The Self-Cure Reaction

in

Nippostrongylus brasiliensis

Infections in the Rat

A Thesis

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by

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GENERAL INTRODUCTION

Although immunity to bacterial and viral diseases of man and animals has been recognised and studied for many years (Topley and Wilson, 1964), the possibility that resistance might also be acquired to helminth parasites received little attention until about thirty years ago (Culbertson, 1941; Chandler, 1953). The reasons for this are obscure, but were probably associated with a preoccupation by parasitologists with taxonomy, with the complexity of life-cycles and with diagnostic tests. It seemed also to be generally assumed that the acquisition of immunity was unlikely since adult animals were frequently infected with helminths.

Over the last few decades and stemming from the early observations on acquired resistance reported by Taliaferro (1929) and Chandler (1952a), the vast amount of literature which has subsequently been published reflects increasing recognition of the significance of the immune response in the epidemiology of helminth diseases in man and animals. There is now such an extensive range of literature available on the immunology of parasitic helminth diseases (see reviews by Chandler, 1953; Urquhart, Jarrett and Mulligan, 1962; Soulsby, 1962 and 1966) that in this review it is proposed to include only those observations which are pertinent to the subject of the thesis.

Much of the current interest and ideas in this particular field stem from the pioneer work of Chandler (1932, 1935, 1936a, 1936b, 1937a, 1937b, 1938) and of Taliaferro and Sarles (1939) on the host parasite relationship of Nippostrongylus brasiliensis and the laboratory rat. The life cycle of this small trichostrongyle parasite is representative of that of a number of intestinal helminths and provides a valuable tool for studying specific aspects of these infections. As this thesis is concerned with certain aspects of N. brasiliensis infection in rats, a brief outline of the life cycle of the parasite is included at the end of this review.

The immune response in animals stimulated by parasitic helminths may be manifested in a variety of ways:-

1) Reduction in the number of mature adult worms developing from a challenge infection

The most frequently recorded demonstration of acquired immunity to parasitic helminths is a significant reduction in the numbers of adult worms which develop from a standard challenge infection in immunised groups of animals. This may be caused either by the death of the developing worms, or by their retardation as larval stages. For example, it has been shown that when rats which have recovered from an initial infection of N. brasiliensis are challenged

with a second infection, many of the developing larvae are trapped and destroyed by cellular reactions in the skin and lungs (Taliaferro and Sarles, 1939; Chandler, 1937b). A similar reaction has been demonstrated in parasitic bronchitis of cattle where a single experimental infection of Dictyocaulus viviparus, of sufficient magnitude, gives rise to a strong resistance to reinfection as measured by the greatly reduced number of adults which develop from the challenge infection (Rubin and Lucker, 1956; Jarrett, Jennings, McIntyre, Mulligan, Thomas and Urquhart, 1959b). A successful commercial vaccine is now marketed against this disease, in which two doses of X-irradiated larvae administered orally a month apart give rise to a very high degree of immunity (Jarrett et al, 1958a, 1958b, 1959a, 1960b; Poynter, Jones, Nelson, Peacock, Robinson, Silverman and Terry, 1960; Jones and Nelson, 1960).

2) Reduction in biotic potential or size of mature worms

When immune rats are challenged with N. brasiliensis, the small number of worms which mature in the small intestine are stunted and lay fewer eggs compared to worms from a primary infection (Taliaferro and Sarles, 1939; Chandler, 1932, 1936b). Stunted adult worms have also been observed at necropsies of calves repeatedly infected with D. viviparus (Jarrett et al, 1959b), and of sheep infected with H. contortus (Roberts, 1957). A similar

result was reported by Sadun (1949) who showed that when passively immunised chickens were challenged with A. galli, the resulting worms were significantly smaller than the controls. In T. colubriformis infections, Stewart and Gordon (1958) state that the worms may develop to maturity in the normal time in immune sheep, but produce very few eggs.

It is interesting that although inoculation of rats with living larvae of Longistriata adunca, a worm closely related to N. brasiliensis, has no effect on the number of adult worms which develop from a challenge infection of N. brasiliensis, it has a decidedly inhibiting effect on reproduction in that the egg output from such rats is only about one-tenth that of controls (Chandler, 1932).

3) Inhibition of development

A third important manifestation of host response to parasitic helminths is inhibition of larval development, as distinct from retardation and stunting of growth previously discussed. Characteristically it arrests the growth of a high proportion of a worm population at a particular stage. Until recently, inhibition was considered to be a direct consequence of an immune response by the host but it is now apparent that other factors can also be involved.

Thus, inhibition may occur in at least three different ways:-

a) The result of an immune response

Two of the best examples of this are those quoted by Michel (1963) and by Dineen, Donald, Wagland and Offner (1965). The former found that calves given repeated doses of O. ostertagi larvae for at least 127 days had large numbers of inhibited fourth larval stages at autopsy (Michel, 1963). Dineen et al (1965) showed that when Merino sheep were given 30 consecutive doses of 100 H. contortus larvae, a high percentage of the worms were inhibited at the fourth larval stage.

b) The result of a single large dose of infective larvae

Examination of the worms from sheep which had received a single dose of 100,000 O. circumcincta larvae showed that up to 75% were inhibited at the early fourth stage (Dunsmore, 1960).

c) The result of a physiological change in the infective larvae

Recent work by Armour, Jennings and Urquhart (1967) has shown that the inhibited development of O. ostertagi at the early fourth stage in calves is associated with physiological changes in larvae on the pasture in the late autumn.

4. The immune expulsion of primary and challenge infections

Immune expulsion of an adult population of worms at the termination of both primary and challenge infections has been reported in several parasitic helminth infections and is particularly characteristic of H. contortus infections in sheep and N. brasiliensis infections in rats. This phenomenon, known as self-cure, was first described by Stoll in 1928 and 1929 in experiments with sheep infected with the stomach worm Haemonchus contortus. Lambs which were lightly infected were placed on a limited pasture area. After some weeks the faecal egg counts rose to high levels and then suddenly dropped, a reasonable indication of loss of the adult infection; the lambs were also found to be highly resistant to subsequent reinfection. It now seems remarkable that this important aspect of acquired immunity received so little further attention at the time, and it was not until the 1950's that the phenomenon was further investigated by Stewart. As a result of his work, it is now known that self-cure in this infection is triggered by the intake of infective larvae on top of an existing adult population of worms (Stewart, 1950b, 1953, 1955).

A similar self-cure reaction has been reported in N. brasiliensis

infections except that in this case the adult worms are expelled at the end of a primary infection. When rats are infected subcutaneously with 500 or more N. brasiliensis larvae, towards the end of the second week of infection they undergo a self-cure reaction manifested by a sudden drop in egg production by the worms, followed over the next few days by their expulsion from the small intestine (Africa, 1931; Schwartz and Alicata, 1934; Graham, 1934).

The fact that self-cure in N. brasiliensis infections is due to an immune response on the part of the host was initially established by Chandler (1936) and Spindler (1936). They showed that if an adult worm population is removed from the host just before self-cure and transferred surgically to the small intestine of a previously uninfected rat, the worms will become successfully established and continue egg production for a further 8-9 days before resistance develops in the new host.

Ogilvie (1964) has recently shown that the self-cure reaction is affected by cortisone. She showed that daily administration of large doses of cortisone, to rats infected with N. brasiliensis, abolishes the self-cure reaction. If the treatment is stopped the worms are quickly expelled.

A number of theories have been advanced to explain the mechanism of self-cure. The first of these was the suggestion by Chandler (1935a) that the immune effect may be nutritional in nature and may be due to the development of anti-enzymes which inhibit the activity of the specialised enzymes by means of which the parasite digests and assimilates host protein. A second possibility was put forward by Stewart as a result of his observations on sheep infected with H. contortus. He suggested that the mechanism of self-cure in this disease might be due not to the direct action of antibody on the parasite, but to a state of specific hypersensitivity in which a local anaphylactic reaction gave rise to conditions which were unsuitable for the worms (Stewart, 1953, 1955). This suggestion was based mainly on four observations.

i) Self-cure was accompanied in 50% of the sheep by a significant rise in blood histamine. In all cases where self cure did not occur, the level of histamine remained unaffected.

ii) The administration of an antihistaminic drug - mepyramine maleate - in some cases prevented the fall in egg count which is characteristic of the self-cure reaction, but it did not interfere with the subsequent rise in titre of the specific H. contortus antibody in the serum.

iii) Histological examination of the abomasum at the time of the increase in the level of blood histamine, showed that self-cure was accompanied by oedema and infiltration of eosinophil leucocytes in the abomasal mucosa. Both of these changes are characteristic of immediate-type hypersensitive reactions. This change was observed only in sheep which had previously been infected with H. contortus.

iv) Sheep which manifested self-cure showed a strong immediate-type local reaction to the intradermal inoculation of H. contortus antigen.

A more detailed resume of the relevant literature is given in the introduction to each experiment.

The Life Cycle of Nippostrongylus brasiliensis

Yokogawa in 1922 first described the life cycle of Heligmosomum muris - now known as Nippostrongylus brasiliensis (Haley and Clifford, 1960). More recently Twohy (1956) and Haley (1962) have given more detailed accounts of this parasite of rats.

Under the correct conditions of temperature and humidity, eggs from infected faeces develop through two larval stages to the infective stage within 3-4 days (Haley, 1962). These third stage larvae normally penetrate the skin and migrate via the blood stream (Yokogawa, 1922) and the lymphatic system (Gharib, 1961) to the lungs. It has also been shown that a few larvae from an oral infection can migrate from the stomach to complete the normal life cycle (Yokogawa, 1922; Africa, 1931; Schwartz and Alicata, 1934; Spindler, 1936).

Little growth occurs until the larvae reach the lungs at about 11-15 hours after infection. Here they feed on blood and tissue cells (Taliaferro and Sarles, 1939) and grow rapidly (Figure 1), until at 32-46 hours they moult to the fourth stage. They then migrate via the trachea and oesophagus to the small intestine, reaching it by about the sixtieth hour.

After the second parasitic moult to the fifth stage at 90-108

hours, the worms quickly reach maturity (Figures 2 and 3) and fertile eggs appear in the rat faeces as early as 6 days after infection (Weinstein and Jones, 1959).

Most of the adult worms are found in approximately the first 12 inches of the small intestine (Brambell, 1965). They lie between the villi and have been reported to feed on tissue cells and blood (Taliaferro and Sarles, 1939; Rogers and Lazarus, 1949) and gut contents (Weinstein and Jones, 1956).

Egg production reaches its maximum between day 6 and day 10, and drops rapidly to zero between day 15 and day 20 (Africa, 1931; Schwartz and Alicata, 1934; Graham, 1934). This sudden drop in egg production is associated with the expulsion of the worms, with a more rapid expulsion of females (Africa, 1931; Sarles and Taliaferro, 1936; Porter, 1935). The rate and timing of expulsion of the worms depends to some extent on the size of the initial infection (Haley and Parker, 1961) in that a very low infection of 10-80 larvae gives rise to a prolonged infection (Hurley, 1959).

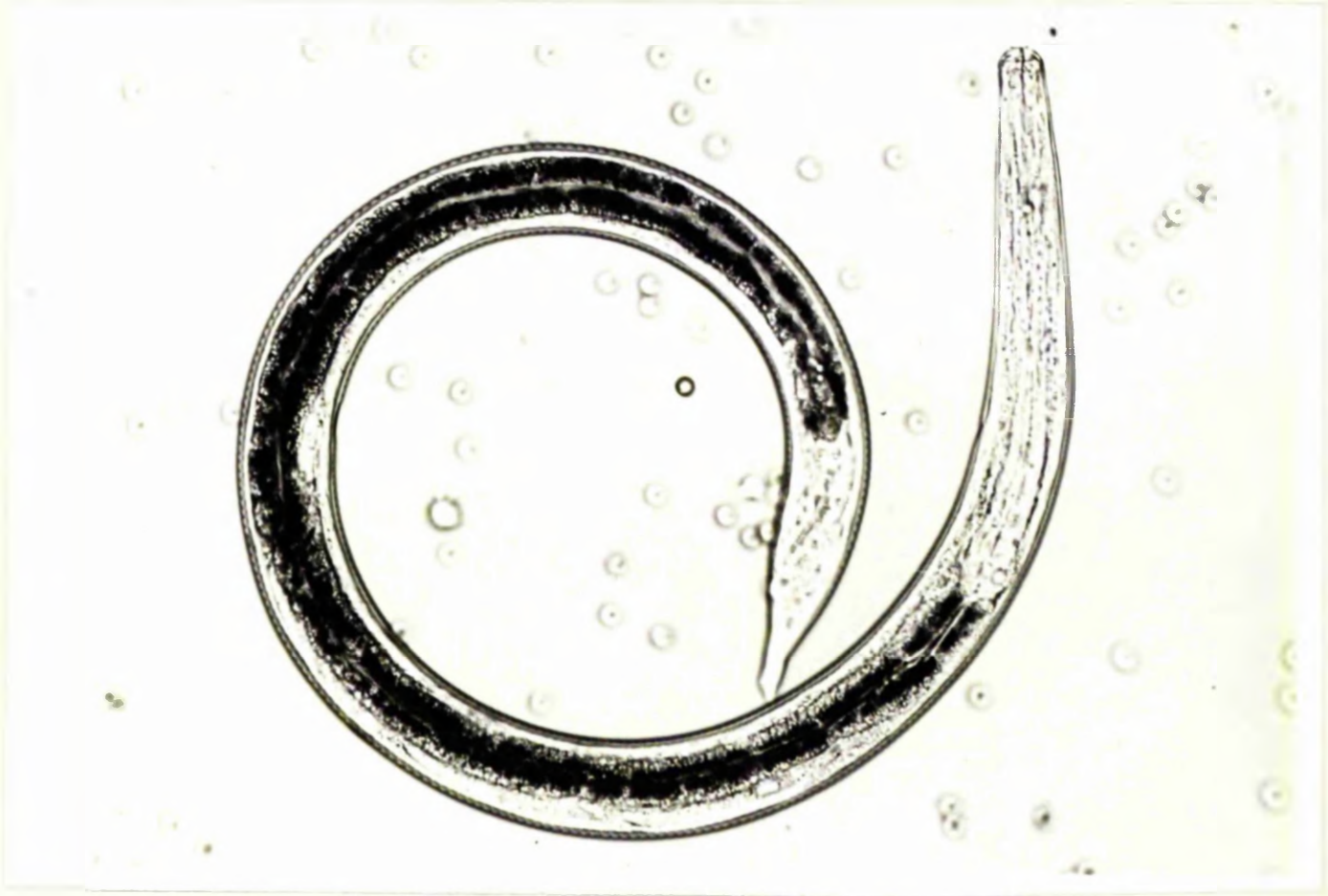


Figure 1. Nippostrongylus brasiliensis. Larva in the first parasitic stage (x 500) taken from the lung of a rat 24 hours after infection.

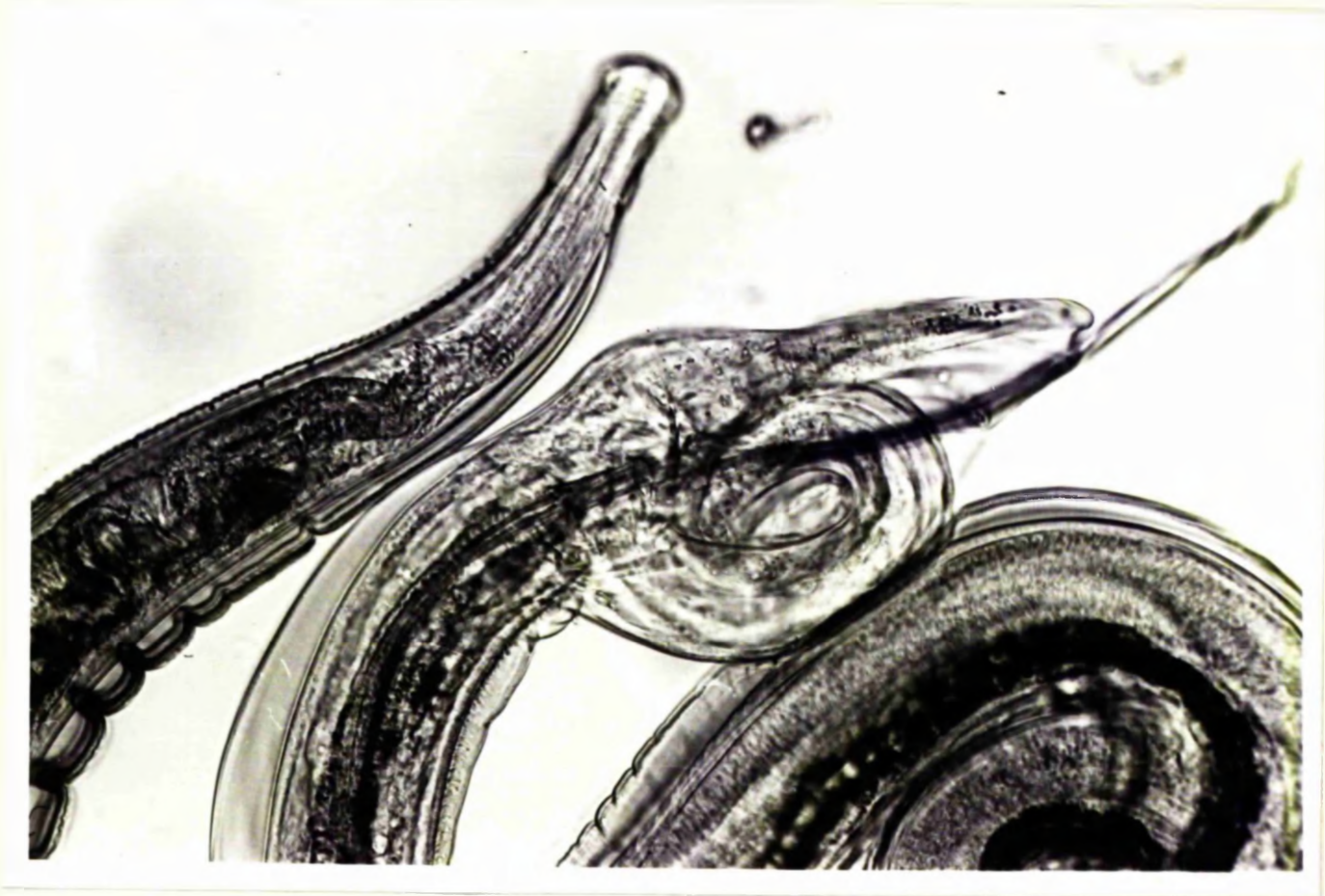


Figure 2. Nippostrongylus brasiliensis. Anterior and posterior ends of a mature male worm (x 500) from the small intestine of a rat 120 hours after infection. Note the fully developed bursa and spicules.

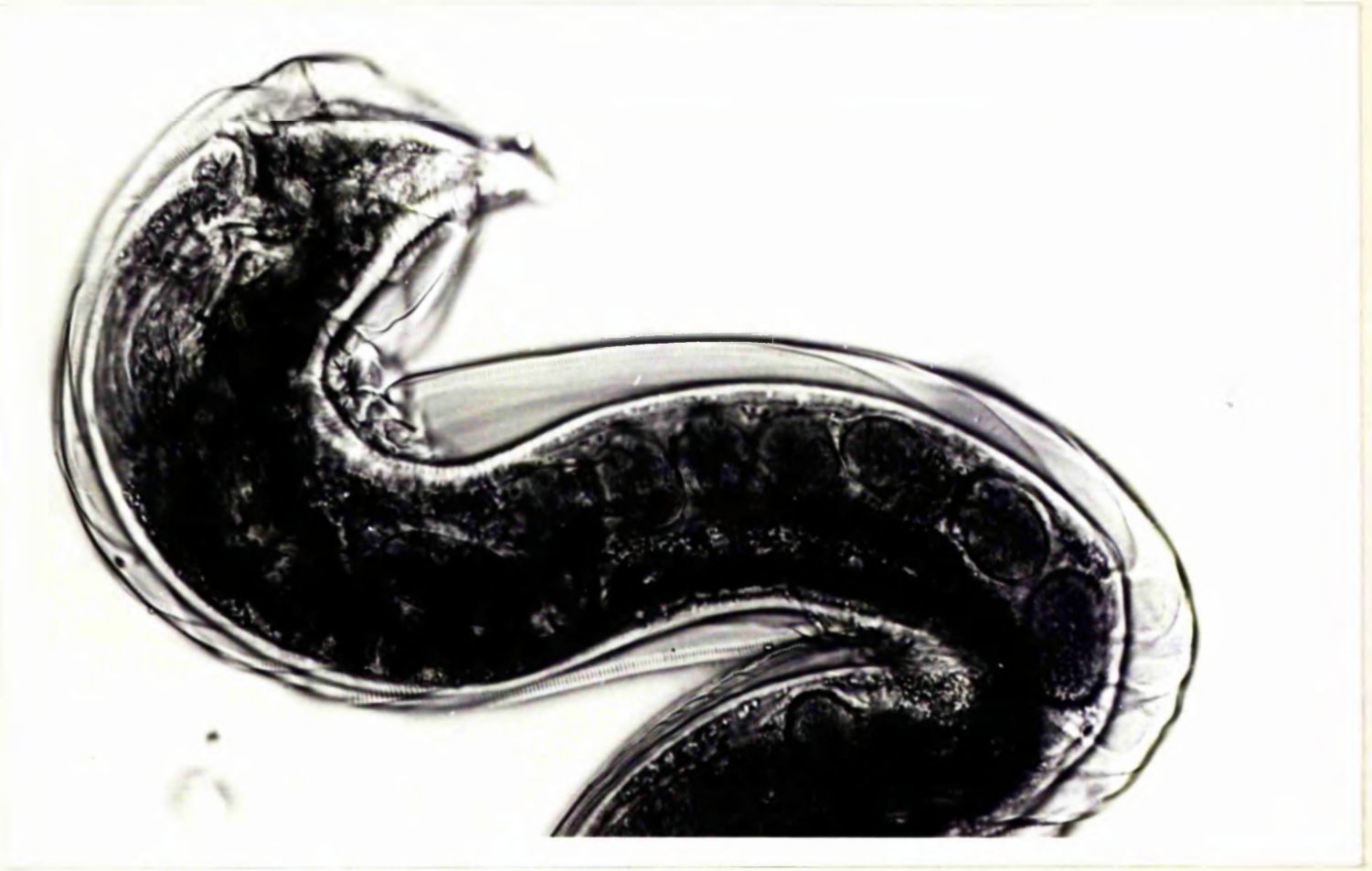


Figure 3. Hippostrongylus brasiliensis. Posterior end of a mature female worm (x 500) from the small intestine of a rat 120 hours after infection, showing the uterus filled with eggs.

General objectives of the research undertaken

The object of the work described in this thesis was to study certain aspects of the self-cure of *N. brasiliensis* infections in the laboratory rat, with a view to elucidating the mechanism of immunity in this host/parasite relationship.

Before undertaking any experiments on the self-cure phenomenon it was thought worthwhile to start by investigating the course of a primary infection of *N. brasiliensis* in a more quantitative fashion than has been previously described, in terms of worm burdens and daily faecal egg counts. Having done this, the remaining experiments described in the first section were designed in an attempt to find the answers to several questions which are fundamental to the understanding of the immune phenomena associated with gastro-intestinal parasites.

1) How does the immune response of the host make the worms' position untenable?
2) What is the role of humoral antibody in self-cure?
3) If antibody is involved, does it act directly on the worms and, if so, how does it get to them?

As already mentioned, Stewart suggested that the production of a state of specific hypersensitivity in the abomasum of sheep infected

with H. contortus was an essential part of self-cure. It may be that a similar phenomenon occurs in the immune expulsion of adult N. brasiliensis from the small intestine of the rat. In Section II therefore, an attempt was made to determine if this was the case, and if so, to investigate the possible role of a local anaphylactic reaction in the mechanism of self-cure.

Further work was then undertaken to find out if self-cure was accompanied by any detectable general or local release of histamine, since this would provide supportive evidence of the occurrence of an anaphylactic reaction. This was done by estimating the total daily output of urinary histamine of rats infected with a single dose of N. brasiliensis larvae (Section III).

GENERAL METHODS AND MATERIALS

Certain techniques common to all the sections are described here.

Experimental Animals

All the rats used were of the hooded Lister strain, more than 6 weeks old, and weighing 150 to 200 gms. at the time of the experiment (Animal Suppliers Ltd., London). The rats were free from helminth parasites with the exception of Hymenolepis spp. which were occasionally noted at autopsy.

They were housed in wire cages suspended in racks, with the wire mesh floor above a sawdust-covered metal tray. This eliminated the possibility of accidental reinfection.

Food (Diet 86, supplied by Wm. Shearer & Co., Glasgow) and water were available at all times.

Parasite

The strain of Nippostrongylus brasiliensis used in all experiments was obtained initially from Dr. Hopkins of the Zoology Department, Glasgow University, and was maintained by regular passage through rats similar to those used for experimental purposes.

Culture Technique

Faeces from culture rats infected by the subcutaneous injection of 2,500 infective N. brasiliensis larvae were collected between days 7 and 12 of the infection. The faeces were set up for culture by a method similar to that described by Bakarat (1951), except that the filter papers were supported by synthetic sponges. The cultures were incubated at 27°C for 5-7 days, by which time larvae had migrated to the edge of the filter paper, and had reached the infective stage. They were harvested by filling the petri dish with water, removing the filter paper and sponge, and concentrating the larval suspension by filtration through a coarse filter paper. The filter paper was then inverted on a fine sieve (60 mesh to the inch) in a Baermann funnel filled with water at 27°C and the larvae collected by sedimentation. This procedure gave a concentrated suspension of highly active larvae almost free from contaminating material.

Infection Procedure

Infective larvae were collected on the day of inoculation. The concentration of the larval suspension was determined by examining at least twenty 0.025 ml. portions of the suspension until a total of 400 larvae was recorded. The total volume was then adjusted so that

the required dose for each rat was contained in 1.0 ml. Several checks were made to ensure that the desired number of larvae ± 10 per cent was present. Penicillin and streptomycin were added to give a concentration of 100 units and 10 μg per millilitre, respectively.

Post-Mortem Procedures

Adult N. brasiliensis were collected from the small intestine by slitting it open, cutting it into three-inch lengths and suspending these in a muslin bag in a beaker of saline at 37°C. After one hour over 95% of the adult worms had collected in the bottom of the beaker.

Faecal Egg Counts

The method used to estimate the number of eggs per gram is a modification of that first described by Gordon and Whitlock (1939).

Faeces from the infected rats were collected over a 24-hour period. A 3 gram sample of faeces was selected at random and homogenised in 42 ml. water. After passing through a 50 mesh sieve, 15 ml. of the filtrate was centrifuged for 3 minutes at 1,500 r.p.m. in a glass centrifuge tube. The supernatant was then discarded and the precipitate resuspended in 15 ml. of saturated sodium chloride

solution. The tube was then inverted several times to ensure an even suspension and both chambers of a McMaster slide were immediately filled by means of a Pasteur pipette, and examined under the microscope for nematode eggs.

The average number of eggs per gram of faeces was calculated by multiplying the total number of eggs in both chambers by 50.

Preparation of Adult Worms for Transplant

Adult N. brasiliensis from a 10-day single larval infection were carefully removed from donor rats by the method described in Post-Mortem Procedures. The resulting worm suspension was washed several times in warm saline, counted, and the total volume adjusted with warm saline until 5 ml. contained the required dose of worms to be transferred to each rat. Aliquots of 5 ml. were then removed with a broad mouthed pipette and placed, in tapered centrifuge tubes, in a water bath at 37°C.

Immediately before transfer to the recipient rat, the worms were carefully drawn up along with 1 to 2 ml. of liquid, into a length of 2 mm. diameter catheter tubing fixed to a 5 ml. syringe. A number 1 needle (B.S.W.G.) was then placed on the free end of the tubing.

Laparotomy Technique

The recipient rat was anaesthetised with "Trilene", and the abdomen shaved and washed. The duodenum was exposed by a small incision in the skin and muscle layers, on the right side, just below the rib cage. The worms were then slowly injected, directly into the duodenum, and the muscle and skin layers each closed with a single nylon suture. Very few deaths occurred using this method and the treated rats usually showed complete recovery by the next day.

Normal Serum

Normal serum was obtained from parasite free rats by cardiac puncture.

Immune Serum

Immune serum was obtained by cardiac puncture from rats infected 20 days previously with 3,500 infective N. brasiliensis larvae.

Hyperimmune Serum

Hyperimmune serum was obtained from rats which had received

4 infections of N. brasiliensis of 3,500, 5,000, 7,500 and 10,000 larvae at fortnightly intervals. Blood was collected 10 to 12 days after the final infection.

All serum was stored at -20°C until required.

Injection of Evans Blue

The dye Evans Blue (T1824) was dissolved in 0.9% NaCl to give a concentration of 0.5%. This solution was injected into the tail vein of the experimental rats at a dose level of 0.5 ml./100 gms./rat, using a 1 ml. tuberculin syringe.

Injection of Evans Blue-Antigen Mixture

Fresh adult worms from 10-day larval infections were suspended in physiological saline at a concentration of 1,000 worms/ml. They were macerated in a Griffiths tube and then subjected to ultrasonic disintegration for 15 minutes, while being cooled in an ice bath. After centrifugation for 30 minutes at 1,000 x g. in a refrigerated centrifuge, the clear supernatant fluid was removed and mixed with an equal volume of 1% Evans Blue in 0.9% NaCl. This mixture was injected into a tail vein at a dose level of 0.5 ml./100 gms./rat.

SECTION I

THE SELF-CURE PHENOMENON

Introduction

The immunology of *Nippostrongylus brasiliensis* infections in rats has been the subject of many investigations, and has already been reviewed in the General Introduction. Most of the work described has been related to the immune phenomena associated with the administration of infective larvae to a resistant host, *i.e.*, to the effect of acquired resistance on the migration, development and establishment of the challenge infection. Of equal interest from the point of view of understanding the mechanisms operating against gastro-intestinal parasites, is the immune expulsion of adult worms from the small intestine, which occurs between approximately the 12th and 16th day of the primary larval infection in rats. In this first section, an attempt has been made to clarify this particular aspect of the host's response to *N. brasiliensis*.

Experiment 1

The Dynamics of the Self-cure Phenomenon in a Primary Infection

Introduction

It is well established that when rats are infected with a large number of *N. brasiliensis* larvae, the resulting adult populations are lost almost completely in 20 days. In a primary infection of this parasite, the faecal egg count of the rats reaches a peak between the 6th and 10th day, falling rapidly to zero at about the 16th day (Africa, 1931; Schwartz and Alicata, 1934; Graham, 1934). Taliaferro and Sarles (1939) showed that this sudden drop in egg production tends to precede the expulsion of the adult worms from the small intestine.

As most of these studies have only been semi-quantitative in nature, and as the period of self-cure varies in different strains of rats, the following experiment was carried out to obtain specific information, in terms of faecal egg counts and worm burdens, before, during and after self-cure in hooded Lister rats infected with *N. brasiliensis*.

Plan of Experiment

A group of 48 rats of both sexes were infected subcutaneously

Table 1

The Mean Worm Burdens and Daily Faecal Egg Counts of Rats Throughout a Primary Infection of 2,500 *N. brasiliensis* Larvae

No. Days After Infection	Mean Worm Burden* ± S.D.	Sex Ratio ♂:♀	Faecal Egg Count in E.P.G.
5	-	-	3,300
6	1,116 ± 311	1:1	35,900
7	-	-	16,600
8	-	-	29,300
9	-	-	66,200
10	1,462 ± 546	3:2	86,600
11	-	-	49,200
12	1,563 ± 240	1:1	24,600
13	1,033 ± 293	1:1	5,000
14	461 ± 394	5:4	200
15	-	-	-ve
16	17.83 ± 18.8	35:1	-ve
17	-	-	-ve
18	2.7 ± 1.8	14:1	-ve
19	-	-	-ve
20	9.5 ± 13.1	18:1	-ve

* The individual worm burdens are given in Appendix Table 1.

with 2,500 N. brasiliensis larvae, and killed in groups of 6 on the 6th, 10th, 12th, 13th, 14th, 16th, 18th and 20th days of the infection. Daily faecal egg counts were done throughout the experiment and the number of adult worms in the small intestine at autopsy were counted.

Results

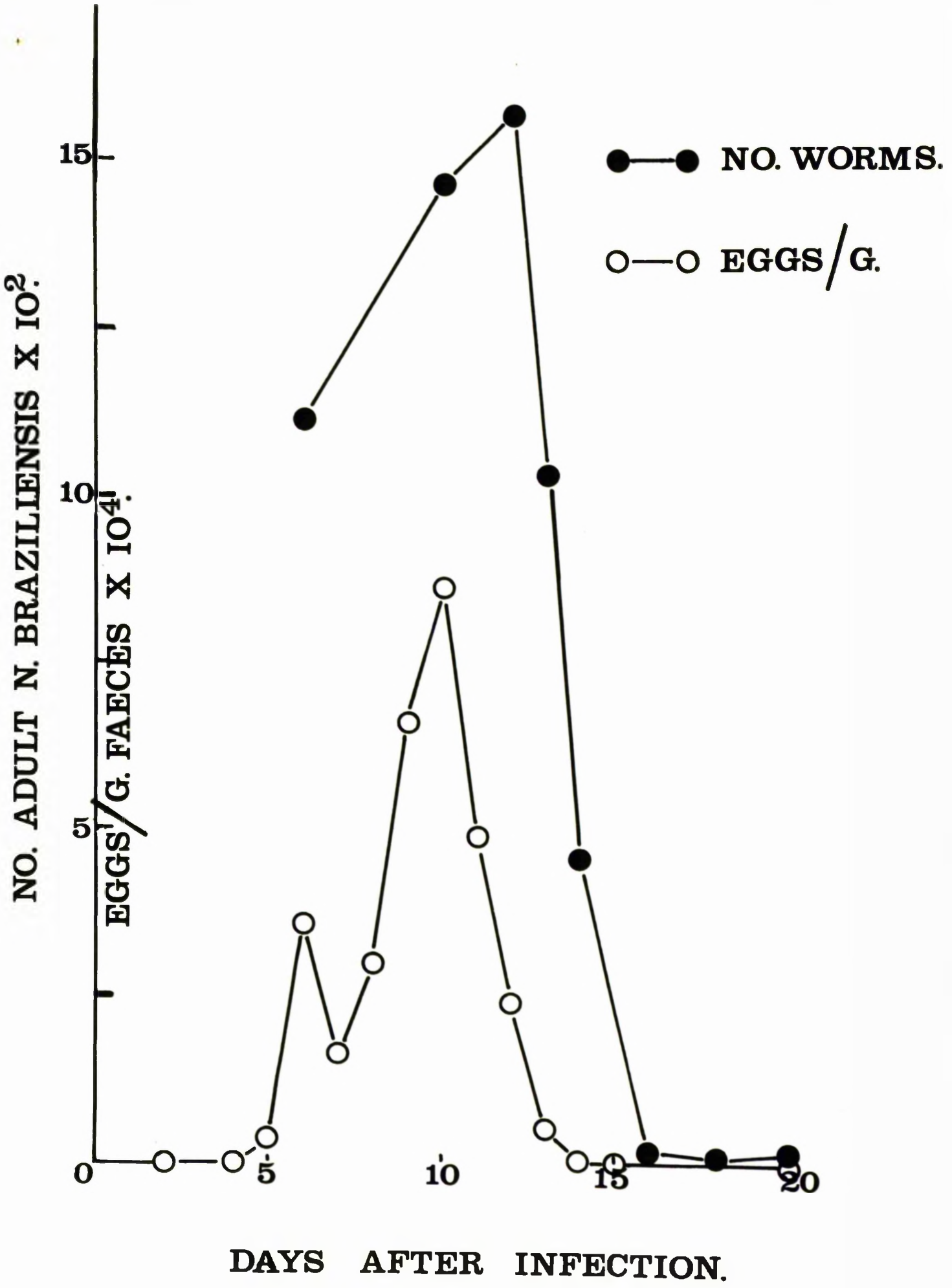
The results of daily faecal egg counts and the mean worm burdens in the small intestine at autopsy are given in Table 1 and are expressed graphically in Figure 4.

Eggs first appeared in the faeces 5 days after infection and reached a maximum output of 86,600 e.p.g. by the 10th day. On the 11th day, the count dropped to 49,200 e.p.g. and fell rapidly to zero by the 15th day.

Of the 2,500 infective larvae, approximately 60% developed to maturity and remained in the small intestine until the 13th day. They were then rapidly expelled, the female worms being eliminated more quickly than the males, until by the 16th day, there were only 17.83 ± 18.8 worms remaining. Further autopsies showed that these remaining worms - mostly males - were still present on the 20th day after infection.

Figure 4

The Mean Worm Burdens and Daily Faecal Egg Counts of Rats Throughout a Primary Infection of 2,500 *N. brasiliensis* Larvae.


NO. WORMS.
EGGS/G.
NO. ADULT N. BRAZILIENSIS X 10^2.
EGGS/G. FAECES X 10^4.
15
10
5
0
5
10
15
20
DAYS AFTER INFECTION.

Discussion

These results give some support to the findings of Taliaferro and Sarles (1939) who stated that the sudden drop in egg production tended to precede the elimination of the adult population of parasites. From Figure 4 it can be seen that there was a period of about 2 days between the sudden drop in the daily egg count and the expulsion of the majority of the parasites in this infection, so the egg output graph reflects not only a fall in the number of adult worms, but also some interference with reproduction due to a suppression of egg production. It was apparently not due to a disproportionate elimination of females since the sex ratio on days 12, 13 and 14 was consistently 1:1.

Proof that the self-cure shown in Figure 4 is due to an immune response on the part of the host depended initially on the results reported by Chandler (1936a). He demonstrated that if the parasites were removed at day 10 of the infection and transferred to parasite free rats, they became established and remain in the small intestine for a further period of 8 to 9 days. This was later confirmed in an experiment, not reported in this thesis (Douthwaite, Jarrett and Urquhart, unpublished observation), in which 1,000 adult worms from 10-day infections were transplanted to each of 10 rats. Two days later, 5 rats were killed and autopsy revealed a mean worm burden

of 591 ± 102; seven days after transplant, there was still a mean of 287 ± 198 worms present. The original hosts by this time would only have had a mean population of about 10 worms.

Experiment 2

The Transfer of 1000 Adult N. brasiliensis to Immune and Normal Rats

Introduction

It has been well established that after recovery from an initial infection rats possess a degree of resistance to reinfection with larvae (Africa, 1931; Schwartz et al, 1934; Graham, 1934; Chandler, 1932) which is manifested by inhibition of development, stunting of growth, and by reduced longevity and fecundity of the resulting adult population (Chandler, 1937b; Taliaferro and Sarles, 1939). Previous studies of resistance to reinfection have involved the immune response of the rat to a further larval challenge. It was considered that a simpler study of the immunological attack on adult worms could be made by introducing an adult population directly into the small intestine of immune rats.

A preliminary experiment was carried out to see whether this transfer of adult worms by laparotomy could be done quantitatively. A group of 21 rats was given 1,000 adult worms by laparotomy and was killed 4 days later. It was found that 67% of the transferred adult worms became established in the new host (a mean worm burden of 672 ± 231) and that the method was therefore sufficiently quantitative to be of experimental value.

Plan of Experiment

To study the effect of humoral antibody on worms *in vivo*, 1,000 adult *N. brasiliensis* parasites from a 10-day infection were transferred by laparotomy to 5 groups of rats immunised as follows:-

Group	No. of rats	Immunising treatment
A	12	Passive immunisation with Immune serum.
B	10	Passive immunisation with Hyperimmune serum.
C	6	Passive immunisation with Normal serum.
D	21	No serum.
E	7	Active immunisation by a 3,500 larval infection 30 days earlier.

Immune, hyperimmune and normal rat serum were prepared as described in General Methods and Materials.

In Groups A, B and C, the serum was given intraperitoneally on a single occasion at a level of 0.03 ml./gm. rat, 2 days before challenge with adult worms.

All groups were killed 4 days after challenge and the worm burdens counted.

Results

Table 2 shows the mean worm burdens at autopsy of the 5 groups

Table 2

Transfer of 1,000 Adult *N. brasiliensis* to Normal and Immune Rats

Group	No. of rats	Immunising treatment	No. of worms recovered day 4 Mean ± S.D.	Significance (p = 0.05) compared to Group D
A	12	Immune serum	416 ± 231	Significant
B	10	Hyperimmune serum	288 ± 77	Significant
C	6	Normal serum	668 ± 169	Not significant
D	21	No serum	672 ± 231	-
E	7	Experimental larval infection	210 ± 149	Significant

of rats, 4 days after challenge with 1,000 adult N. brasiliensis.

It is clear that normal serum (Group C) had no protective power against this challenge, as the mean worm burden of this group (668 ± 169) was almost identical to that of the control Group D (672 ± 231). Although active immunisation with a previous larval infection resulted in the smallest mean worm burden of 210 ± 149 (Group E) passive immunisation with either immune or hyperimmune serum (Groups A and B) also conferred significant protection. The worm burdens of Groups A and B were 416 ± 231 and 288 ± 77 respectively, showing that immunisation with hyperimmune serum gave better results than immune serum.

Discussion

No satisfactory explanation has yet been given as to how the immune mechanisms of the host operate against the adult stage of a gastro-intestinal parasite. The problem is clearly exemplified in the 'self-cure' of a primary infection of N. brasiliensis in rats infected with 5,000 larvae. On day 10 of the infection, these rats may be supporting a burden of approximately 1,000 worms; egg output is at a maximum and conditions for the parasite are clearly favourable. Yet within 2 days the egg count begins to drop rapidly and by the 15th to 20th day after infection this population

of worms has largely disappeared. In this experiment an attempt was made to study the effect of passively acquired serum antibody on a population of adult worms surgically transplanted into the intestine of previously uninfected rats. It was clearly shown that both immune and hyperimmune serum interfered significantly with either the establishment or the longevity of the challenge population.

Experiment 3

A Quantitative Assessment of the Ingestion of Whole Blood by Adult *N. brasiliensis*

Introduction

Having shown that humoral antibody interferes with the establishment of adult *N. brasiliensis*, the questions remain as to whether antibody has any direct action on the worms, and if so, how does it get to them? Ever since Taliaferro and Sarles (1939) reported the presence of red blood cells in the gut of *N. brasiliensis*, it has been assumed that the parasites feed on blood and on tissues of the host (Weinstein and Jones, 1956; Haley, 1962). If this is the case, the ingestion of blood might provide a means whereby serum antibodies, in adequate quantities, could gain close access to the adult worms. Humoral antibody might then act directly on the worms, in some way debilitating them, so as to make them prone to expulsion. It is possible that this method of feeding could also facilitate the acquisition of worm antigens by the host, especially if secretory enzymes, as suggested by Chandler (1957), are important as antigens.

This experiment was designed to obtain a quantitative measure of the amount of blood consumed by a population of adult worms, by the injection of Cr^{51} labelled red cells.

Materials and Methods

Labelling of Red Blood Cells

Approximately 0.5 ml. samples of venous blood, from the tail of each rat, were collected separately into heparinised tubes and washed several times with cold isotonic saline.

After the addition of about 100 μc of Cr^{51}, as sodium chromate, the bloods were incubated at 37°C, with frequent mixing, for 60 minutes. As the uptake of Cr^{51} by red cells is rapid (Mollison and Veall, 1955), a one hour incubation period allowed maximum uptake of label. The labelled red cells were then washed 3 times with 5 ml. of saline each time, and finally suspended in 1.0 ml. of saline for injection.

Each rat received its own red cells intravenously in the tail.

Experimental Animals

Three groups of rats were selected:-

Group	No. of rats	Stage of Infection
A	3	5,000 *N. brasiliensis* larvae 5 days previously.
B	4	5,000 *N. brasiliensis* larvae 12 days previously.
C	3	Uninfected controls.

Four hours after the injection of the Cr^{51} labelled cells, the rats were anaesthetised with "Trilene" and exsanguinated by cardiac puncture. The samples of blood from each rat were delivered into heparinised tubes. 0.1 ml. aliquots of each sample of whole blood were then measured into separate counting tubes and laked with normal sodium hydroxide. The radioactivity of each sample was assessed twice for periods of 100 seconds, and the mean calculated.

At autopsy the small intestines of the rats were removed and the worms collected into warm saline, by a modified Baermann technique. The worms from each rat, where present, were collected with the intestinal debris, into a broad mouthed Pasteur pipette, washed once with saline, and placed in a glass counting tube. The radioactivity of each sample of worms was measured in a well type scintillation counter. The number of worms present in each sample were then counted microscopically. By comparing these two figures it was then possible to calculate the amount of "blood" ingested by the worms in each case during the 4 hour period, using the following formula:-

$$\text{Vol. blood } (V\mu l) = \frac{\text{Net worm sample count rate}}{\text{Net 1.0 } \mu l \text{ blood count rate}}$$

Results

The radioactivity of the worms was, in fact, too small to be

Table 3

Amount of "Blood" Found in Adult *N. brasiliensis* 4 Hours after the Injection of Cr^{51}-Labelled Red Cells

Group	Rat No.	Count rate of 0.1 ml. blood	Count rate of worms & debris	No. of worms	"Blood" volume in µl
A (5 days after infection)	1	73,240	0	1,000	0
	2	82,600	22	1,100	0.03
	3	112,700	70	1,200	0.06
B (12 days after infection)	1	93,800	9	1,310	0.01
	2	46,600	25	1,200	0.05
	3	78,640	48	1,300	0.06
	4	93,220	62	1,600	0.07
C (non-infected rats)	1	98,650	0	0	0
	2	61,700	31	0	0.05
	3	82,100	16	0	0.02

determined with any degree of accuracy. It can be seen from Table 3 that the count rates of all three groups do not differ, nor are they significantly greater than the background count given by the equipment.

Correspondingly, the worms present in Groups A and B possess no more radioactivity than the intestinal debris obtained in Group C.

Discussion

It was shown in the previous experiment that humoral antibody was important in interfering with the establishment or persistence of the parasites in the small intestine. This raises the question as to whether this effect is due to a direct action of antibody on the worm, or to a more subtle mechanism. For a direct action hypothesis, it is necessary to explain how the antibody comes into contact with the parasite in sufficient quantity, and having done so, what effect it has on the worm.

From the results of this experiment it is clear that no appreciable quantity of blood was ingested by N. brasiliensis during the experimental period. The result is even more clear-cut if a comparison is made between N. brasiliensis and a known blood-ingesting parasite such as Fasciola hepatica (Jennings, Mulligan and Urquhart, 1956). To make

such a comparison it is necessary to express the result in terms of nitrogen to correct for the different sizes of the parasites. The mean blood consumption for N. brasiliensis based on the figures in Table 3 and the nitrogen content of the parasite is 0.06 µl/mg. N for the 4 hour period; a value based on earlier experiments with F. hepatica is 30 µl/mg. N in the same time (Jennings, Mulligan and Urquhart, 1955). It would appear that blood sucking can be largely ruled out as a method for bringing about the contact between adult N. brasiliensis and appreciable amounts of antibody from the host.

Experiment 4

The Effect of Immune Serum on the *in Vitro* Oxygen Uptake of Adult *N. brasiliensis*

Introduction

The respiratory metabolism of parasitic helminths has been reviewed by several authors, and most recently by Rogers (1962). It has been found that all the parasites examined so far respire aerobically when a supply of oxygen is available. There is nevertheless a wide range in respiratory rates. This might result from a difference in the importance of the various metabolic routes involving oxygen, or be due to the fact that not all parasitic helminths possess pigments capable of transporting oxygen (Lazarus, 1950).

Nematodes in general do not have specialised respiratory surfaces and it is thought that oxygen is obtained by diffusion through the general body surfaces. A high concentration of haemoglobin, having a different spectrum from that of the rat, is present in the pseudocoel of adult *N. brasiliensis* (Davenport, 1949). It has been shown by Rogers (1949a) that this pigment is important in the transport of oxygen, and is well adapted to take up oxygen at the low

oxygen tensions, which exist close to the mucosa of the host's intestine.

Schwabe (1957) studied the respiratory rates of free-living and parasitic third stage N. brasiliensis larvae, suspended in normal and immune rat serum. He showed that immune serum would depress the uptake of oxygen by the free-living stage, but not that of the parasitic larvae. However, on the addition of glucose to the sera, the respiratory rates of both the free-living and parasitic larvae tended to be depressed by the immune serum. He suggested that these results gave some support to the enzyme-antienzyme theory of immunity to parasitic worms, first proposed by Chandler (1936).

The respiratory rate of adult N. brasiliensis suspended in a balanced salt solution has also been investigated (Rogers, 1949b; Roberts and Fairbairn, 1965), but as yet no attempt has been made to find out if immune serum interferes with normal respiration.

This experiment was carried out to find if immune serum had any effect on the oxygen uptake of adult worms, and to relate the result to the 'self-cure' phenomenon of the parasite.

Materials and Methods

Normal and Hyperimmune Serum was prepared as described in Experiment 2.

Krebs-Ringer Phosphate Buffer was prepared after the method of Umbriet, Burris and Stauffer (1957) and was made up fresh on the day required. The pH of the sera and Krebs-Ringer phosphate solution were adjusted to 7.4 just before use.

Preparation of Adult Worms

Adult N. brasiliensis were isolated from the small intestines of rats on the 10th day of a single infection with 3,500 larvae and washed three times in warm sterile physiological saline. An aliquot was counted, and the volume of the suspension of worms was adjusted so that 5.0 ml. contained 500 worms.

As each Warburg flask was to contain 500 worms, 5.0 ml. aliquots of the worm suspension were pipetted into individual 10 ml. calibrated centrifuge tubes and allowed to settle to the bottom of the tubes. The supernatant was removed and the worms resuspended in either normal serum, immune serum or Krebs-Ringer phosphate buffer, plus antibiotics (250 units penicillin + 25 mgs. streptomycin/ml.) to give a final volume of exactly 3.0 ml. This was then removed using a broad-mouthed Pasteur pipette and placed in the appropriate Warburg flask. During this operation, care was taken to maintain the temperature of the worms at 37°C and to expose them to as little mechanical damage as possible.

Measurement of Oxygen Uptake

0.5 ml. of M/5 KOH was placed in the outer trough of each Warburg flask and the 3.0 ml. of worm suspension put in the central compartment. The flasks were then rapidly fitted to the manometer and placed in the Warburg bath set at 37°C. The apparatus was adjusted to give a shaking frequency of 80 strokes/minute, each stroke being 2 cm. After 15 minutes equilibrium, readings were taken every hour for the first 3 hours. The flasks were left overnight with the manometer stopcocks open, and readings were resumed for a second 3 hour period the next day. Thus the 'initial period' was 0-3 hours and the 'final period' 20-23 hours.

At the end of the experiment, worms plus media from each flask were removed separately and the worms washed thoroughly with saline. The total nitrogen content of each sample of worms was determined by the micro-Kjeldhal method. The volume of oxygen in microlitres consumed per hour per milligram of worm tissue nitrogen ($Q0_2N$) was calculated for each flask.

Results

From the results shown in Table 4, it can be seen that over the initial 3 hour period, there was no difference in the oxygen uptake

Table 4

Oxygen Consumption Rates of Adult *N. brasiliensis* in Various Media*

Medium	Number of determinations	Initial Period Hour			Final Period Hour		
		1	2	3	21	22	23
Normal serum	12	9.7	8.2	7.8	3.7	3.0	2.4
Hyperimmune serum	6	9.9	8.7	8.5	1.0	0.4	0.5
Krebs-Ringer phosphate buffer	14	6.5	5.4	5.3	4.5	5.1	4.7

* µl oxygen/mg. dry weight/hour; Atmosphere = air; Temperature = 37°C

between worms suspended in either normal serum, immune serum or Krebs-Ringer phosphate solution. In the final period, after a gap of 20 hours, it is clear that the oxygen consumption rate was lower in immune serum than in both normal serum and Krebs-Ringer phosphate buffer. There was also an indication that there was some depression of oxygen uptake with worms in normal serum when compared with Krebs-Ringer phosphate solution. As the adult worms aged, their respiratory rate decreased. After 20 hours incubation in Krebs-Ringer phosphate buffer, the respiratory rate had decreased by about 20%.

Discussion

Whether oxygen consumption is a suitable parameter to study the effect of antibody on N. brasiliensis is open to argument. Although this experiment showed that the oxygen uptake of adult worms suspended in hyperimmune serum was significantly less over a twenty hour period than that of worms in normal serum, it is possible that the parasite depends little on aerobic pathways as the oxygen tension in its environment, close to the mucosa of the small intestine, is relatively low. The apparent inhibition of oxygen uptake after the parasites had been in contact with immune serum overnight is difficult to interpret. This may have nothing to do with a specific inhibition of oxygen uptake, but simply indicates

that a high proportion of the worms are dead or moribund. Such a difficulty of interpretation is inherent in any attempted study of the impairment of metabolism by immune serum. For this reason, it may be preferable to look for anti-metabolic effects of immune serum by using worm homogenates rather than intact parasites.

It may be that the importance of antibody lies, not in direct action on the parasites, but in inducing a state of hypersensitivity in the gut. Reactions between 'fixed' antibody and antigenic materials from the worm could then give rise to a local anaphylactic reaction, which might render the parasite's environment 'unsuitable'. Such a suggestion was made by Stewart (1953), to explain the 'self-cure' of an adult Haemonchus contortus infection by the intake of a new batch of larvae.

The two suggestions discussed above for the role of antibody in the 'self-cure' phenomenon are not mutually exclusive. The increased capillary permeability associated with the local anaphylactic reaction may be important as a means of allowing significant amounts of plasma protein and thus antibody into the gut. This antibody may then act directly on the worms.

General Summary

Section I

1. The adult population of worms which develop from a single dose of 2,500 N. brasiliensis larvae in rats, are rapidly expelled from the small intestine between the 13th and 16th days of the infection and are almost eliminated by the 20th day.

2. A sharp fall in egg production precedes the elimination of the adult population of N. brasiliensis from the small intestine.

3. By transferring adult worms to:-

   a) Rats passively immunised with immune or hyperimmune serum

   b) Rats immunised by prior larval infection

   c) Control rats

it was shown that humoral antibody is important in interfering with the persistence of the parasites in the small intestine.

4. With the aid of Cr^{51} labelled erythrocytes it was shown that N. brasiliensis adults do not consume whole blood in any significant quantity. Blood sucking can therefore be eliminated as a means of bringing antibody into contact with the worm on the mucosa of the lumen of the small intestine.

5. Using the Warburg technique, it was found that the oxygen consumption of adult N. brasiliensis suspended in hyperimmune serum was significantly less over a 20 hour period than that of worms in either normal serum or Krebs-Ringer phosphate buffer.

SECTION II

THE ROLE OF INTESTINAL ANAPHYLAXIS

Introduction

The experiments described in Section I have shown that the passive transfer of immune serum to rats will confer a significant degree of protection against the establishment of a transplanted population of adult *N. brasiliensis*. It was suggested that antibody may not act directly on the parasites but may be important in that it produces a state of hypersensitivity in the small intestine. Subsequent anaphylaxis, stimulated by antigens either secreted or excreted by the adult worms (Thorson, 1953), may render the environment in the small intestine unsuitable for the parasites.

This present section described a series of experiments in which the possible role of intestinal anaphylaxis, in the production of self-cure, was studied.

Experiment 5

The Injection of Evans Blue into Rats at Different Stages of Infection with *N. brasiliensis*

Introduction

The intravenous injection of dyes such as Evans Blue (T1824), which become attached to plasma proteins, can be utilised to demonstrate localised regions of increased capillary permeability and is used in the study of passive cutaneous anaphylaxis (Kabat and Mayer, 1961, p. 311). It therefore seemed that this might be a useful way to demonstrate local anaphylactic reactions in the small intestines of rats injected with *N. brasiliensis*. Two experiments were carried out. In the first, Evans Blue alone was used to demonstrate the oedema associated with natural infection. In the second, Evans Blue was injected together with adult worm antigen to find if a more striking lesion could be produced in the small intestine.

Materials and Methods

The injection of Evans Blue dye and Evans Blue-antigen mixture, and the preparation of both normal and hyperimmune serum are described in General Methods and Materials.

Plan of Experiments

Experiment A

The infected rats consisted of four groups of five, harbouring 5, 10, 14 and 19 day infections of 5,000 *N. brasiliensis* larvae.

A control group of five uninfected rats was also included. All 5 groups of rats were injected with Evans Blue dye via a tail vein and were killed 30 minutes later for examination of their small intestines.

Experiment B

Evans Blue-antigen mixture was injected intravenously into 7 groups of rats. These were:-

Group 1 - Uninfected controls.

Group 2 - 7th day of a single infection of 2,500 *N. brasiliensis* larvae.

Group 3 - 21st day of a single infection of 2,500 *N. brasiliensis* larvae.

Group 4 - 26th day of a single infection of 2,500 *N. brasiliensis* larvae.

Group 5 - 7 days after 3 infections of 2,500, 5,000 and 7,000 larvae at fortnightly intervals.

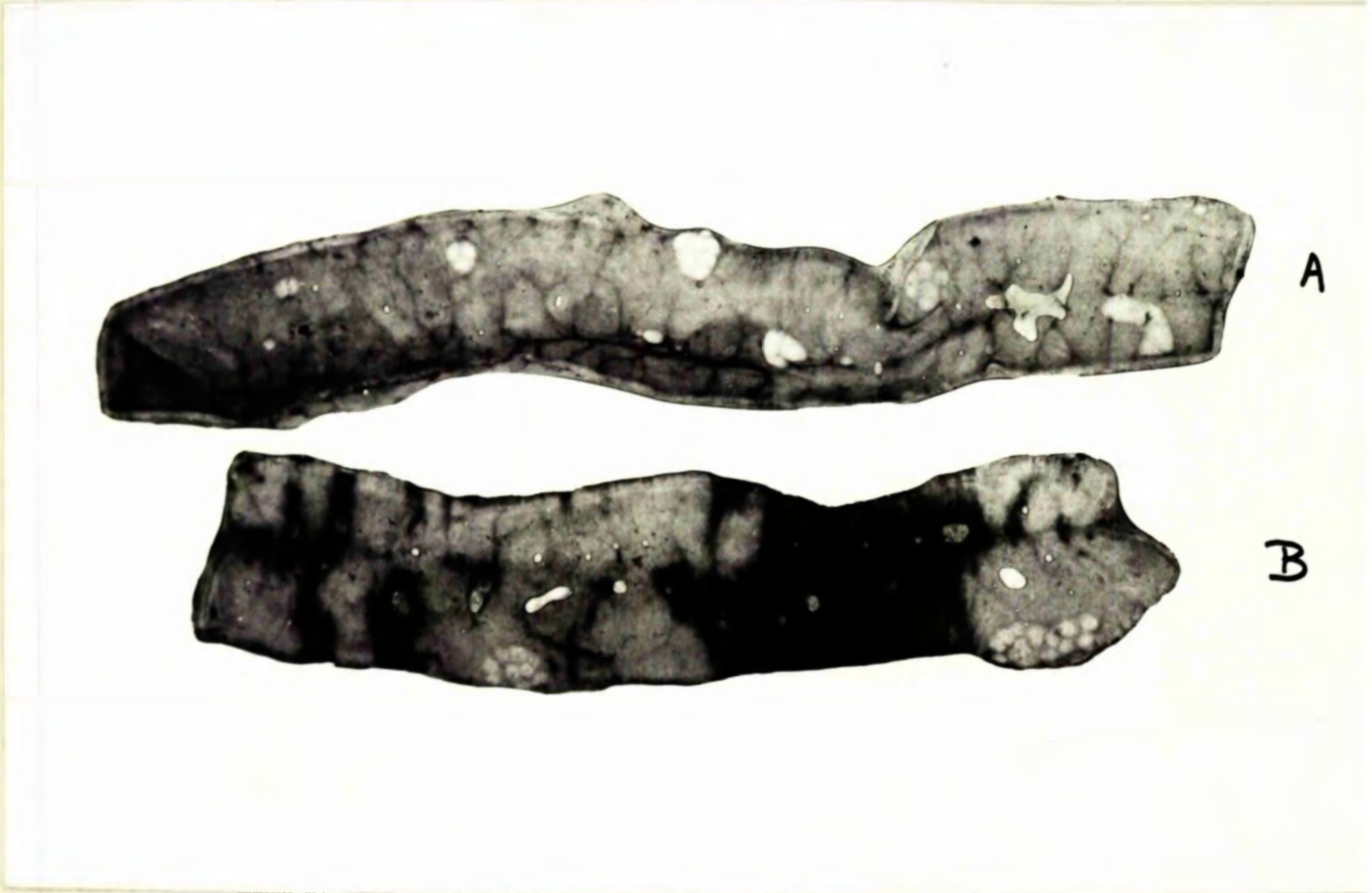


Figure 5. Small intestine from rats 30 minutes after the intravenous injection of Evans Blue.

A. Normal rat

B. 10-day infection.

Group 6 - Passively immunised with hyperimmune serum.

Group 7 - Passively immunised with normal serum.

In Groups 6 and 7 serum was administered intraperitoneally at a dose rate of 3 ml. serum/100 gm. body weight. All the rats were killed 30 minutes after the injection of the Evans Blue-antigen mixture and their small intestines were removed and examined.

Results

Experiment A

In the 5 uninfected control rats the small intestines had a pale blue background with no intense blue spots (Figure 5A).

In the 5, 10 and 14 day infections, speckles and patches of dark blue corresponding to areas of increased capillary permeability were found (Figure 5B). These mostly occurred where worms were found and the intensity of bluing seemed roughly proportional to the concentration of parasites. The rats infected 19 days prior to the experiment had lost their worm burdens and their small intestines were similar to those of the control rats.

Experiment B

Apart from the controls (Group 1), the small intestines of all

Table 5

Changes in the Small Intestine Produced by the Injection of Evans Blue-antigen Mixture into a Group of Normal Rats and Groups of Rats at Different Stages of Infection with *N. brasiliensis*

Group No.	Stage of Infection	Rat No.	Manifestation of reaction		
			Intensity of blue colouration (intestine & mucus)	Amount of Mucus	Hyperaemia
I	Control (uninfected) Rats	1	0	0	0
		2	1	0	0
		3	1	0	0
		4	0	0	0
		Mean	0.5	0	0
2	7-day Infection	1	2	1	0
		2	4	1	1
		3	2	0	0
		4	3	1	0
		Mean	2.8	0.8	0.3
3	21-day Infection	1	4	1	1
		2	6	4	0
		3	4	3	2
		4	4	1	5
		Mean	4.5	2.3	2
4	26-day Infection	1	6	4	8
		2	8	8	8
		3	5	4	7
		4	9	8	10
		Mean	7	6	8
5	Hyperimmune Rats	1	10	4	1
		2	5	9	5
		3	4	10	10
		4	9	4	6
		Mean	7	7	5.5

the rats in Groups 2 to 5 showed very marked reactions, a characteristic feature of anaphylaxis in the rat (Kabat and Mayer, 1961, p. 287). These consisted of extensive blueing of the mucosa, a macroscopically apparent hyperaemia and an increased amount and fluidity of deeply stained mucus. An arbitrary score between 0 and 10 was assigned to each feature of the gut reaction and the results are summarised in Tables 5 and 6.

From Table 5 it is clear that by all criteria the reaction increased in intensity in the order: Control, 7th, 21st and 26th day infection. The hyperimmune animals (Group 5) gave reactions in most cases comparable to those of the 26th day infections.

The results in Table 6 show that all the features of the anaphylactic reaction can be passively transferred in the serum.

Discussion

It cannot be assumed that the increased capillary permeability demonstrated in Experiment A was necessarily due to a local anaphylactic reaction in the small intestine, rather than to irritation caused by the worms or their products. However, the result of Experiment B indicates that as early as 7 days after infection the intestinal mucosa is capable of undergoing anaphylaxis when exposed to adult N. brasiliensis antigen. This suggests that

Table 6

Results of Injecting of Evans Blue-antigen Mixture into Passively Immunised and Normal Rats

Group No.	Rat No.	Manifestation of Reaction		
		Intensity of blue colouration	Amount of Mucus	Hyperaemia
6 - Hyperimmune Serum	1	8	6	5
	2	5	6	0
	3	7	7	0
	4	7	6	3
	5	7	8	4
	Mean	6.8	6.6	2.4
7 - Normal Serum	1	0	0	0
	2	0	0	0
	3	0	0	0
	Mean	0	0	0

anaphylaxis may occur in a localised form during the self-cure reaction, at points where the antigenic stimulus is being supplied by the worms themselves. The physical changes associated with anaphylaxis, i.e., fluid and mucus passing into the gut, may then be responsible for the expulsion of the adult population by creating an unsuitable environment for the parasites' survival. Alternatively, if humoral antibody were to have a direct action on the parasites, the increased capillary permeability associated with a local anaphylactic reaction may provide the means whereby antibody escapes in quantity on to the mucosal surface of the small intestine.

Experiment 6

The Effect of Antihistamine and Anti-5-hydroxytryptamine Drugs on the Hypersensitivity of Rats Infected with N. brasiliensis

Introduction

In the last experiment it was shown that local anaphylaxis occurred in the small intestine of immune rats, following the intravenous injection of adult N. brasiliensis antigen. This reaction was manifested by fluid and mucus passing into the gut as a result of increased capillary permeability. In order to assess the relevance of this reaction to the self-cure, it was decided to investigate the use of drugs which might abolish this reaction and to study their effect on self-cure.

The inhibiting materials tested were the antihistaminic drugs mepyramine maleate(1) and promethazine hydrochloride(2), and the anti-5-hydroxytryptamine compound lysergic acid(3). The latter was tested because of the suggestion that 5-HT is more important than histamine in anaphylaxis in the rat (Page, 1958; Parratt and West, 1957; Medakovic, 1959).

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(1) Anthisan: May & Baker Ltd., Dagenham.
(2) Phenergan: May & Baker Ltd., Dagenham.
(3) Delysid: Sandoz Products Ltd., London.

Plan of Experiments

Three experiments were completed. These were:-

Experiment A

A preliminary experiment on the short term effects of mepyramine maleate (Anthisan) and lysergic acid (Delysid) on rats 22 days after a single infection of 5,000 *N. brasiliensis* larvae was carried out as follows:-

Group 1 - 22-Day Infection

Group 2 - 22-Day infection + 100 μ gms. lysergic acid/rat

Group 3 - 22-Day infection + 1 mg. mepyramine maleate/rat

Group 4 - Controls - no infection.

The injections of Anthisan and Delysid were given subcutaneously 20 minutes prior to a shocking dose of Evans Blue-antigen mixture. The rats were all killed 30 minutes after being shocked and their small intestines were examined.

Experiment B

In this experiment, the long term effects of lysergic acid and mepyramine maleate on a single infection of 2,000 *N. brasiliensis* larvae was studied.

Three groups of 5 rats were treated daily as follows:-

Group 1 - 50 µgms. lysergic acid/rat, beginning on day 5.

Group 2 - 250 µgms. mepyramine maleate/rat beginning on day 5.

Group 3 - Controls - saline.

Egg counts were carried out daily on each group from the 9th to the 19th day of the infection.

Experiment C

Here the antihistaminic drug, promethazine hydrochloride (Phenergan), was studied because of its more lasting effect compared with mepyramine maleate. Four groups of infected rats were treated daily as follows:-

Group 1 - 2.5 mgs. promethazine HCl beginning on day 10.

Group 2 - Controls - saline beginning on day 10.

Group 3 - 2.0 mgs. promethazine HCl twice daily beginning on day 10.

Group 4 - Controls - saline twice daily beginning on day 10.

Daily faecal egg counts were carried out and the rats were killed on the 16th day of the infection of 2,000 N. brasiliensis larvae, and the worm burdens counted.

Results

Experiment A

From the results shown in Table 7 it is clear that neither lysergic acid nor mepyramine maleate, in the amounts used, interfered significantly with intestinal anaphylaxis, when administered 20 minutes prior to the shocking dose of antigen. Mepyramine maleate did however appear to abolish hyperaemia in this experiment.

Experiment B

The results of daily faecal egg counts of rats treated with lysergic acid and mepyramine maleate from the 9th to the 19th day of the infection showed that by day 19 the egg output in all 3 groups had fallen almost to zero. Treatment with these drugs, in the amounts used, had no effect on the self-cure reaction when estimated by faecal egg counts.

Experiment C

Tables 8 and 9 show the results of daily faecal egg counts and worm burdens at autopsy on rats treated with promethazine hydrochloride. Although self-cure did not appear to be influenced by the antihistamine when the faecal egg count was used as a criterion, the worm counts at autopsy in Group 3, where promethazine

Table 7

The Effect of a Single Dose of Lysergic Acid and of Mepyramine Maleate on the Reaction Produced by Intravenous Injection of Adult Antigen and Evans Blue into 22-day Infected Rats

Manifestation of Reaction	Group 1 22-Day Infection	Group 2 22-Day Infection + Lysergic Acid	Group 3 22-Day Infection + Mepyramine Maleate	Group 4 Control (no infection)
Colour	7	9	5	0
Amount of Mucus	6	10 (watery)	5	0
Hyperaemia	3	9	0	0

The results are expressed as the mean of the 3 rats in each group.

Table 8

The Daily Faecal Egg Counts of Rats Infected with 2,000 *N. brasiliensis* Larvae during Treatment with Promethazine Hydrochloride

Days after infection	Group 1 2.5 mgs. Phenergan per day	Group 2 Control	Group 3 2.0 mgs. Phenergan twice daily	Group 4 Control
7	NS	NS	45,500	70,100
8	NS	NS	52,100	41,800
9	80,000	46,200	49,500	37,200
10	43,700	39,000	43,000	52,400
11	36,400	15,850	51,000	65,000
12	34,800	6,200	7,250	34,500
13	22,400	10,800	3,300	8,100
14	4,850	5,700	32,150	5,250
15	2,150	250	2,250	1,300
16	50	50	NS	NS

NS = No sample

Table 9

The Worm Burdens at Autopsy of Rats Infected with 2,000 *N. brasiliensis* Larvae after Treatment with Promethazine Hydrochloride

Rat No.	Group 1 2.5 mgs. Phenergan per day	Group 2 Control	Group 3 2.0 mgs. Phenergan twice daily	Group 4 Control
1	926	20	1,186	82
2	1,230	1,790	740	652
3	650	66	1,000	13
4	1,120	486	506	540
5	650	416	1,326	960
6	970	130	426	146
7	610	80	1,086	380
8	340	90	620	546
9			606	640
10			986	240
Mean	812 ± 298	385 ± 593	848 ± 304	420 ± 300
♂:♀	3:2	3:2	3:2	3:2
P	-	$0.1 > P > 0.05$ Not significant	-	$0.01 > P > 0.001$ Significant

HCl was given twice daily, showed that a significantly greater number of worms were present. It therefore seemed that promethazine hydrochloride had interfered with the full expression of the self-cure reaction.

Discussion

Although it is now generally accepted that both histamine and 5-hydroxytryptamine are released during anaphylaxis in the rat (Austen, 1965) the exact contribution, if any, of these compounds to this reaction has not yet been ascertained.

Despite the fact that Sanyal and West (1958) were unable to protect rats significantly against systemic anaphylaxis by the use of an antihistamine or a specific anti-5HT drug, other workers have reported positive evidence that these drugs will interfere to some extent with the anaphylactic reaction (Mota, 1957). For example, Halpern (1955) showed that promethazine hydrochloride (Phenergan) protected rats from almost all the symptoms of shocking with dextran.

The mechanism involved in the self-cure reaction of N.brasiliensis may have much in common with the sudden loss of T. spiralis from the intestine of the mouse at the end of the second week of the infection. It is interesting that Campbell, Hartman and Cuckler (1963) were able

to prolong the intestinal phase of trichinosis in the mouse by the use of antihistamine or anti-5HT compounds.

The failure of mepyramine maleate and lysergic acid to block the anaphylaxis caused by the intravenous injection of antigen described in Experiment A, could perhaps be due to an inability to provide a sufficient concentration of the antagonist at the actual site of the reaction (Mongar and Schild, 1962). In support of this theory, is the fact that twice daily doses of promethazine hydrochloride, given throughout a primary infection of N. brasiliensis, resulted in significantly more worms at autopsy on the 16th day compared to the untreated control group, whereas one daily dose did not.

A second theory which may explain the failure of antihistamines and anti-5HT drugs to block the self-cure reaction, is that some agent other than histamine or 5HT may be involved in anaphylaxis in the rat.

It must be remembered however, in the interpretation of these results, that the partial inhibition of self-cure by promethazine hydrochloride is not necessarily proof that an anaphylactic reaction occurred. It may be that histamine or 5HT was released purely as a result of inflammation or trauma caused by mechanical damage to the mucosal surface of the small intestine by the parasites. However, it can be said that these results are not incompatible with the theory first described in Section I that a local anaphylactic reaction is an essential part of self-cure in N. brasiliensis infections.

Experiment 7

The Effect of a Cortisone Derivative on the Self-cure of *N. brasiliensis* Infection and the Hypersensitivity Reaction

Introduction

It was shown by Weinstein (1955) that the administration of cortisone to white rats during the course of a primary infection with *N. brasiliensis* resulted in the suppression of the inflammatory response in the skins of these animals when they were subsequently challenged with infective larvae. All the larvae successfully penetrated this barrier but many were then trapped in cellular reactions in the lungs, liver and peritoneal membranes during their migration. The resulting adult population was however greater in number and larger in size than those of a non-treated immune group. In a further experiment, rats which were first immunised with *N. brasiliensis* and then treated with cortisone a few days prior to a larval challenge, showed a reduction in the immune response, which was manifested by a suppression of the cellular reaction in the skin, but not in the internal organs of the rat. In this case, there was no difference in the numbers of the resulting population of adults, but the worms were larger as compared to the non-treated immune controls.

In view of these results, it was thought that it would be interesting to follow on from this point in Weinstein's work and study the effect of cortisone on the self-cure reaction, particularly as at this time Ogilvie (personal communication) reported that cortisone prevented self-cure of a primary infection of N. brasiliensis, even when administration was delayed as late as the 9th day of the infection.

Plan of Experiment

This experiment was divided into two parts, A and B.

Experiment A

In this first experiment the effect of cortisone on the self-cure reaction of a primary infection of N. brasiliensis was studied.

Two groups of 8 rats were infected with 2,000 N. brasiliensis larvae. On the 9th, 11th and 14th days of the infection, one group of rats was given 0.1 mg. betamethazone * - a proprietary cortisone derivative - together with 2.5 mgs. Terramycin, subcutaneously. The control group was given saline.

Daily faecal egg counts were carried out and the rats were killed on day 16 and the worm burdens determined.

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* Betsolan: Glaxo Laboratories, Greenford, Middlesex, England.

Experiment B

In the second part of the experiment the effect of cortisone on an artificially induced anaphylaxis was studied.

A group of 5 rats, which had undergone self-cure from a primary infection of 2,000 N. brasiliensis larvae 3 weeks previously, were given 0.2 mgs. of betamethazone, subcutaneously, on two occasions, 18 and 2 hours prior to an intravenous injection of Evans Blue-antigen mixture.

Results

Experiment A

The worm burdens at autopsy and the daily faecal egg counts are shown in Tables 10 and 11 respectively. It can be seen that on the 16th day of the infection the cortisone treated rats had a mean worm burden of 1,388 ± 304 N. brasiliensis as compared with 385 ± 593 in the untreated controls. It is clear then that cortisone not only prevented the sudden drop in egg production (Table 11) associated with self-cure, but also blocked the expulsion of the worm population.

Experiment B

Clinical signs of shock, following the injection of Evans Blue-

Table 10

Effect on the Worm Counts at Autopsy, on the 16th Day, of Betamethazone Subcutaneously Administered to Rats on the 9th, 11th and 14th Days of a Primary Infection of 2,000 *N. brasiliensis* Larvae

No. of Rat	Cortisone treated Rats	Control Rats
1	1,910	20
2	1,010	1,790
3	1,580	66
4	1,380	486
5	1,300	416
6	960	130
7	1,270	80
8	1,290	90
Mean	1,388 ± 304	385 ± 593
♂:♀	1:1	2:1
	$P < 0.001$ Significant	

Table 11

Effect on the Faecal Egg Count of Betamethazone Subcutaneously Administered to Rats on the 9th, 11th and 14th Days of a Primary Infection of *N. brasiliensis*

Day of Infection	Cortisone treated Rats (e.p.g.)	Control Rats (e.p.g.)
9	47,800	46,200
10	48,400	39,000
11	33,400	15,850
12	78,200	6,200
13	76,600	10,800
14	49,500	5,700
15	89,800	250
16	23,200	50

antigen mixture, were slight, and when the rats were killed 30 minutes later, the small intestines appeared almost normal. In contrast, two control rats of similar immunological status which were not treated with cortisone before the injection of antigen, had very hyperaemic and deeply blue stained small intestines.

Discussion

Mast cell concentration has been correlated with the histamine content of tissue (Riley and West, 1953; Ehrich, 1953; Smith, 1958) and it has been shown that systemic anaphylaxis in the rat is accompanied by mast cell disruption and histamine release (Mota, 1962 and 1963a). It was reported in 1951 by Cavallero and Bracceini that when adult rats were treated with cortisone over various periods of time, they constantly showed a marked reduction in mast cell counts. More recently, by studying the quantity of histamine mobilised by various histamine releasors and the speed at which tissue histamine was replenished, Neveu (1960) showed that cortisone had an inhibitory action on the biosynthesis of histamine. As yet, however, the mechanism of the action of cortisone is still obscure.

More recent work by Ogilvie (1965) has shown that by giving large

doses of cortisone to immune rats, the effects of acquired resistance to N. brasiliensis could be totally suppressed. If the cortisone treatment was then stopped, expulsion of the adult worm population quickly ensued.

A similar observation was reported by Coker (1956) with T. spiralis infections in mice. He showed that the expression of immunity to reinfection was blocked on the administration of cortisone, which greatly suppressed cellular reactions in immune animals. That cortisone did not affect antibody production was shown by the fact that precipitating antibodies were present before the cellular reactions appeared and the titres of these antibodies were not apparently suppressed.

In summary, the results of this experiment confirm the work of Ogilvie - subsequently published in 1964 - that cortisone treatment of rats during an initial infection of N. brasiliensis results in a suppression of the immune expulsion of the parasites at self-cure. The role of cortisone in blocking the artificially induced anaphylaxis described in Experiment B, may possibly be associated with its property as an antagonist to certain permeability factors (see discussion by Rose, 1958, p. 600). However, in spite of the obscure role of cortisone, these results do provide supportive evidence that local anaphylaxis in the intestinal mucosa may be a necessary component of the self-cure reaction in N. brasiliensis infections in rats.

Experiment 8

The Fate of Adult N. brasiliensis Transplanted to the Small Intestine of Rats which had recently Undergone Self-cure

Introduction

In the first section of this thesis it was suggested that a local anaphylactic reaction in the intestinal mucosa of the rat may be an essential part of the immune expulsion of the adult worm population in the second week of a primary infection of N. brasiliensis larvae. Evidence in support of this theory has been presented up to this point in Section II and may be summarised as follows:-

1) Rats which had recently undergone self-cure of a single larval infection were susceptible to anaphylaxis when injected intravenously with N. brasiliensis antigen. This reaction was principally expressed in gross alterations in the small intestine, manifested by increased capillary permeability, hyperaemia and increased fluidity and amount of mucus.

2) The administration of the antihistamine promethazine hydrochloride partially inhibited the self-cure reaction.

3) The use of a cortisone derivative completely blocked both the natural self-cure and an artificially induced anaphylaxis.

4) When hyperimmune serum was administered to normal rats, it had two effects. It conferred protection against a transplanted adult infection and also produced susceptibility to intestinal anaphylaxis.

In view of these results it was considered important to determine the fate of the adult worm population transferred to the small intestine of an actively immunised rat. If a hypersensitive reaction is involved in self-cure, it was considered that this might be more rapidly evoked in an actively immunised rat. Accordingly, the following experiment was carried out.

Plan of Experiment

1,000 adult N. brasiliensis from 10-day infections were collected as described in Methods and Materials, and transferred by laparotomy to the small intestine of each of 8 rats which had thrown off a single larval infection of 2,000 larvae, three weeks previously, and to each of 8 uninfected control rats.

Four rats from each group were killed at 20 and 72 hours after receiving the worms, and the number of N. brasiliensis in the small intestines at autopsy were counted.

Faecal egg counts were carried out daily during the 72 hours of the experiment.

Table 12

Worms Recovered from Immune and Normal Rats at Different Times after Receiving 1,000 Adult *N. brasiliensis*

Group	Rat Nos.	Worms Recovered			
		20 hours		72 hours	
		Total	♂:♀	Total	♂:♀
Immune Rats	1 - 4	547 ± 120	10:9	-	-
	5 - 8	-	-	161 ± 134	7:2
Normal Rats	1 - 4	594 ± 66	10:7	-	-
	5 - 8	-	-	507 ± 105	10:9
Significance of difference between test and control groups		N.S.		$P < 0.01$	

Results

It is clear from the results in Table 12 that there was no difference in the worm burdens of the two groups of rats 20 hours after challenge. However, after 72 hours, there was a significant difference in the number of parasites remaining in the small intestine, the immune rats having 161 ± 134 worms as compared to 507 ± 105 in the control group. The sex ratios in the two groups had also changed showing that there had been a preferential expulsion of females in the immune rats.

The daily faecal egg counts shown in Table 13 show that although there was no difference in worm burdens at 20 hours, egg production by the parasites in the immune group was only 1,000 e.p.g. as compared to 14,000 e.p.g. in the control group at this time. The count in the former group then fell to 200 e.p.g. by 72 hours while the control group had reached a value of 23,000 e.p.g.

Discussion

The results of this experiment, summarised in Tables 12 and 13, show that the expulsion of the adult worms introduced surgically into the small intestines of immune rats had not begun within 20 hours of the transfer. This is contrary to the usual speed of a

Table 13

Daily Faecal Egg Counts of Immune and Normal Rats after Receiving 1,000 Adult *N. brasiliensis*

Group	Mean eggs per gm. faeces		
	Day 0 - 1	Day 1 - 2	Day 2 - 3
Immune	1,000	800	200
Normal	14,000	21,000	23,000

reaction associated with anaphylaxis. On the other hand, it may be that this time was required for sufficient antigen to be absorbed, or for a secondary antibody response to occur, or alternatively, for the worms to be finally dislodged: in the latter connection it is possibly significant that there appeared to be a selective effect on the females in that egg production was specifically inhibited from the time of transplant and the females were also preferentially expelled.

Proof that the physical changes of anaphylaxis alone are not responsible for self-cure is established by the recent work of Barth, Jarrett and Urquhart (1966). They showed that when rats were infected with an adult population of N. brasiliensis, by laparotomy, and then subjected to intestinal anaphylaxis induced by an unrelated antigen-antibody system (ovalbumin and Haemophilus pertussis) the physical changes associated with anaphylaxis did not result in the expulsion of the parasites. On the other hand, anaphylaxis induced by this system in passively immunised rats produced a significant reduction in their transplanted population of adult worms, compared to rats which were passively immunised alone, or to rats subjected to ovalbumin induced anaphylaxis alone. Ogilvie (1964), using rats infected subcutaneously with N. brasiliensis larvae, also failed to accelerate self-cure of a primary infection by the production of ovalbumin-induced anaphylaxis alone.

More recently Jones and Ogilvie (1967) have reported that passive transfer of immunity to N. brasiliensis with pooled antiserum from immune rats was neutralised in vivo by the intravenous injection of small amounts of a saline extract of adult worms. This inhibition of protection was associated with systemic anaphylaxis. On first examination this result appears to conflict with that reported by Barth et al (1966) but it may be explained by assuming that protective antibodies, as well as anaphylactic antibodies, were neutralised when specific worm extract was injected intravenously into passively immunised rats. (The nature of these antibodies will be reviewed in Section III.)

General Summary

Section II

1. The intravenous injection of Evans Blue dye into rats harbouring a patent single infection of N. brasiliensis, resulted in intense blueing of the intestinal mucosa corresponding to areas of increased capillary permeability. The intensity of blueing was roughly proportional to the concentration of parasites.

2. As early as 7 days after an initial infection the intestinal mucosa of rats is capable of undergoing anaphylaxis when exposed to adult N. brasiliensis antigen injected intravenously. The intensity of anaphylaxis is increased in post-patent or hyperimmune rats.

3. Neither lysergic acid nor mepyramine maleate, in the amounts used, interfered significantly with intestinal anaphylaxis induced by the intravenous injection of N. brasiliensis antigen. Mepyramine maleate did however appear to abolish hyperaemia.

4. Treatment of rats with lysergic acid and mepyramine maleate from the 9th to the 19th day of a single infection had no effect on the self-cure reaction, when estimated by faecal egg counts.

5. Treatment of infected rats with promethazine hydrochloride had no effect on the faecal egg count at the time of self-cure, but resulted in a significantly greater number of worms being present in the small intestine at autopsy on the 16th day.

6. Cortisone treatment of rats during an initial infection of N. brasiliensis resulted in the suppression of the immune expulsion of the parasites at the time of self-cure.

7. The administration of cortisone, 18 and 2 hours prior to an injection of Evans Blue-antigen mixture into immune rats, blocked the usual anaphylactic reaction.

8. There was no difference in the number of worms present in the small intestine of immune as compared with non-immune rats, 20 hours after receiving a transplant of 1,000 adult N. brasiliensis, but at 72 hours there were significantly less parasites in the immune group.

Egg production by the parasites was specifically inhibited in the immune rats from the time of transplant.

9. These results are compatible with the possibility that a local anaphylactic reaction in the intestinal mucosa is an essential part of the self-cure phenomenon in rats infected with N. brasiliensis.

SECTION III

HISTAMINE IN THE SELF-CURE REACTION

Introduction

Stewart in 1953 suggested that the self-cure phenomenon in sheep infected with Haemonchus contortus depended not on any direct reaction of antibody on the parasite, but on a state of specific hypersensitivity, in which a local anaphylactic reaction in the abomasal mucosa gave rise to conditions which were unsuitable for the parasites' survival. It was suggested in Section I of this thesis that a similar type of phenomenon may be wholly or partly responsible for the self-cure reaction in Nippostrongylus brasiliensis infections in the rat. Observations in support of this theory, that anaphylaxis may be involved in self-cure, were presented in Section II of this thesis. These were as follows:-

1) As early as 7 days after an initial infection rats could be shocked by the intravenous injection of N. brasiliensis antigen. This reaction was principally expressed in gross alterations in the small intestine of the rat, which is known to be the target organ for shock (Sanyal and West, 1958; West, 1959; Mota, 1963a). It was manifested by increased capillary permeability, hyperaemia and increased amount and fluidity of mucus in the intestinal lumen. The reaction increased in intensity in post-patent or hyperimmune rats and the anaphylactic lesions produced resembled the lesions observed at self-cure.

2) Susceptibility to intestinal anaphylaxis was passively transferred to normal rats by the injection of hyperimmune serum prior to shocking with antigen.

3) Twice daily doses of the antihistamine promethazine hydrochloride interfered significantly with the expulsion of an adult N. brasiliensis population at self-cure, but did not prevent the rapid drop in egg production by the parasites.

4) The administration of cortisone to rats completely blocked the self-cure of a primary infection and abolished systemic anaphylaxis induced in immune rats by the intravenous injection of N. brasiliensis antigen.

Furthermore, it has been shown that reaginic antibodies, similar to human reagins (Stanworth, 1963) appear in the serum of rats 1-2 weeks after the self-cure of a primary N. brasiliensis infection (Ogilvie, 1964). It has since been demonstrated that a saline extract of the adult worms contains an allergen specific for these antibodies (Jones and Ogilvie, 1967).

Further support of the theory that an anaphylactic reaction may be an important factor in the self-cure reaction is the finding that anaphylaxis induced by an unrelated antigen-antibody system (ovalbumen adjuvanted with the reagin-producing B. pertussis) caused the

accelerated expulsion of adult worms transplanted to passively immunised rats, as compared with rats passively immunised alone (Barth et al. 1966).

Although it has not yet been shown conclusively that anaphylaxis does occur at self-cure, these results do provide strong evidence in support of the theory.

If an anaphylactic reaction is involved in the phenomenon, the question arises as to which mediator or mediators are involved. The rat is known to have 2 types of mast cells. The first type occur in the connective tissue of most organs, except the mucus membranes lining the gastro-intestinal tract, and are most numerous in the tongue and skin. They have been shown to contain both histamine (West, 1955; Barrnett, Hagen and Lee, 1958; Humphrey, Austen and Rapp, 1963) and 5-hydroxytryptamine (Benditt, Wong, Arase and Roeper, 1955b; Cass, Marshall and Riley, 1958; Benditt, Holcemberg and Lagunoff, 1963). The second type of mast cell is found in the gastro-intestinal tract and differs both morphologically and histochemically from the first type (Enerback, 1966a). It contains a catecholamine, possibly dopamine, but little if any 5-HT (Enerback, 1966d). It has been established that the small intestine has a high histamine content (Mota, Beraldo, Ferri and Junqueira, 1955) but whether it

occurs in the mast cells or not has yet to be shown. In support of the opinion that it may be of mast cell origin is the observation that whole body X-irradiation caused a virtual disappearance of globule leucocytes (intestinal mast cells) from the small intestine of the rat (Kent, Baker and Pliske, 1956) and reduced the histamine content to 7% of the normal value (Eisen, Ellis and Wilson, 1956). While it is possible that the histamine may be bound to cells other than intestinal mast cells, these two findings may be linked.

It has been established that systemic anaphylaxis in rats induced by various antigen-antibody systems and the anaphylactoid syndromes induced by certain histamine liberators is accompanied by the release of histamine (Spuzic and Halpern, 1958; Gozsy and Kato, 1962a and 1962b; Mota, 1957, 1958, 1962 and 1963a; Cody, Code and Kennedy, 1963). It would seem reasonable therefore to assume that if a local anaphylactic reaction were to occur during the period of self-cure, it would be accompanied by a release of histamine. However, one of the problems in determining if this type of reaction is involved is that, if it does occur, it probably proceeds in relatively small areas of the intestinal mucosa and might be spread over several days, in contrast to the grossly visible and immediate response to the intravenous injection of N. brasiliensis antigen. As the estimation of urinary histamine may be used as an

index of the amount of histamine released in the whole body at any one time (Wilson, 1954; Gaddum, 1955), it was considered that it might be possible to detect possible increases in histamine excretion at self-cure, using this technique.

Since an increase in histamine excretion might result purely from inflammation or trauma in the intestinal mucosa, urinary histamine output was estimated before, during and after the approximate period of self-cure.

Materials and Methods

Culture of Larvae
Infection of Rats
} were as described in General Methods and Materials.

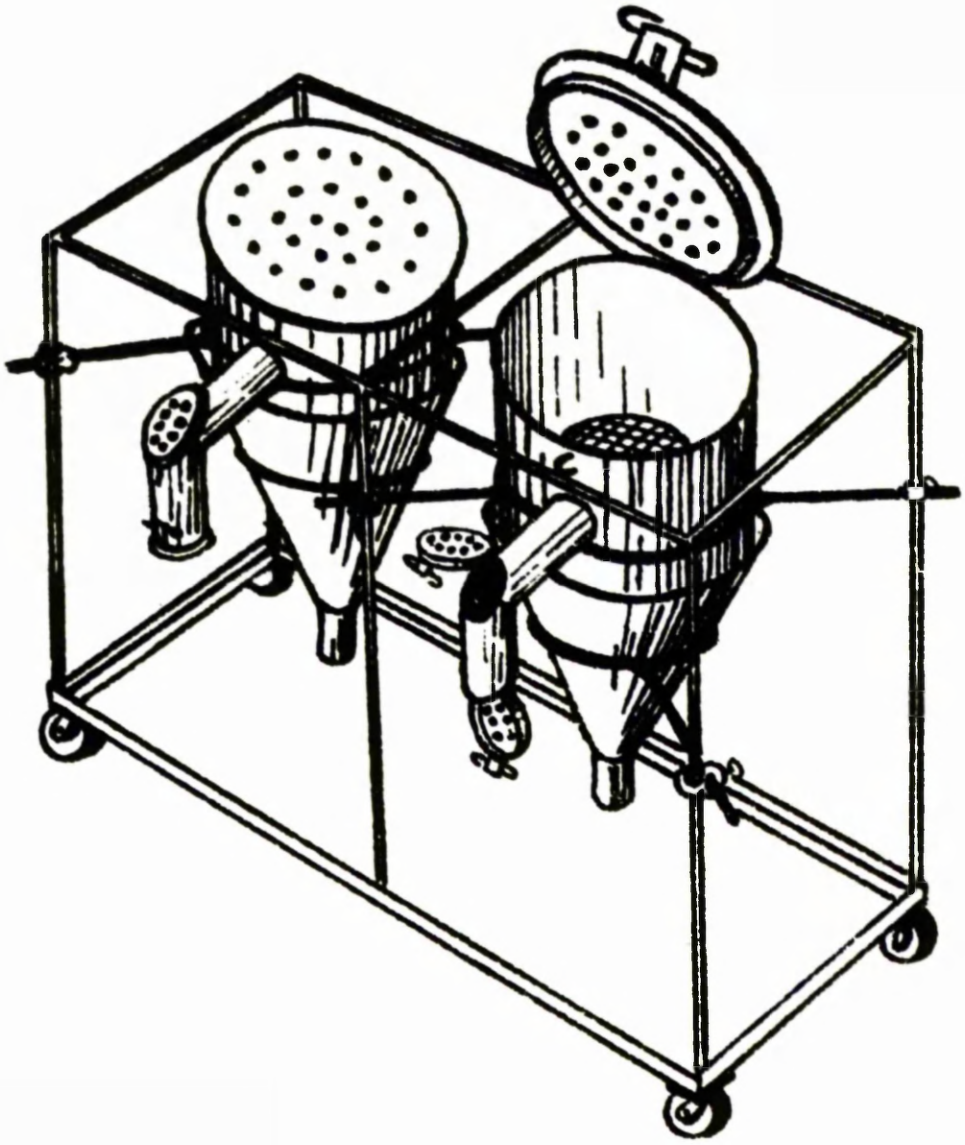
Experimental Animals

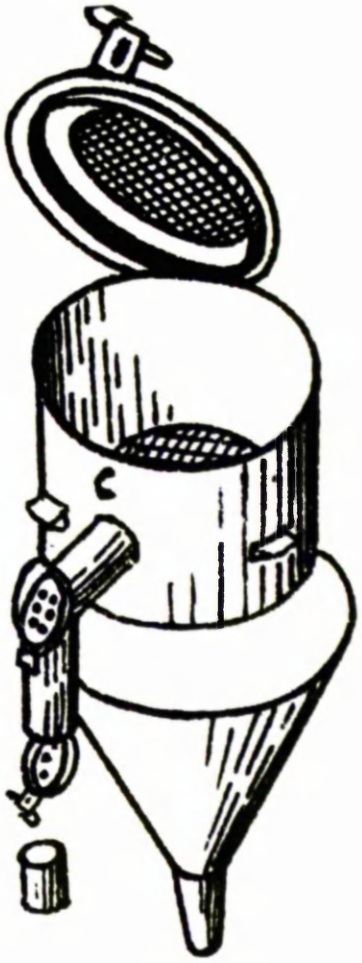
All of the rats used were of the hooded Lister strain and were 3 months old at the start of the experiments.

In Experiment 9 the rats were kept in standard cages. In Experiments 10 and 11, they were housed in individual metabolism cages (Figure 6) which enabled the entire daily output of urine from each rat to be collected free from contamination with food and faeces. In all cases, the rats were put into the cages 3 days before the first histamine estimations were made, to acclimatise them to their surroundings.

Figure 6

Metabolism Cage - Type No. B.7. (E.K. Bowman Ltd., London).





Diet

Food (Diet 86) and water were available at all times. In Experiments 10 and 11 the weight of food consumed/rat/day was measured, as a marked increase in food consumption on a particular day could result in an increased excretion of conjugated histamine (Gaddum, 1955).

Estimation of Free and Total Histamine

The 24-hour urine output of each rat was collected directly into a conical flask containing 2 ml. of N/3 HCl. Each sample was then centrifuged at 2,000 r.p.m. for 5 minutes and the supernatant divided into two equal parts. To the first part, N/3 HCl was added to adjust the pH to less than 4.0 and it was heated in a boiling water bath at 100°C for 1 hour. The samples were then maintained at -20°C until bio-assay could be carried out. The free histamine concentration was determined by bio-assay on the guinea pig ileum preparation at 37°C in Tyrode solution by the method described by Wilson (1954).

The second aliquot was subjected to acid hydrolysis to convert conjugated histamine to the free form. This was done by adding 1 ml. of concentrated HCl and boiling for 1 hour in a conical flask. Distilled water was added during boiling to maintain the volume. The sample was then stored as above until

required. By this method the total histamine concentration was determined by bio-assay in Tyrode solution containing 0.2 µg/ml. atropine sulphate.

During bio-assay the nature of the contracting substance was confirmed by the addition of mepyramine maleate, a specific antagonist to histamine.

Estimation of Tissue Histamine

Weighed tissue samples were ground, using a mortar and pestle, in N/3 HCl with washed sand. The homogenates were boiled for 30 minutes to release histamine and stored at sub-zero temperature until bio-assay could be carried out.

Just prior to bio-assay, samples were neutralised with 1N NaOH and were diluted and assayed on the atropinised guinea-pig ileum preparation.

In all cases, the nature of the contractions was checked by the use of the specific histamine antagonist, mepyramine maleate.

Experiment 9

The Daily Urinary Histamine Output of a Group of Male Rats Infected with *N. brasiliensis*

Introduction

As the estimation of urinary histamine may be used as an index of the amount of histamine released in the whole body at any one time (Wilson, 1954; Gaddum, 1955), it was considered that this would be a useful method of determining whether or not self-cure in *N. brasiliensis* infections in rats was accompanied by a detectable release of histamine.

Rats normally have a high daily output of urinary histamine which is in two forms, as free histamine and as conjugated histamine (Anrep, Ayadi, Barsoum, Smith and Talaat, 1944). Wilson (1954) has shown that histamine absorbed from the gut (exogenous histamine) appears in the urine in a conjugated form, whereas histamine given by injection or released from tissue stores by a histamine liberator (endogenous histamine) appears partly in the free form and partly in the conjugated state. To ensure therefore that any release of endogenous histamine was detected, both free and total histamine concentrations were determined in this experiment.

Plan of Experiment

A group of 6 male rats were infected subcutaneously with 2,500 *N. brasiliensis* larvae. The daily output of urine was collected for the same 4-hour period each day, from the 1st to the 20th day of the infection.

Faecal egg counts were carried out daily throughout the experiment.

Free and total histamine concentrations were carried out by the technique described in Methods and Materials at the beginning of Section III.

Results

The results of daily estimations of total and free urinary histamine are shown in Table 14 and Figure 7. It can be seen that there was a fairly wide variation in the daily excretion of both free and total histamine before day 11, the highest concentrations being 5.6 and 9.7 µg histamine/ml. respectively, on the 2nd day after infection. On day 11, however, the amount of free and total histamine excreted began to increase rapidly, reaching values of 11 and 19.5 µg/ml. by the 14th day of the infection, when the self-cure reaction, as assessed by the sudden drop in faecal egg count (Table 15), was at its height.

As individual rats do not necessarily self-cure on the same days an arbitrary period of five days, from the 12th to 16th day after infection, was selected as the period of self-cure. These particular days were chosen as it has already been shown in Section I of the thesis that the daily faecal egg count begins to drop rapidly from the 12th day and the expulsion of the worm population is practically complete by the 16th day.

From Table 14 it can be seen that when the histamine concentrations during this period were tested for significance against those of the preceeding days, using Student's "t" test, there was a significant rise in the concentration of free histamine in the urine during self-cure. Although the concentration of total histamine also reached maximum values at this time, the difference in this case proved to be just below the accepted level of significance.

Discussion

It has already been shown in Section I that there is a delay of about 1 or 2 days between the onset of the sudden drop in egg production by the parasites and their subsequent expulsion from the small intestine of the rat. This means that on day 14 of this experiment, when the excretion of both free and total histamine had reached peak concentration, the expulsion of the worm population would probably be beginning.

With the reservation that in this experiment it was not possible to estimate the entire 24 hour output of urinary histamine, it is interesting that a significant increase in free histamine concentration occurred specifically at self-cure. In view of this promising result it was considered that further and more detailed experiments should be undertaken.

Table 14

The Mean Concentration of Total and Free Urinary Histamine in a Group of 6 Male Rats During a Primary Infection of N. brasiliensis

	DAYS AFTER INFECTION													
	2	3	4	6	8	11	12	13	14	15	16	17	18	19
* Total Histamine	9.7	8.2	4.5	8.1	7.1	7.1	14.1	10	19.5	14.2	4.2	14.9	10.3	13
* Free Histamine	5.6	3.7	1.4	5.7	3.8	3.9	7.3	6.8	11.0	11.3	3.6	7.7	8.6	4.0

Table 14 (contd.)

	Mean histamine concentration during self-cure	Mean histamine concentration of preceeding days	P value
* Total Histamine	12.4 ± 5.69	7.45 ± 1.60	> 0.05 Not Significant
* Free Histamine	8.0 ± 3.19	4.01 ± 1.57	< 0.05 Significant

* µg/ml. urine

⊢——⊣ Period of self-cure.

Figure 7

The Concentration of Total and Free Urinary Histamine in a Group of 6 Male Rats During a Primary Infection of *N. brasiliensis*.

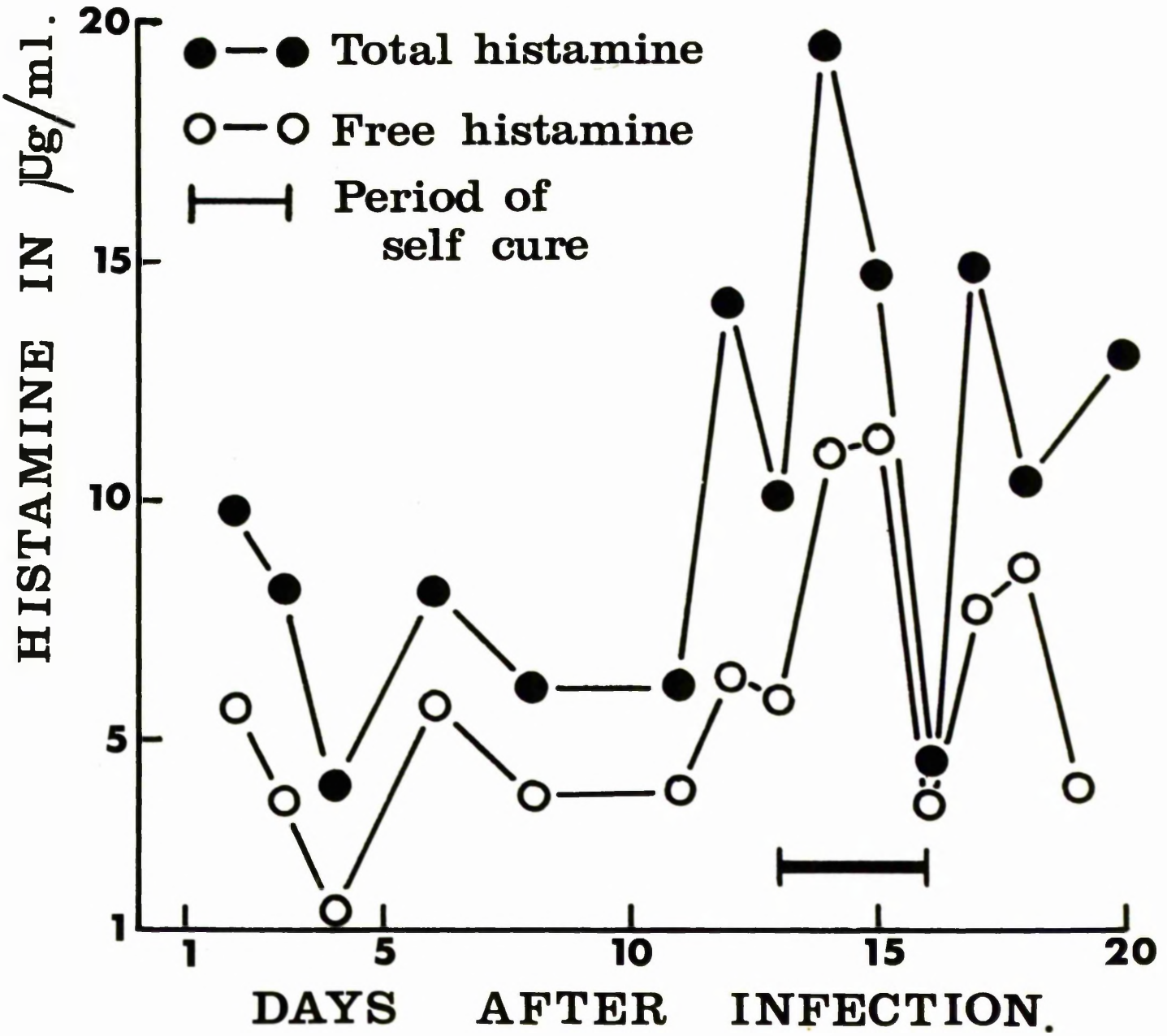

HISTAMINE IN /Ug/ml.
20
15
10
5
1
Total histamine
Free histamine
Period of
self cure
1
5
10
15
20
DAYS AFTER INFECTION.

Table 15

The Daily Faecal Egg Count of a Group of 63 Rats Infected with 2,500 *N. brasiliensis*

No. of Days after Infection	Daily Faecal Egg Count in Eggs/g.
6	11,650
7	93,000
8	
9	55,300
10	
11	27,500
12	12,900
13	2,250
14	300
15	100
16	-ve
17	-ve
18	-ve
19	-ve
20	-ve

Experiment 10

The Daily Urinary Histamine Outputs of 12 Male Rats Infected with *N. brasiliensis*

Introduction

Although it was shown in the previous experiment that a significant rise in urinary free histamine excretion occurred at the time of self-cure in a primary *N. brasiliensis* infection, the interpretation of this result was limited for two reasons. The first is that the result was based purely on the daily variations in urinary histamine concentration during a 4-hour period each day. Secondly, the result was expressed on a group basis. As all rats do not necessarily self-cure on the same day, it was thought advisable to show that individual rats would also show this rise in urinary histamine output specifically at self-cure. In the following experiment therefore, the entire daily output of urinary histamine was estimated for each individual rat throughout a primary infection of this parasite.

Plan of Experiment

In this experiment the rats were housed in individual metabolism

cages which enabled the daily output of urine of each rat to be collected free from contamination with food or faeces. Due to the limited number of cages available the experiment was divided into two parts - A and B - each involving 6 male rats infected with 2,500 N. brasiliensis.

Group A

Both total and free histamine outputs were estimated daily from 3 days before infection until 18 days after.

Group B

Histamine estimations were determined from the 11th to the 20th day after infection.

Daily faecal egg counts were carried out during the patent period of infection for each rat.

Results

Group A

The daily estimations of total and free urinary histamine output are shown in Tables 16 and 17 respectively, together with the approximate periods of self-cure. The latter were assessed on the rapid drop in the individual daily faecal egg counts which are given in the Appendix, Table 2.

Table 16

The Daily Output of Total Urinary Histamine of 6 Male Rats During a Primary Infection of *N. brasiliensis*

GROUP A

Rat Number	DAYS AFTER INFECTION														
	-1	+1	3	5	7	9	10	11	12	13	14	15	16	17	18
1	24.5*	57	20	25	51	28	28	21	30	40	32.5	47	75.5	24	NS
2	14.5	NS	19.5	11.5	31.75	18.5	NS	43.25	40.75	23.25	23.5	NS	14.25	NS	22.25
3	16	24	13	15	30.5	15	27.5	31.5	53	47	120	NS	NS	NS	NS
4	14.75	6.5	2.5	11.5	16.5	NS	NS	NS	13	8	8	10	20	1	NS
5	17.25	25.5	17.75	34.25	NS	NS	NS	NS	15	43.75	NS	NS	40	NS	24
6	14.5	19	14	16	13	NS	NS	8	25	69.5	20.5	18.5	25	3.5	NS
Mean	16.96	26.4	14.46	18.87	28.55	20.5	28	25.9	29.5	38.6	40.9	25.2	34.9	9.5	23.1

Table 16 (contd.)

Rat Number	Mean during period of self-cure	Mean on preceeding days	Probability
1	51.6 ± 21.9	32.45 ± 12.7	$P > 0.1$
2	35.75 ± 10.9	19.15 ± 7.7	$P < 0.05$ Significant
3	73.3 ± 40.5	21.5 ± 8.08	$P < 0.001$ Highly Significant
4	12.67 ± 6.43	9.09 ± 5.15	$P > 0.25$
5	29.37 ± 20.3	23.69 ± 8.0	$P > 0.25$
6	36.3 ± 28.9	15.6 ± 5.3	$P > 0.1$
Mean	32.50 ± 6.84	21.96 ± 6.36	$P < 0.02$ Significant

* Total histamine in µg/24 hours.

⊢——⊣ Approximate period of self-cure based on egg counts.

NS No sample.

Table 17

The Daily Output of Free Histamine in µg/24 hours of 6 Male Rats During a Primary Infection of *N. brasiliensis*

GROUP A

Rat Number	DAYS AFTER INFECTION																
	-3	-1	+1	3	5	6	7	9	10	11	12	13	14	15	16	17	18
1	11.5	12.5	12.5	2	9	7.5	7.5	4	10	7.5	9.5	15.5	10.5	8.5	18	11.5	16.
2	NS	9	6	4	5.5	NS	3	8.5	11.5	21.5	11.5	30.25	23.5	NS	11.25	NS	9.
3	NS	12.5	NS	6.5	6.5	NS	7	13.5	9.5	17	28	24.5	17.5	NS	14.5	NS	9.
5	3.75	4.75	3.75	1.5	3.25	NS	1.75	3.75	3.5	4.25	3.25	23.5	4.5	NS	6.25	NS	3.
Mean	7.62	9.68	7.42	3.5	6.06	7.5	4.81	7.44	8.62	12.56	13.06	36.50	14.0	8.5	12.50	11.5	9.

Mean Histamine Output during Self-cure	Mean Output on preceding days	Probability
16.19 ± 10.15	6.96 ± 1.89	$P < 0.05$ Significant

|——| Period of self-cure.

NS No sample.

There was a wide variation in the output of total urinary histamine (Table 16) before the onset of the self-cure reaction, but in each rat, the highest recorded value occurred during the approximate period of self-cure. Unfortunately statistical treatment of the individual results revealed that only two rats (numbers 2 and 3) showed increases in histamine excretion during the period of self-cure; nevertheless, when treated as a group, a significant increase in total histamine output was observed at this time, as compared with the preceeding days.

From Table 17 it is clear that a similar result was observed in the excretion of free histamine, in that the mean output during self-cure was significantly greater than that of the preceeding days. In rats 4 and 6, the concentration of free histamine was too low to assay accurately, i.e., it was below 5 µg/24 hours.

Group B

As the amount of free histamine excreted in this group of rats was in most cases too low to assay, only total histamine determinations were made. These results are shown in Table 18 together with the approximate periods of self-cure. The individual daily faecal egg counts are given in the Appendix, Table 3.

Of the six rats in this group, only three (numbers 8, 10 and 11)

Table 18

The Daily Output of Total Histamine of 6 Male Rats During a Primary Infection of *N. brasiliensis*

GROUP B

Rat Number	DAYS AFTER INFECTION										Mean during self-cure period	Mean on days following self-cure
	11	12	13	14	15	16	17	18	19	20		
7	6*	28	23	27	74	44	15	38	35	4	26	34.4
8	18	34	42	14	NS	23.5	14.5	25.5	16	14.5	30	18.7
9	24.5	47	34.5	21.5	58.5	75	14.5	NS	NS	NS	34.3	49.3
10	23	26	17.5	36	31	34	15.5	45	34	8	28.2	27.3
11	NS	54	25.5	28	109	NS	21.5	37	5.5	7	52.5	17.8
12	NS	36	NS	41	66	44	62	52	34	13	38.5	45
Mean	17.88	37.5	35.6	27.92	67.7	44.1	23.8	38.9	24.9	9.3	42.18 ± 17.52	28.2 ± 13.7
											P > 0.1 not significant	

* Total histamine in µg/24 hours.

|—— Period of self-cure.

NS No sample.

had maximum daily histamine output during the arbitrary period of self-cure. The remaining rats did however reach peak histamine excretion within two days of this time. There was no significant rise in the mean histamine output during self-cure as compared with the following days, i.e., days 16 to 20.

The mean output of total histamine of both groups of rats are further summarised in Figure 8.

Discussion

This period of self-cure shown in Tables 16, 17 and 18 is purely arbitrary and was selected to include the 3 days when the egg count was dropping rapidly to zero. As this dramatic drop in egg production by the parasites is known to slightly precede the elimination of the worms from the small intestine (Section I of this thesis), at the end of the selected period of self-cure there was probably a large proportion of worms still present in the rat. This delay might explain why in Group B, three rats did not reach peak histamine output until two days after this approximate period of self-cure.

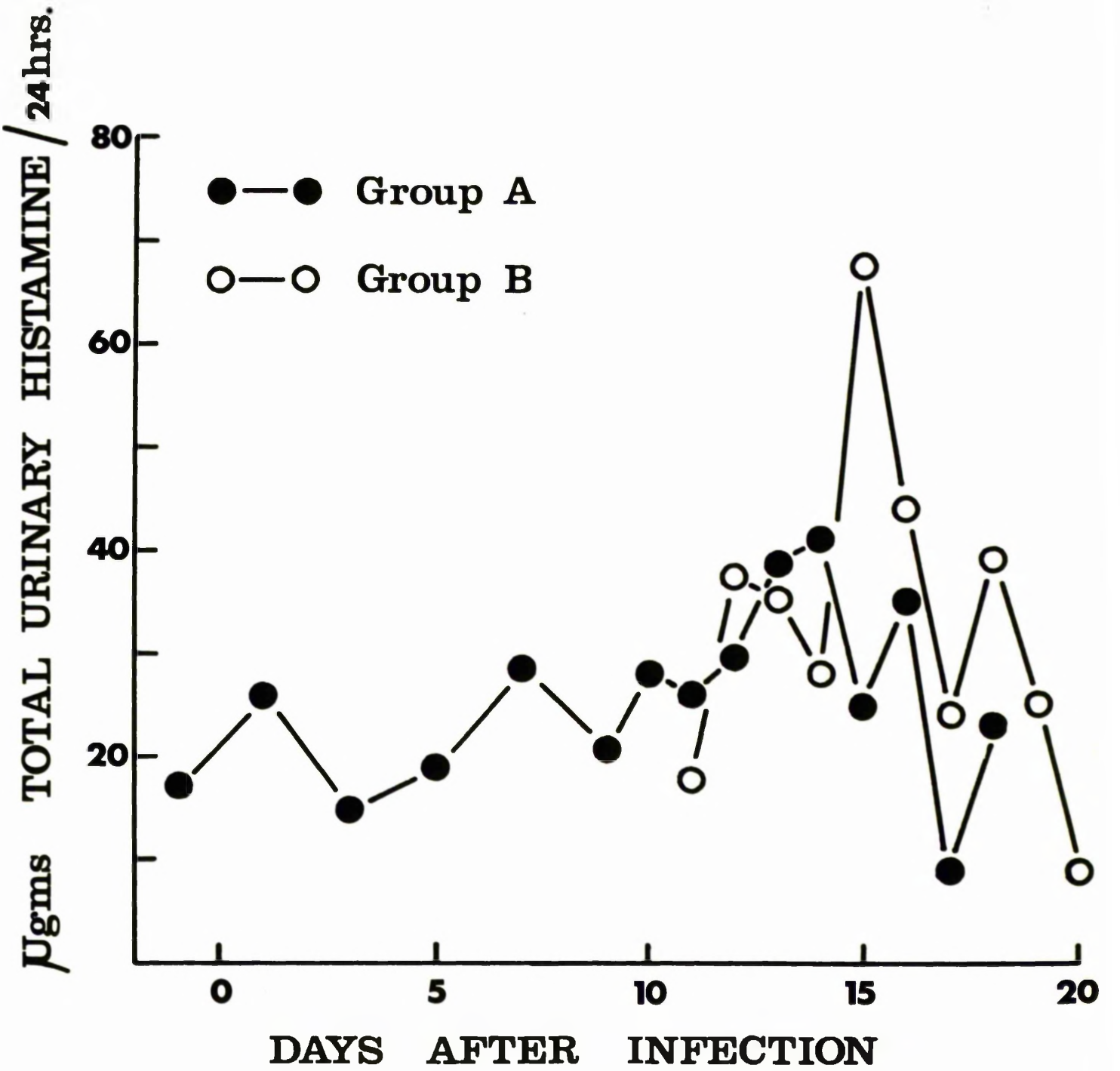
The fact that in Group B there was no significant rise in the mean histamine output during self-cure as compared with the output on the following days, suggests that the situation may be more

complex than originally thought. Increases in urinary histamine output may occur for some reason, even after the majority of the worms have been expelled from the small intestine.

In summary, the fact that a significant increase in the excretion of both free and total histamine was observed during self-cure in Part A of this experiment, provides further evidence that histamine release may be associated with the self-cure reaction.

Figure 8

The Mean Daily Output of Total Urinary Histamine of Two Groups of 6 Male Rats During a Primary Infection of *N. brasiliensis*


γgms TOTAL URINARY HISTAMINE / 24 hrs.
80
60
40
20
Group A
Group B
0
5
10
15
20
DAYS AFTER INFECTION

Experiment 11

The Urinary Free Histamine Outputs of Female Rats During a Primary Infection with *N. brasiliensis*

Introduction

The results up to this stage in the present series of experiments have shown that there is a significant increase in the excretion of free urinary histamine during the self-cure period of a primary infection of *N. brasiliensis*. One of the groups of six rats also showed a significantly increased output of total histamine specifically at this time. In a further group of rats, the increased output was just below the accepted level of significance.

In all these experiments the rise in histamine output, in each rat, during the period of self-cure was compared with its own output on the preceeding days. Whether this was an adequate control or not could be open to criticism. It was chosen for the following reasons:-

1) Each experiment had to be limited to six rats.
2) There is a very wide variation in the amount of histamine excreted by individual rats. Consequently, if uninfected control rats were included, then with the small numbers of rats involved in each group, any rise in histamine excretion during the period

of self-cure might be obscured by the normal daily variations.

3) As the experiments were designed purely to detect possible increases in histamine output at self-cure, it was considered that this method of comparison was the most suitable.

In the following experiment it was thought necessary to include a group of uninfected rats to act as a further control, before it could be finally stated that urinary histamine output increased significantly during the period of self-cure.

As this resulted in the groups being very small, it was decided to use littermate rats, of the same strain as in all previous experiments, in the hope that this might help to eliminate the wide variation in histamine excretion between the individual rats.

Furthermore, as the work up to this point has involved only male rats, it was considered advisable to show that similar results could be observed in female rats. They excrete mostly free histamine in contrast to male rats in which most of the urinary histamine is in the conjugated form (Westling and Wetterquest, 1962). Consequently, only free histamine was estimated in the following experiment.

Plan of Experiment

This experiment was also divided into two parts, A and B; each

involved 6 female rats. Three rats in each experiment were infected with 2,500 N. brasiliensis larvae, and the corresponding 3 littermates kept as uninfected controls.

Daily faecal egg counts were carried out during the patent period of the infection, and the faeces of the control rats were checked every second day to ensure that they had not become infected.

Results

Experiment A

The results of daily estimations of urinary free histamine are shown in Table 19 and Figure 9. The faecal egg counts are given in the Appendix, Table 4.

From these results it is clear that in this experiment there was no indication of any increase in histamine excretion during self-cure as compared with either the output on the days preceeding it, or with the corresponding excretion of free histamine of the control rats at this time.

Experiment B

Free histamine estimations are shown in Table 20 and Figure 10, and the faecal egg counts in the Appendix, Table 5.

At first sight, the histamine outputs of the infected rats in Experiment B during the period of self-cure, are more encouraging

Table 12

e Daily Output of Urinary Free Histamine of 3 Female Rats Infected with N. brasiliensis and of 3 Female Control R

Experiment A

at mber		DAYS AFTER INFECTION															
		-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
.	INFECTED	3.45*	11.44	2.52	2.75	6.0	8.04	21.7	4.88	4.5	6.83	9.05	9.94	2.73	15.4	1.62	8.74	11
2		4.45	8.12	9.0	1.6	2.02	8.12	13.28	4.51	6.3	7.6	3.17	18.9	5.76	3.76	13.65	3.9	13
3		2.78	6.48	8.75	18.50	11.48	35.8	7.96	8.68	6.27	5.7	2.99	5.82	3.68	4.1	14.5	8.16	8
an		3.56	8.68	6.75	7.61	6.5	17.32	14.31	6.02	5.69	6.71	5.07	11.55	4.05	7.75	9.92	6.93	11
4	CONTROLS	2.0	3.88	6.8	10.08	9.01	11.77	6.82	6.82	8.05	9.5	11.48	5.35	2.31	8.85	2.18	NS	10
5		8.39	7.25	9.59	15.98	6.24	12.54	12.83	4.85	10.45	6.99	8.44	11.96	3.6	1.62	7.08	1.43	3
6		7.1	3.02	4.91	NS	4.55	4.62	4.2	3.62	19.2	NS	6.72	16.6	3.48	8.58	4.43	3.75	3
an		5.83	4.71	7.1	13.03	6.6	9.64	7.95	5.09	12.56	8.24	8.88	11.3	3.13	6.35	4.56	2.59	5

Table 19 (contd.)

Rat Number		Mean of 3-day self-cure period	Mean of preceeding days
1	INFECTED	8.59	7.21
2		7.11	7.14
3		8.92	9.61
Mean		8.2	7.99
4	CONTROLS	7.24	
5		7.78	
6		6.52	
Mean		7.18	

* Urinary free histamine in µg/24 hours.

Period of self-cure.

Figure 9

Group A

The Daily Output of Urinary Free Histamine of a Group of 3 Female Rats Infected with 2,500 *N. brasiliensis*, and of 3 Female Control Rats.

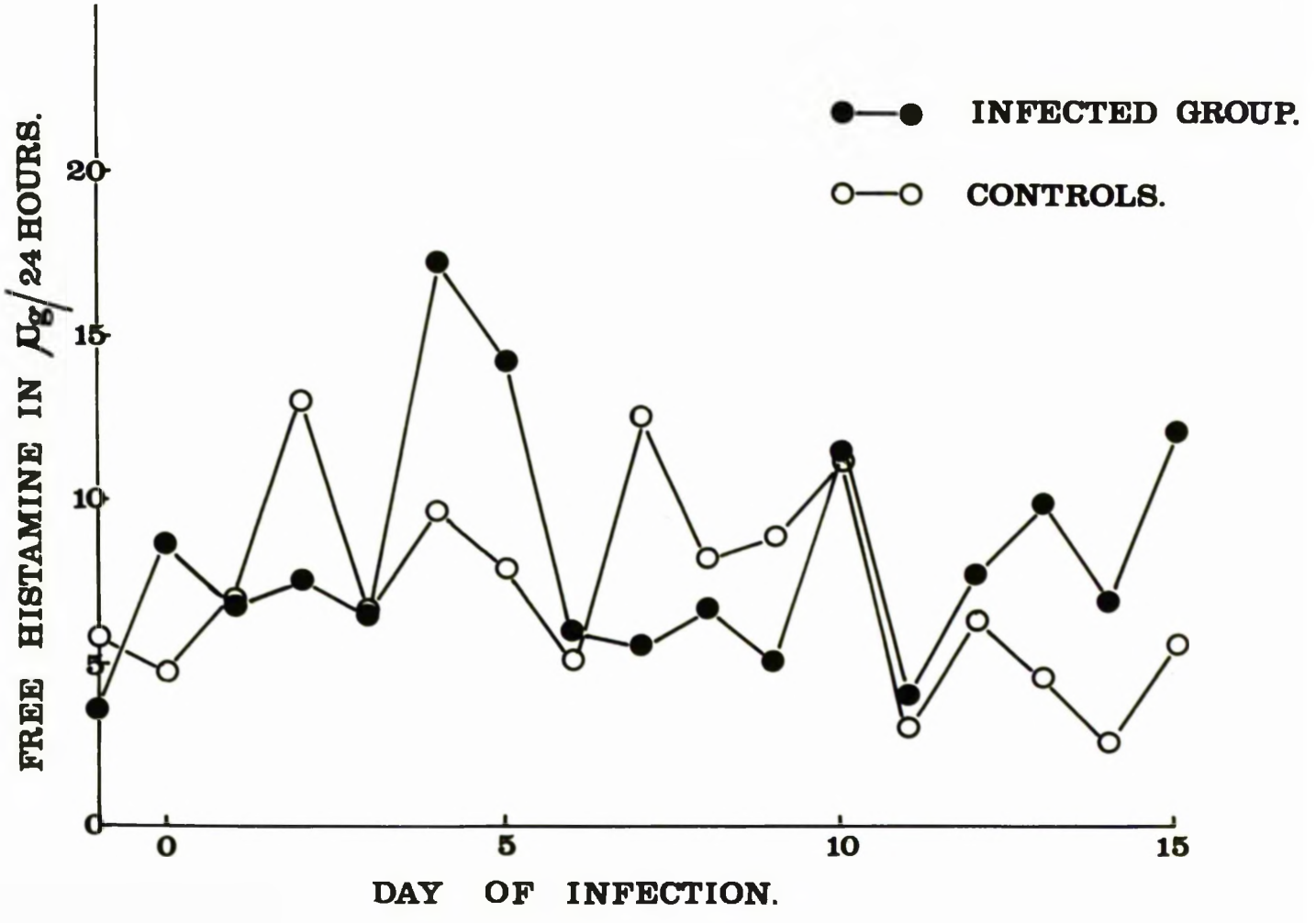

INFECTED GROUP.
CONTROLS.
FREE HISTAMINE IN γg/24 HOURS.
20
15
10
5
0
0
5
10
15
DAY OF INFECTION.

Table 20

The Daily Output of Urinary Free Histamine of 3 Female Rats Infected with 2,500 *N. brasiliensis* and of 3 Female Control Rats - Experiment B

Rat Number		DAYS AFTER INFECTION 9	10	11	12	13	14	15	16	17	18	Mean During Period of Self-cure	Mean During Preceeding Days	
7	INFECTED	*24.3	9.5	16.7	48.4	7.7	51.7	17.6	31.96	26.35	23.5	35.93	16.8	
8		24.4	20.4	21.8	31.9	115.3	26.4	55.0	33.12	34.32	28.84	65.56	24.6	
9		7.4	3.1	17.6	19.8	21.2	25.6	47.6	35.36	25.65	29.87	31.43	11.9	
Mean		18.7	11.0	18.7	33.3	48.1	34.5	40.1	33.48	28.77	27.4	39.00	16.13	P<0.01 H.S.
10	CONTROLS	23.1	30.6	5.8	14.6	44.9	44.5	44.1	12.0	3.33	NS			
11		37.7	31.6	16.2	21.8	92.2	31.2	47.9	33.0	24.1	NS			
12		25.4	11.25	19.9	23.2	33.8	22.6	24.0	19.0	12.32	NS			
Mean		28.7	24.48	10.6	19.9	56.9	32.8	38.7	21.3	13.25	NS			

* Urinary Free Histamine in µg/24 hours

NS No sample

Period of Self-cure.

than the results in Experiment A. All the rats (numbers 7 to 9) showed maximum output of free histamine during this period, and the mean output was significantly greater than the output on the days before self-cure. However, it is clear that the control rats (numbers 10 to 12) also showed maximum histamine excretion during the same period, although faecal egg counts showed that the rats did not become infected at any time. Furthermore, statistical comparison of the mean histamine output of the infected rats during days 12 to 15, inclusive, with the output of the control rats on the same days, showed that there was no difference in histamine excretion between the groups.

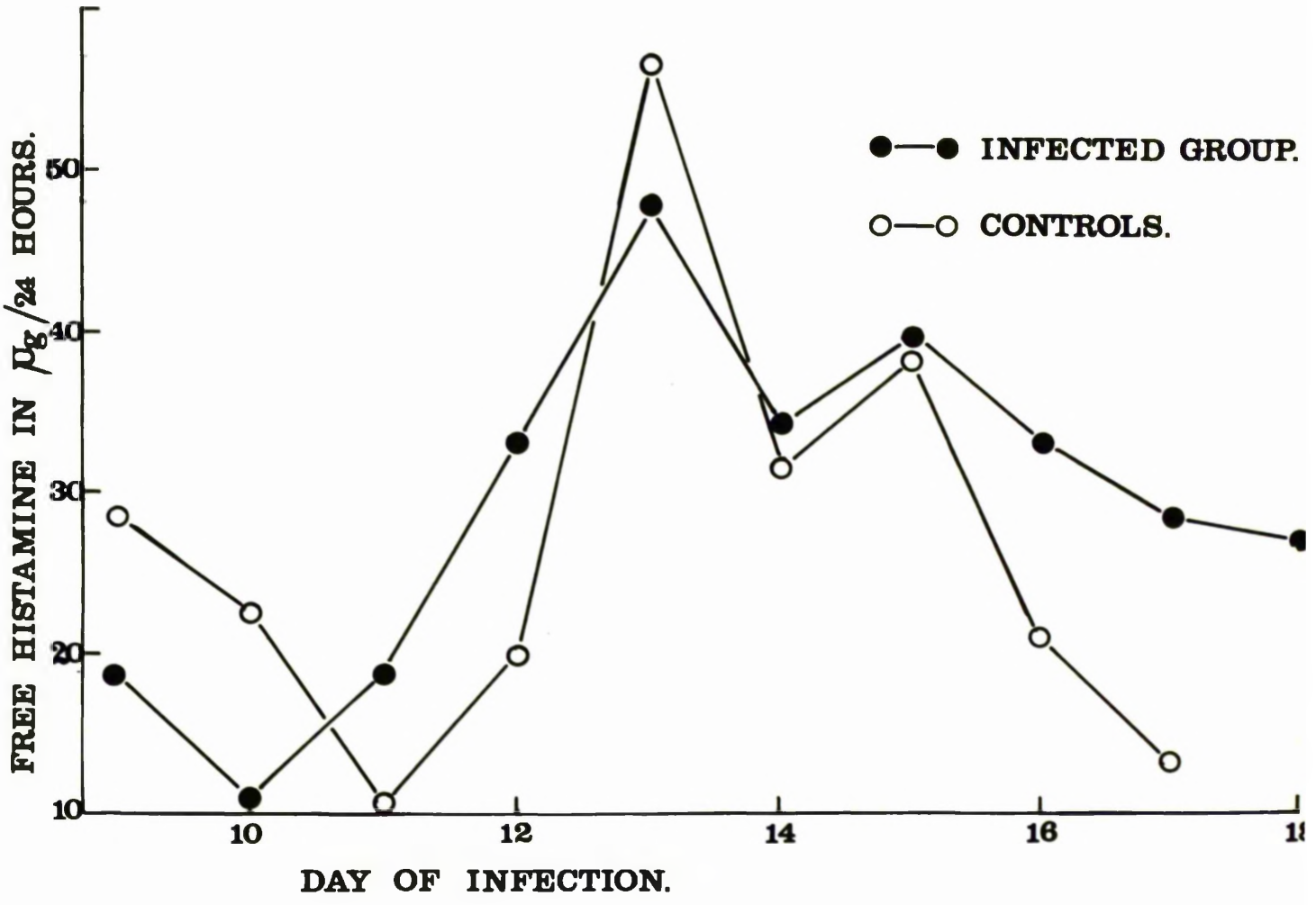
Discussion

From these results two interesting facts emerge. The first is that in Experiment A there was no indication of any increase in urinary free histamine output in the infected female rats during the period of self-cure. This result is in complete contrast to the significant increases in free histamine excretion reported during this period in infected male rats (Experiments 9 and 10). Secondly, in Experiment B there was a significant increase in free histamine excretion at self-cure when compared with the mean output on the preceeding days ($P < 0.01$). However, the fact that all 3 uninfected control rats in this experiment showed a similar increased

Figure 10

Group B

The Daily Output of Urinary Free Histamine of a Group of 3 Female Rats Infected with 2,500 *N. brasiliensis*, and of 3 Female Control Rats.


FREE HISTAMINE IN µg/24 HOURS.
50
40
30
20
10
10
12
14
16
DAY OF INFECTION.
INFECTED GROUP.
CONTROLS.

histamine output at the same time, makes the interpretation of these results rather difficult.

It was perhaps inadvisable to have used female rats in this type of experiment because of their high and variable output of free histamine. If a rise in histamine output had occurred as a result of anaphylaxis associated with the self-cure reaction, it would have been obscured by the normal variations in whole body metabolism of histamine.

Although histamine output in male rats differs from that in females in that most of the histamine is excreted in the conjugated form, in view of the results reported in the present experiment it would be necessary to repeat Experiment 10 and include a group of uninfected rats as a further control. If it could be shown that a significant rise in free histamine output occurred specifically at self-cure using the uninfected rats as a control, it would then be reasonable to conclude the increase was associated with the self-cure reaction.

Experiment 12

The Estimation of Tissue Histamine Concentrations in Rats Infected with *N. brasiliensis*

Introduction

In the last experiment it was suggested that in female rats the estimation of urinary free histamine output might be an unsuitable technique for detecting histamine release during the period of self-cure in *N. brasiliensis* infections. If anaphylaxis were to occur during this period, the amount of histamine released might be small compared to the whole body histamine values. As there is a wide variation in the daily output of free histamine in the urine of female rats, this comparatively small proportion of the daily output might be undetected by this method.

At this stage therefore it seemed that a more suitable method of detecting the occurrence of a hypersensitive reaction at self-cure might be to estimate the histamine content of certain tissues before and after this period.

Plan of Experiment

Six male rats were infected with 2,500 *N. brasiliensis* larvae.

Three were killed on the 10th day of the infection, before self-cure. The remainder were killed on the 14th day after infection, when the daily faecal egg count had reached zero.

The rats were killed by a single blow on the head. Samples of tissue were removed immediately from the ears, mesentery, omentum and various regions of the upper jejunum and placed in N/3 hydrochloric acid prior to histamine estimation. The first three types of tissue were selected because they are known to have a high concentration of histamine containing mast cells.

As the adult parasites do not distribute themselves uniformly in the small intestine (Brambell, 1965), on the 10th day duplicate samples of tissue were selected from areas of high and low worm density. The former, i.e., the areas of high worm density, are the sites of greatest vascular permeability (Jarrett, W.F.H.; personal communication). In the rats killed on day 14, after self-cure, sites of previous high and low worm density were selected by the amount of tissue damage and epithelial thickening.

Results

The histamine concentrations shown in Table 21 are the mean of two estimations. It can be seen that there was no indication of a reduction in the histamine content of the ears, mesentery and omentum, after self-cure.

Table 21

The Concentration of Tissue Histamine in µg/g tissue in Rats Infected with *N. brasiliensis*, Before and After Self-Cure

No. Days after Infection	Rat No.	Mesentery	Omentum	Ears	SMALL INTESTINE	
					High Worm Density	Low Worm Density
10	1	10.22	5.5	32.7	3.22	4.35
10	2	10.1	21.6	32.6	2.15	5.74
10	3	8.8	13.2	19.7	1.34	10.6
Mean		9.71 ± 0.79	13.43 ± 8.05	28.33 ± 7.48	2.23 ± 0.94	6.89 ± 3.28
14	4	14.3	22.38	52.5	5.47	2.00
14	5	22.52	7.76	57.75	8.60	3.52
14	6	8.71	33.08	31.40	13.86	4.79
Mean		15.18 ± 6.95	21.07 ± 13.01	47.21 ± 13.9	9.31 ± 4.24 (P = 0.05 S.)	3.43 ± 1.4 (P > 0.1 N.S.)

In the small intestine the areas of high worm density had a mean concentration of 2.23 ± 0.94 µg histamine per g. tissue before self-cure, compared with a concentration of 9.31 ± 4.24 µg histamine after self-cure. This increase in histamine content was significant (P = 0.05). There was no significant change in histamine concentration in the area of low worm density over this same period.

Discussion

The mesentery, omentum and ears are tissues with a high density of mast cells so it is reasonable to assume that a general anaphylactic reaction at self-cure would involve a release of histamine from these sites. There was no indication of this in the above experiment. However it is clear that considerably larger groups of rats would have to be used to reach a definite conclusion on this point because of the wide range in histamine content in these tissues.

The small intestine is known to have a high histamine content (Nota et al. 1955) but as it has only recently been established that it also has a high concentration of a specific type of mast cell (Enerback, 1966a), it has yet to be proved whether or not the histamine is bound in these cells. It has recently been suggested (Jarrett, Miller and Murray, 1967) that during infection these mast cells migrate through the basement membrane into the intestinal epithelium, with the

subsequent loss of at least some of their amine content, to become the globule leucocytes which are so commonly seen after parasitic infections (Kirkman, 1950; Sommerville, 1956). Furthermore, it was shown that the number of mast cells in the intestinal wall fell after infection and remained at a low level until about the tenth day, whereupon there was a dramatic rise in their numbers. At this point they migrated into the intestinal epithelium, as described above, and self-cure began 2 days later. After the worms had been expelled, the mast cells, or globule leucocytes, appeared compact and well granulated (Jarrett, Jarrett, Miller and Urquhart, 1967). In view of these findings the results of the present experiment are of particular interest in that the reduced histamine content of the highly infected areas of the small intestine, before self-cure, apparently coincides with the period when there are few mast cells present. Also, the significant increase in histamine content in the same areas after self-cure coincides with the dramatic increase in mast cell count reported by these authors.

The results reported in this preliminary experiment are based on too few animals and estimations to enable definite conclusions to be reached. Nevertheless the results suggest that the estimations of the histamine content of portions of the small intestine may be a useful tool in studying the role of anaphylaxis during self-cure.

It would also be useful to parallel these estimations with mast cell counts in these areas of the small intestine.

General Summary and Conclusions

Section III

1. Total and free urinary histamine was determined in a group of 6 male rats for a period of 4 hours each day, from the 2nd to the 19th day after a primary infection of 2,500 *N. brasiliensis* larvae.

It was found that there was a significant increase in the concentration of free histamine over the period of self-cure, when compared with the concentration on the preceeding days ($P < 0.05$).

The concentration of total histamine also increased at self-cure, but the difference in this case proved to be just below the accepted level of significance ($0.1 > P > 0.05$).

2. The entire daily output of total and free urinary histamine was determined in 2 groups of 6 male rats infected with 2,500 *N. brasiliensis* in Group A from 3 days before infection until 18 days after, in Group B from the 11th to the 20th day after infection.

The following results were observed:

Group A

Only 2 out of 6 rats showed significant increases in free and total histamine output during self-cure. On a group basis however, both total and free histamine output increased significantly during this period when compared with the mean output on the preceeding days ($P < 0.02$ and $P < 0.05$ respectively).

Group B

Three rats out of 6 showed maximum output of total histamine during the arbitrary period of self-cure. The remaining 3 rats reached peak values within 2 days of this time. On a group basis there was no significant difference in the output of total histamine when compared with the output on the remaining days ($P > 0.1$).

3. The free histamine output of 2 groups (A and B) of 6 littermate female rats was determined. Each group consisted of 3 uninfected control rats and 3 rats infected with a primary infection of 2,500 N. brasiliensis larvae.

The following results were observed:

Experiment A

There was no increase in urinary free histamine output during the period of self-cure.

Experiment B

Although the 3 infected rats showed increased free histamine output at self-cure when compared with the output on the preceeding days, the increase was not significantly different from the output of the uninfected control rats at the same period.

4. The results of these experiments rather suggest that the output of urinary free histamine is increased during the period of self-cure.

This is in general agreement with the previous observations in support of the theory that anaphylaxis may occur at self-cure. Final proof that histamine excretion increases during this period would require a further experiment utilising male rats, in which the output of urinary free histamine in infected rats was compared with that of uninfected control rats over a prolonged period.

5. The histamine content of the small intestine was estimated before and after self-cure. It was found that in areas of high worm density there was an apparently significant increase in histamine content after self-cure.

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Appendix

Table 1

The Individual Worm Burdens of Rats Throughout a Primary Infection of 2,500 *N. brasiliensis* Larvae

Day	Rat Number						
	1	2	3	4	5	6	Mean ± S.D.
6	964	1,190	671	1,091	1,159	1,621	1,116 ± 311
10	975	1,874	1,951	653	1,895	1,427	1,462 ± 546
12	1,180	1,775	1,781	1,647	1,633	1,368	1,564 ± 240
13	1,065	932	1,013	1,333	535	1,317	1,033 ± 293
14	16	211	925	315	832	467	461 ± 394
16	7	37	2	46	12	3	17.83 ± 18.8
18	1	1	3	4	2	5	2.7 ± 1.8
20	35	3	0	4	12	3	9.5 ± 13.1

Appendix

Table 2

The Individual Daily Faecal Egg Counts of 6 Male Rats Infected with 2,500 N. brasiliensis

GROUP A

No. Days after Infection	Rat Number 1	2	3	4	5	6	Mean
7	*63,000	43,350	89,100	65,850	32,200	67,800	60,210
8	25,800	16,500	100,200	68,700	44,800	83,700	56,610
9	52,900	19,100	37,800	108,900	36,000	31,500	47,700
10	53,400	37,800	30,100	72,000	41,200	72,600	51,100
11	7,700	5,200	4,800	18,600	2,750	16,100	9,190
12	31,900	3,750	6,300	42,300	8,050	34,400	21,100
13	25,400	100	1,200	33,400	2,500	9,700	12,050
14	7,800	300	-ve	8,200	100	300	2,800
15	4,000	-ve	-ve	3,500	-ve	100	1,270
16	200	-ve	-ve	500	-ve	-ve	11
17	100	-ve	-ve	-ve	-ve	-ve	17
18	-ve	-ve	-ve	200	-ve	-ve	34

* eggs per gram faeces.

Appendix

Table 3

The Individual Daily Faecal Egg Counts of 6 Male Rats Infected with 2,500 *N. brasiliensis*

GROUP B

No. Days after Infection	Rat Number 7	8	9	10	11	12	Mean
7	*159,000	77,200	48,700	52,300	100,200	68,100	84,250
8	76,200	60,900	48,900	53,700	89,100	57,600	64,400
9) 10)	29,900	33,800	23,800	18,100	49,800	43,800	33,200
11	15,600	22,900	22,600	42,100	87,000	66,000	42,700
12	2,800	5,200	7,650	20,250	31,200	11,900	13,160
13	500	2,200	1,600	14,300	6,800	1,500	4,480
14	-ve	-ve	-ve	100	1,100	-ve	600
15	-ve	-ve	-ve	-ve	-ve	-ve	-ve

* eggs per gram faeces.

Appendix

Table 4

GROUP A

The Daily Faecal Egg Counts of 3 Female Rats Infected with 2,500 *N. brasiliensis*

No. Days after Infection	Rat Number 1	2	3	Mean
7	22,500	29,100	34,200	28,600
8	18,900	13,650	13,200	15,250
9	53,100	28,200	28,300	36,530
10	6,500	8,550	10,500	8,500
11	14,400	5,900	8,700	9,670
12	2,700	1,150	1,850	1,900
13	300	50	450	260
14	-ve	-ve	-ve	-ve
15	-ve	-ve	-ve	-ve

Appendix

Table 5

GROUP B

The Daily Faecal Egg Counts of 3 Female Rats Infected with 2,500 *N. brasiliensis*

No. Days after Infection	Rat Number 7	8	9	Mean
8	56,700	42,000	21,000	39,900
9	2,250	10,900	18,800	10,650
10	16,800	24,300	1,950	14,350
11	5,100	12,500	8,400	8,660
12	4,800	9,100	5,700	6,530
13	1,800	6,600	22,500	10,300
14	300	850	11,100	4,080
15	300	1,200	-ve	500
16	50	-ve	-ve	16
17	50	-ve	150	65
18	-ve	-ve	450	150

The Self-cure Reaction in *Nippostrongylus brasiliensis* Infection of the Rat

A summary of a thesis submitted for the degree of Master of Science of the University of Glasgow, by Rosalind Mary Douthwaite.

This work was undertaken in an attempt to determine the mechanism of the "self-cure reaction" in Nippostrongylus brasiliensis infections in the laboratory rat. In this reaction the immune mechanisms of the rat cause the rapid expulsion of the adult worms from the small intestine at the end of the second week of a primary larval infection.

As a starting point it was necessary to obtain more quantitative information on the kinetics of self-cure, in terms of worm burdens, sex ratios and faecal egg counts, than had previously been described in the literature. Following a primary infection it was shown that egg output by the parasites reached a maximum on the 10th day; thereafter it dropped rapidly to around zero by the 15th day. By the 12th day the expulsion of the parasites had begun and by the 16th day only about 1% of the original worm burden remained.

In order to facilitate the study of the in vivo effect of antibody on the parasites a surgical technique was developed in which serum and adult worms were transferred to a previously uninfected rat. Using this technique it was shown that both immune and hyperimmune serum interfered significantly with either the establishment or the longevity of a transplanted adult population.

In an attempt to assess if humoral antibody has any direct effect on the adult parasites, the oxygen consumption of worms in hyperimmune serum was compared with that of worms in normal serum. It appeared

that the former consumed less oxygen over a 20-hour period than the latter. The significance of this observation was discussed.

If antibody does have a direct effect on the parasites, in some way debilitating them so as to make them prone to expulsion, it is necessary to demonstrate how it comes into contact with the worms. By the use of ^{51}Cr labelled red cells it was shown that the ingestion of blood by the parasites could be ruled out as a means of bringing significant quantities of antibody into contact with the worms.

Alternatively, it was considered possible that antibody may reach the worms as a result of a worm-induced hypersensitive reaction in the intestinal mucosa. The capillary permeability associated with such a reaction might allow appreciable quantities of antibody into the intestinal lumen where it could act directly on the parasites.

In support of this theory are the findings of the experiments reported in Sections II and III, which were designed to find if anaphylaxis did occur and was an essential part of the self-cure reaction. These results were as follows:

1) As early as 7 days after an initial infection rats could be shocked by the intravenous injection of N. brasiliensis antigen.
2) This reaction was principally expressed in gross alterations in the small intestine which resembled those present at self-cure.

3) Susceptibility to intestinal anaphylaxis could be passively transferred to normal rats by a prior injection of hyperimmune serum.

4) The antihistaminic promethazine hydrochloride interfered significantly with the expulsion of the adult population at self-cure.

5) Cortisone completely blocked the self-cure of a primary infection and abolished the anaphylactic reaction induced by the intravenous injection of N. brasiliensis antigen.

6) There was a significantly increased output of urinary free histamine in male rats during the period of self-cure as compared with the output on the preceeding days.

7) There were indications that the concentration of histamine in heavily infected areas of the intestinal mucosa of infected rats increased after self-cure.

These results are discussed in relation to recent publications by other workers in this field. It was concluded that intestinal anaphylaxis is probably an essential part of the self-cure reaction and that its role may be to facilitate the passage of antibody into the lumen of the small intestine.

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