
	1	2	3	4	5	6	7	8
Larvae recovered from lungs	108 45 270 151 279	697 223 119 198 175	16 145 11 133 168	20 60 24 44 57			10 8 16 8	
Mean	171	282	95	41			11	
S.D.	102	235	72	18			4	
Worms recovered from guts	19 5 257 97 112	261 210 320 230 120	258 318 170 829 996	749 290 1073 108 973	436 204 704 726 1120	257 498 269 271 265	197 272 72 18	50 59 17
Mean	98	228	514	639	638	312	140	42
S.D.	100	73	372	423	344	104	100	22
Geometric Mean (log_{10})	1.68	2.34	2.61	2.68	2.75	2.48	1.96	1.57
S.D.	0.48	0.15	0.33	0.43	0.29	0.12	0.6	

APPENDIX 7 (Table 19)

DOSE RESPONSE IN YOUNG RATS (EXP. 1). WORMS RECOVERED FROM INTESTINES AT INTERVALS AFTER INFECTION WITH A SINGLE DOSE OF LARVAE

Dose	500 larvae			1000 larvae			2000 larvae		
Days	12	24	48	12	24	48	12	24	48
	103	179	118	444	515	443	1201	1230	404
	105	158	127	405	327	176	858	580	337
	125	61	150	282	304	350	790	510	335
	118	119	163	279	300	218	402	408	317
	84	40	208	575	262	108	104	350	218
	83	86	94	338	210	279	770	235	211
	64	153	70	129	156	208	860	205	279
	49	42	74	104	127	170	500	179	176
	129	10	138	62	88	157	820	130	189
	159	111	69	77	252	111	644	264	178
						72			155
									234
Mean	102	96	121	269	254	208	696	409	253
S.D.	38	57	46	175	122	111	369	323	80
Geometric Mean ($\log_{10}$)	2.01	1.98	2.08	2.43	2.40	2.32	2.84	2.61	2.40

APPENDIX 8 (Table 21)

DOSE RESPONSE IN YOUNG RATS (EXP. 2). WORMS RECOVERED FROM INTESTINES AT INTERVALS AFTER INFECTION WITH A SINGLE DOSE OF LARVAE

Dose	800 larvae				1,600 larvae				3,200 larvae			
Days	10	25	50	70	10	25	50	70	10	25	50	70
	553	9	124	22	980	100	198	186	1900	260	192	269
	355	229	122	179	2050	208	170	195	1060	184	305	265
	342	213	221	144	890	168	355	59	1340	290	264	117
	416	198	212	203	650	370	153	111	1070	232	105	60
	451	212	102	13	735	244	202	16	1400	162	265	36
	28	354	245	67	353	121			873	269		83
			72									58
			59									34
			58									41
			198									108
			75									
			146									
Mean	357	202	171	103	776	202	216	113	1273	233	226	107
S.D.	178	110	62	67	255	98	81	78	363	50	79	89
Geometric Mean ($\log_{10}$)	2.55	2.31	2.23	2.01	2.89	2.31	2.33	2.05	3.10	2.37	2.35	2.03

APPENDIX 9 (Table 22)

COMPARISON OF LP2 IN YOUNG AND ADULT RATS INFECTED WITH 2,500 LARVAE

	Days after infection				
	9	11	13	15	17
Worms recovered from baby rats	1820	1080	1580	940	1006
	2060	1880	1160	1220	1760
	1460	1160	980	1340	712
	740	1480	920	780	286
Mean	1520	1400	1160	1070	941
S.D.	570	363	298	256	607
Geometric Mean (log_{10})	3.15	3.14	3.05	3.02	2.89
S.D.	0.31	0.11	0.11	0.13	0.33
Worms recovered from adult rats	1960	1420	480	225	159
	860	1080	560	238	311
	1240	1200	980	820	65
	1200	1400	1180	680	134
Mean	1315	1275	800	491	223
S.D.	462	163	335	305	103
Geometric Mean (log_{10})	3.10	3.10	2.87	2.62	2.16
S.D.	0.24	0.06	0.18	0.29	0.28

APPENDIX 10 (Table 23)

INFECTION OF YOUNG RATS WITH 2,500 LARVAE. WORMS RECOVERED OVER THE LOSS PHASE 2 PERIOD

	Days after infection										
	10	13	14	15	16	17	18	19	20	21	22
Worms recovered from small intestine	980	760	960	1260	520	720	652	211	209	513	521
	1490	1080	1100	1280	1320	360	190	840	449	486	232
	960	1500	640	1060	960	1480	600	920	601	189	201
	1950	1560	1240	520	1120	1120	740	680	524	207	175
	2100	1820	1360	1200	720	220	1160	640	786	396	397
Mean	1498	1340	1060	1064	936	780	668	658	514	358	305
S.D.	509	421	278	316	317	523	347	277	211	153	149
Geometric Mean ($\log_{10}$)	3.15	3.11	3.01	3.01	2.93	2.80	2.76	2.78	2.67	2.52	2.45
S.D.	0.16	0.15	0.12	0.16	0.16	1.08	1.35	0.26	0.22	0.20	0.21

APPENDIX 11 (Table 30)

CHALLENGE EXPERIMENT 1. ADULT WORMS IN DIFFERENT GROUPS OF RATS CHALLENGED WITH 1,000 LARVAE

	8 days after infection					23 days after infection				
Groups*	1	2	3	4	5	1	2	3	4	5
Worms recovered from intestine	8	7	32	543	395	16	7	15	0	89
	13	24	10	347	399	5	2	30	0	29
	2	241	45	163	615	0	0	48	15	9
	6	12	31	438	276	7	3	91	65	18
	3	9		902	395				6	
Mean	6	58	23	478	416	7	3	46	17	34
S.D.	4	102	14	275	123	7	3	34	27	36

* Group treatment before challenge

1 3,200 larvae when adult
2 1,600 larvae when adult
3 3,200 larvae when 4 weeks old
4 1,600 larvae when 4 weeks old
5 None

APPENDIX 12 (Table 32)

CHALLENGE EXPERIMENT 2. ADULT WORMS IN RATS CHALLENGED WITH 1,000 LARVAE, FOLLOWING AN INFECTION OF 2,000 LARVAE GIVEN WHEN YOUNG

	Worms recovered from intestine			
Days after infection	5	9	15	20
Group 1 (infected with 2,000 L. when young)	339	275	0	68
	200	129	1	1
	690	209	5	2
	84	166	74	32
	525	157	112	2
	497	16	4	4
	259	202	368	0
Mean ± S.D.	370 ± 211	165 ± 81	81 ± 142	16 ± 28
Group 2 (not previously infected)	309	137	2	1
	162	41	0	3
	61	272	16	7
	622	513	13	0
	395	684	90	
	436	264	65	
	255	107	4	
Mean ± S.D.	320 ± 185	288 ± 232	29 ± 38	3 ± 3

APPENDIX 13 (Table 34)

CHALLENGE EXPERIMENT 3. ADULT WORMS IN RATS CHALLENGED WITH 500 LARVAE FOLLOWING AN INFECTION OF 500 LARVAE GIVEN WHEN YOUNG

	Worms recovered from intestine		
Days after infection	5	8	15
Group 1 (infected when young)	97 167 253 147 314 127 321	150 103 246 214 83 186 188	46 5 3 6 8 3 2
Mean ± S.D.	203 ± 91	181 ± 61	10 ± 16
Group 2 (not previously infected)	134 57 59 254 263	36 102 205 241 279	18 42 21 50 27 24 34
Mean ± S.D.	153 ± 101	172 ± 100	31 ± 11

APPENDIX 14 (Table 35)

THE FATE OF A TRANSPLANTED ADULT WORM INFECTION IN UNRESPONSIVE RATS AFTER REMOVAL OF THE ORIGINAL POPULATION WITH THIBENZOLE

	Days after infection					
	7		14		21	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
	21	190	136	25	33	11
	290	128	151	9	7	0
	4	42	165	3	6	2
	257	23	98	66	27	28
	157	211	5	37	15	6
					13	35
Mean	146	119	111	28	14	18
S.D.	131	85	64	25	12	21
t test			p = 0.05			

IMMUNITY TO NIPPOSTRONGYLUS INFECTION IN THE RAT

A summary of a thesis submitted for the degree of Doctor of Philosophy in the Faculty of Medicine, the University of Glasgow, by Ellen Elizabeth Evelyn Jarrett, 1968.

Infection of the rat with the nematode parasite Nippostrongylus brasiliensis provides a convenient laboratory model for the investigation of many aspects of the relationship between nematode parasites and their mammalian hosts. This is particularly so in the field of immunity where it is well established that primary infections with this parasite are terminated abruptly by an immunological reaction called the 'self-cure' and that subsequently rats are relatively immune to reinfection.

Three aspects of the immunological response have been studied by the author. First the kinetics of worm populations in immune and normal rats, secondly the mechanisms responsible for the self-cure reaction and thirdly the characteristics of the immune response in immature rats.

In Section 1 the quantitative aspects of establishment and expulsion of Nippostrongylus brasiliensis in the normal and immune rat are described It is proposed that the kinetics of a primary infection may be described in 4 phases. These are:

1) Loss Phase 1 (LP1). After infection a proportion of the infecting dose of larvae fails to develop and is immobilised or destroyed before or during the course of migration via the lungs to the intestine.

2) The Plateau Phase. The remainder of the larvae develop to maturity in the intestine, their number remaining constant for a defined period of days.

3) Loss Phase 2 (LP2). This is synonymous with the self-cure reaction. During this phase the worms are expelled from the intestine of the host by an immunological mechanism. The onset of the expulsion is sudden but the process once initiated is exponential. Its rate is measurable in terms of the regression coefficient. This phase terminates when a threshold of worm numbers is reached.

4) Threshold Phase. This describes the residual population, usually of approximately 40 worms, which is not expelled and which survives for a fairly prolonged period.

These four phases also occur in immune animals subjected to second or third infections but there are quantitative differences. With increasing numbers of infections the proportion of larvae destroyed during LP1 becomes greater, the Plateau is shortened and the expulsion rate of LP2 increases. It is suggested that the rate coefficient of this third phase is an accurate measure of the immune status of the animal. The Threshold Phase is of a similar order in first, second and third infections. These results are discussed with regard to their significance as measurements of the immune status of the host, their implication for the mechanism of helminth expulsion and their relevance to other helminth infections.

Experiments undertaken to elucidate the mechanism of worm expulsion from the intestine are described in Section 2. It is thought that the sudden onset of LP2, and the subsequent exponential kinetics, indicate that the mechanism whereby adults are expelled might be a two stage reaction, depending first on the presence of antiworm-antibody and secondly on a specific factor which permits the sudden expression of this antibody. It was considered that the specific factor might be related to the development of a state of permeability of the vascular and/or epithelial tissues which facilitates the passage of antibody from the blood or connective tissue of the villi on to the intestinal mucosa. A lesion which gives rise to such a state was shown to exist. It consists basically of the development of a large fluid-filled space between the blood vessels and the epithelium of the villus. The presence of intravascularly injected Evans blue in the sub-epithelial spaces and in the intestinal lumen indicates that it is a specific site of extravascular and extra-epithelial permeability to macromolecules.

In an experiment in which the lesion was produced by a heterologous system, its presence appeared to facilitate the passage of passively transferred antibody into the intestinal lumen, causing the premature expulsion of a transplanted adult worm population. By itself the lesion had no effect on the worms.

Although these results support the concept that the leak lesion facilitates the passage of antiworm-antibody into the intestinal lumen the observation that the lesion is present from as soon as four days after infection suggests that the mechanism whereby antibody gains access to the worms in the natural infection is in fact more complex. The subject is discussed with particular reference to recent findings concerning the presence of intestinal mast cells in N. brasiliensis infection.

Section 3 reports a study of N. brasiliensis infection in young rats. Preliminary experiments indicated that if rats were infected when under 6 weeks of age with 500 larvae they did not undergo during the second week the characteristic self-cure reaction (LP2) that is a feature of the response of adult rats. Instead these animals underwent a prolonged infection that extended well into adult life indicating that its duration could not be solely attributed to immunological immaturity.

Subsequently the pattern of primary N. brasiliensis infections of different sizes was followed in young rats. It is shown that when the number of adult worms which becomes established in a young rat from the infecting dose is around 250 or less, worm expulsion as seen in adult rats does not occur and the infection persists until weeks after the rat becomes adult. If considerably more than 250 worms are established

worm expulsion occurs but is incomplete in comparison with that of an adult rat in that it starts later, proceeds at a slower rate and terminates when the worm population falls to a mean of approximately 200. This residual worm infection also persists into the adult life of the rat.

Using passive protection tests it is shown that the serum of rats infected when young with larger numbers of worms contains protective antibodies. Such antibodies could not be demonstrated in the serum from rats infected with small numbers of worms.

When rats which have been infected when immature are challenged when adult, the immune response tends to be directly proportional to the size of the original adult worm infection and may be of a primary or secondary nature or a reaction intermediate between the two. During the reaction to the challenge infection the previously tolerated worm burden is also expelled.

It is considered most likely that immunological immaturity either in terms of antibody production or in the development of an effector mechanism is responsible for the reduced response of the young rat. This however does not explain the subsequent failure to eliminate the persisting worm burden when the rats become adult. For various reasons it is considered that immunological tolerance is unlikely to be involved and a possible alternative explanation is discussed.