THE DISTRIBUTION OF *NIPPOSTRONGYLUS BRASILIENSIS* (TRAVASSOS, 1914) IN RESISTANT AND SUSCEPTIBLE RATS.

by

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INTRODUCTION.

INTRODUCTION

Nippostrongylus brasiliensis is a nematode which spends its adult life in the small intestine of rodents, especially the rat Rattus norvegicus. It has a direct life cycle, there is no intermediate host. The eggs are passed out in the faeces and the worm develops as a free living organism until it reaches the infective stage. Infection of a rat takes place by the nematode larvae actively penetrating the skin. From the skin the larvae pass to the lungs and thence, through the air spaces, up the trachea and into the digestive tract. On reaching the small intestine development to the adult stage is completed.

Rats which have already been infected by this worm are more resistant to subsequent infections than rats which have not already had this experience. This means that the first infection induces changes in the rat which, directly or indirectly, make it a less favourable environment for the worm. What these changes are; how they are induced, and how they manifest themselves against the worm are questions which cannot as yet be fully answered.

The work in this thesis was based at its outset on the following question:

Are any of the changes induced by a first infection which affect the course of a second infection, localized in the particular tissues that had been previously parasitized, or are these changes distributed generally throughout the body of the rat?

Now if these changes which follow a primary infection are

regularly established in a particular definable region of the small intestine, then it is to be expected that primary and secondary infections would have different distributions within that organ.

The analysis of this supposition leads to the formulation of the following subsidiary questions:

i) Can a particular region of the small intestine be defined?

ii) How are the worms of a primary infection distributed; and are they distributed in a predictable manner?

iii) How are the worms of a secondary infection distributed?

iv) What effect does the life of the parasite in the skin and lungs have on the distribution within the small intestine?

v) To what extent are the reactions against the worm governed by 'normal' immune processes?

The work described in this thesis deals mainly with the first four of these questions, although some of the preliminary work on the fifth question is also described.

REVIEW OF LITERATURE.

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SYSTEMATICS.

Travassos (1914) described a nematode which he named Heligmosomum brasiliense.

Yokogawa (1920) described a nematode which he named Heligmosomum muris. Lane (1923) proposed that as the genus Heligmosomum typically has a symmetrical bursa this worm belonged to a different genus, for which he coined the name Nippostrongylus. It was under the name of Nippostrongylus muris that this worm became well known to helminthologists and when Travassos and Darriba (1929) pointed out that Heligmosomum brasiliense and N. muris were the same species little notice was taken. The rules of nomenclature automatically demand that the adjective agrees with the noun and the name should have become Nippostrongylus brasiliensis. However, many workers continued to use the old name of N. muris. Haley (1961a) was unable to obtain the type material used by Travassos or Yokogawa, but from measurements made on fresh material obtained from Brazil and Maryland, where the original isolations had been made, he confirmed that they were from the same species. He redescribed the species under the name Nippostrongylus brasiliensis (Travassos, 1914) Lane 1923. There was some confusion over the spelling of brasiliensis, but Haley has followed Travassos in his later papers and substituted an s for the earlier z. In this thesis Haley's name has been used, even when referring to the work of authors who were under the

impression that the worm was named N. muris.

POSITION OF WORMS IN THE SMALL INTESTINE.

Travassos (1914) and Yokogawa (1920) both described N. brasiliensis as being a parasite of the upper part of the small intestine, near the duodenum, of rats. Haley (1961) describes it as being a parasite of the duodenum and jejunum of rats. Several workers have reported that at the height of a primary infection the worms are found in the jejunum but that as the infestation ages the worms that remain are in the duodenum (Porter, 1935; Chandler, 1935). Sarles (1939) noted that in a normal infection the worms were in the anterior third of the intestine at 14 days. Porter and Sarles appear to have assessed the position of the worms by naked eye. Chandler was more precise; he removed one to three inches of intestine at a time and examined the contents. However, he did not publish the numerical results he obtained, only some of the impressions he had gained. Sarles and Taliaferro (1936) and Chandler (1935) also noted that worms in a secondary infection are not distributed in the same way as in a primary infection.

There are, therefore, sufficient grounds in the existing literature to assume that the distribution of N. brasiliensis varies with time and with the degree of immunity.

Little work has been done on the distribution of nematodes within the small intestine of the host. As has been indicated above the work on N. brasiliensis is of a sketchy nature. Gursh (1949) studied the distribution of Trichinella spiralis in rats. He divided the

small intestine into four parts and counted the adult worms in each. Tetley (1935, 1937), Andrews (1937), Davey (1938) and Sommerville (1963) all divided the small intestine of sheep into multiples of linear feet and counted the numbers of several species occurring in each section. Bull (1953) counted the numbers of Trichostrongylus retortaeformis in 5 cm. lengths of formalin-fixed rabbit small intestine. None of these last named authors allowed for differences in the total length of the small intestine and did not express their findings in terms of proportions of the total length of the organ.

Holmes (1961) stretched the intestines of rats in a long pan and plotted the positions of attachment by cestodes. He expressed his results as proportions of the total length of the organ.

Wells (1962) devised a grid on which the small intestine of rats could be divided into proportional parts. The intestine was first stretched by hand and laid onto the grid in air.

The type of distribution has not been well studied. Tetley (1937) described two types; normal and non-normal; by which he mean compact and diffuse. Sommerville (1963) has supported his findings. Gursch (1949) described the distribution of T. spiralis as linear.

PREPARASITIC STAGES.

Yokogawa (1922) had thought that only one moult took place between the hatching of the egg of N. brasiliensis and the infective stage. Lucker (1936) was able to show that two moults took place in this period and that the worm did not differ from other nematodes,

and had four moults.

Yokogawa (1922) described the early development of the worm. Eggs are laid at the 4 - 16 cell stage and become blastomeres while still in the intestine. The morula stage is reached in about five hours and the tadpole stage reached soon afterwards. The first rhabditiform larvae appear in about twenty hours and this is closely followed by hatching. Lucker (1936) found that these larvae moulted between 36 and 48 hours after the faeces were passed. Both the first and second stage larvae appear to be active feeders. They migrate over suitable surfaces (usually upwards) away from faeces (Boardman, 1933). On reaching an edge they cease to migrate further and cement themselves by their tails to the substrate (Africa, 1931). Moulting takes place between 72 and 96 hours (Haley, 1962). The third stage, filariform, infective larvae remain in the second stage sheath, which is complete except for having lost the head end. The larvae are stimulated to leave the sheath by being touched or warmed (Parker and Haley, 1958).

Yokogawa (1922) cultured larvae to the infective stage on damp filter paper. Lucker (1936), Leigh (1956), mixed faeces with moist charcoal to obtain infective larvae. Barakat (1951) modified the damp filter paper method by placing the filter paper on a water permeable pillar of cotton wool, thus keeping the edges of the paper free of water, though still damp.

COURSE OF PRIMARY INFECTIONS

Yokogawa (1920) and Africa (1931) showed that the best route of infection is through the skin and it is generally assumed that

this is the natural route.

Yokogawa (1920) stated that after infection the larvae could be found in the blood, lungs, trachea and intestine, reaching the lungs in 14 to 20 hours after beginning to penetrate the skin, leaving the lungs after 50 to 65 hours and reaching the intestine very quickly after this. The third stage larvae moulted in the lungs and became fourth stage larvae. These moulted after they had reached the intestine about 90 to 108 hours after infection.

Twohy (1955) showed that the skin phase of the life cycle was not essential to the normal development of the worm and larvae injected into the portal vein became normal adults after passing through the lung into the intestine. In 1956 he showed that the phase of skin penetration could be bye-passed by sub-cutaneous injection of larvae. The effect of this was to cut one hour off the time taken for the larvae to reach the lungs. He found the first larvae in the small intestine after 41½ hours. Gharib (1955) thought the route of migration from skin to lungs was by way of the lymphatic system, on the basis of having found a few larvae in lymph tissue. He did not take into account the relative speed of transit that is likely through blood and lymph vessels. By the time the larvae reach the lungs they measure 690 μ long and 27 μ wide (Yokogawa, 1922). The mean diameter of a pulmonary capillary is probably less than 10 μ (Schafer, 1938). Thus larvae being suspended in blood will become arrested in the capillary bed. The literature does not reveal if the larva migrates out of the capillary before or after moulting, but in view of the closeness of

the capillaries to the air spaces it is probable that they remain where they are until after the moult, when they migrate into the air spaces.

By 60 hours after infection most of the worms have reached the intestine (Twohy, 1956). The course of the infection within the intestine is dependent on the size of the initial dose, the age of the rat, the nutritional state of the rat and the immunological experience of the rat. Africa (1931) showed that heavy infections did not last as long as light infections. Africa (1931) and Graham (1932) showed that worms in younger rats produce more eggs than those in older rats. Rats on a lower nutrition harbour more worms for longer than do rats on normal diets (Porter, 1935; Riley, 1943; Watt, 1944; Wells, 1962). There are slight differences between strains of worms and rats (Graham and Porter, 1934). Rats which have already had experience of the worm show resistance to further infection (Africa, 1931; Schwartz, Alicata and Lucker, 1931).

Normal rats aged more than a few weeks and given between 1,000 and 6,000 infective larvae behave in a reasonably consistent manner. In such rats Africa (1931) found that eggs appeared in the faeces from five to six days and the numbers reached a peak two to five days later, after which there was a rapid decline and eggs disappeared by 11 to 19 days. He noted that lighter infections lasted longer than heavier ones. He also noted that male worms survived longer than females. The number of worms in the small intestine rises sharply reaching a plateau by the fourth day after infection and remaining at this level until the ninth or tenth day, Sarles and

Taliaferro (1936). Haley and Parker (1961) showed that whatever the size of the initial dose there were only about 30 or 40 worms remaining in the small intestine by the twentieth day. In their presentation of their results they have apparently overlooked the finding of more or less equal numbers from their variously dosed groups and have concentrated on the fact that the percentage fall between the tenth and twentieth day has been in crude proportion with the initial dose of larvae.

Africa (1931), Porter (1935), Chandler (1935) and Sarles and Taliaferro (1936) all noted that in old primary infections the worms are almost exclusively males.

PATHOGENESIS OF THE INTESTINAL PHASE

Porter (1935) described the intestinal stages as occurring in clumps in the anterior part of the small intestine; the heads of the worms being thrust deeply between villi. Sarles and Taliaferro (1936) also inferred that in normal rats the heads of the worms were thrust deeply between villi. Porter (1935) thought the worms may feed on glandular secretions and stated that they eroded as far as the muscular coat of the small intestine. Lee (1964) has shown that the worms erode the host tissue by the abrasive action of the cuticle in the head region. Davenport (1949) showed that the red pigment of the adult worms is haemoglobin but that it is spectrographically distinct from the host's haemoglobin. Taliaferro and Sarles (1942) described seeing red blood cells in the intestine of some worms.

Symons (1957, 1960a, 1960b, 1961) has studied the biochemical pathology of the condition caused by this worm. He also saw the worms clumped together in the small intestine in light infections. The infected part of the intestine increased in diameter and there is an increase in the amount of blood and tissue fluids present. There is a net efflux of saline into the jejunum due to interference with absorption. Digestion of proteins in the small intestine is lowered; this being associated with a reduction in the amount of enteric enzymes and a relative reduction in the amount of pancreatic enzyme (Symons and Fairbairn, 1963). Taliaferro and Sarles (1939) described the inflammatory reaction that developed around the site of attachment. They considered that it was a similar reaction to that seen in the skin and lungs except that, as the worms were not surrounded by tissue, they could not be enclosed, and no nodules were formed. The worms damage the epithelium of the villi and this results in increased mitosis in the crypts. The lamina propria becomes inflamed, with an increase of globular leucocytes (plasma cells) and mast cells. The inflammation subsides after the worms are eliminated. Wells (1962) showed that the number of mast cells in the lamina propria fell during the first fifteen days and that over the same period the number of eosinophils increased. She considered this to be indicative of a continual release of histamine by the mast cells and of its detoxication by the eosinophils.

SECONDARY INFECTIONS

Africa (1931) and Schwartz, Alicata and Lucker (1931) found

that rats which had had previous infections of N. brasiliensis are refractory to further infections. The effect of the hosts' resistance is to inhibit growth and egg laying of the worms as well as the peak number of worms being passed by the fifth day (Chandler, 1932; Sarles and Taliaferro, 1936). Africa (1931), Graham (1933) and Hurley (1959) have shown that the course of secondary infections is dependent on the size of the primary infection. Secondary infections following small primary infections are more drawn out than after larger primary infections. Chandler (1935) found that the rats showed the maximum resistance to secondary infections when they were reinfected 14 or 15 days after the start of the primary infection and that by 30 days the resistance was slight.

Chandler (1936) showed that worms which had ceased laying eggs in an old primary infection (i.e. about 18 days after infection, and therefore possibly equivalent to the condition of worms in a secondary infection) could be stimulated to resume egg-laying and growth, thus indicating that the effect on the worms was not permanent.

Spindler (1934, 1936) and Chandler (1936) found that adults transferred to the small intestine of previously uninfected rats were able to stimulate a partial resistance to reinfection.

Taliaferro and Sarles (1937a, 1937b, 1939) described the pathology of secondary infections in the small intestine of rats. In essence it was the same as in primary infections but more rapid in onset and less widespread (this being because less worms are involved), though reaction lasts longer. An intense inflammation involving the 'hematogenous' cells of the lamina propria adjacent to the worms

takes place (i.e. involving leucocytes). At first the cells are largely made up of eosinophils, but these are almost wholly replaced by monocytes. After the worms have gone the inflammation subsides leaving an increased number of globular leucocytes (type of plasma cell) and mast cells. Wells (1962) did not find the number of eosinophils increased over those that followed a first infection but she did find an increase in the number of mast cells.

Spindler (1933), Donaldson and Otto (1946) and Wells (1962) all showed that the development of resistance was less in rats on deficient diets.

ROLE OF ANTIBODIES

Circulating antibody plays a large part in the resistance of rats to secondary infections. Although Chandler (1934) failed to demonstrate the passive transfer of immunity, Sarles and Taliaferro (1936) and Chandler himself (1937) showed that serum from previously infected rats conferred some resistance on recipient rats after passive transfer. Sarles and Taliaferro (1938) showed that this resistance was directly related to the amount of serum transferred and was inversely related to the interval between infection and transfer of the serum to a new host. Sarles (1939) showed that when given to rats with five day-old primary infections serum from resistant rats could cause the removal of worms from the intestine. Taliaferro and Sarles (1942) examined the pathology of passively immunized rats at intervals after infection. The inflammatory reaction was intermediate in its intensity between that seen in a primary and in a secondary infection. They considered the changes observed after seven days

were due to the host having acquired an active immunity. Before this time the reaction involved at first a slight exudation of lymphocytes, monocytes and eosinophils; this is followed by the appearance of masses of precipitate and fibrin. Apart from the early appearance of masses of precipitate the pathological picture was similar to that seen in the early stages of a primary infection.

Sarles had earlier (1937) shown that larvae of this worm developed precipitates round the mouth, excretory pore and anus when placed in serum from a previously infected rat but not when placed in normal serum. Thorsen (1951) showed that the secretions and excretions of larvae were able, on injection into rats, to stimulate resistance to subsequent challenge with the worms.

Watt (1943) and Thorsen (1953) showed extracts of larvae could also stimulate resistance when injected into rats. Thorsen (1953, 1954) showed that the antigens in the secretion and excretion preparations could be absorbed by immune serum; and that the antibodies in immune serum could be absorbed by saline extracts of lyophilized larvae.

Weinstein (1955) treated rats to various regimes of cortisone while actively immunizing rats with larvae. He found that there was a reduction in the inflammatory reaction at the skin when challenged with a secondary infection, but that this was not parallelled in the lungs and intestine, though the adult worms recovered were larger than in the untreated immune control rats. There was not a significant difference in the number of the adults recovered from the cortisone treated rats and the untreated immune controls. He

did not measure antibody quantitatively, but found qualitative evidence for it being present in substantial amounts in all the immunized rats, whether treated with cortisone or not.

Hunter and Leigh (1961b) tried to transfer the immunity adoptively by taking lymph and spleen cells from rats which had been infected by the worm and transferring them to previously uninfected animals and then challenging with infective larvae. They did not show any evidence of having transferred immunity; but as with Chandler's original attempt to transfer immunity passively it may yet prove that their failure was due to technical shortcomings.

Harrison and Banvard (1947) have shown that in alimentary Salmonella infections in man antibody appears in the faeces before serum antibody appears, thus showing that antibody is being formed in the alimentary tract independently of the rest of the body. It is not known how local the areas of antibody production in the tract can be.

Jackson (1960) labelled antibodies with fluorescein isocyanate and other fluorescent agents. He immersed infective larvae, moulting fourth stage larvae and adults in the serum and found that precipitates in the digestive and reproductive tracts of adults, the digestive tract of larvae and between the cuticles of the moulting stages to be stained. This confirms the view that the secretions and excretions of the digestive and reproductive tracts of the worms are the common antigens, and also supports the view put forward for Haemonchus contortus by Soulsby, Sommerville and Stewart (1959) that moulting fluid is also highly antigenic.

The experiments on passive transfer only produced a partial immunity. Sarles and Taliaferro (1936) found large numbers of worms surviving at 28 days after a challenge infection, though the egg production had been much affected earlier.

The experiments in which transferred intestinal stages were used to stimulate an active immunity also only produced partial resistance. Spindler (1936) recovered similar numbers of adult worms from his experimental and control groups.

The worms in old primary infections and in secondary infections have been described as occupying different positions in the small intestine from those occupied at the height of a primary infection.

The alimentary tract has been shown to possess some degree of independence from the rest of the body in the manifestation of immunity.

It is probable, therefore, that the small intestine of rats will show differences along its length in its reactions to N. brasiliensis and these differences will affect the way in which the worms are distributed.

EXPERIMENTAL OUTLINE.

EXPERIMENTAL OUTLINE

Before starting on the main experiments of this work two experiments were performed to make egg-counts more useful in assessing the activity of the adult worm populations. Experiment 1 was designed to apply the now standard McMaster method to the eggs of Nippostrongylus brasiliensis. Experiment 2 was aimed to provide information on the transit time of ingesta through the host animal so that egg counts made on faeces collected at the anus could be related in time to the parasite population in the small intestine.

In Experiment 3 the method designed to divide the small intestine into proportional parts was tested for reproducibility.

The distribution of the worms in primary infections was first studied in Experiment 4 when rats which had been given 1800 larvae were killed on successive days after infection. In order to eliminate any possibility that at doses of over 1000 larvae the distribution of worms is markedly dose-dependent the distributions following doses of 1000, 2500 and 6250 larvae were compared in Experiment 5.

In Experiment 6 rats which had been given a primary dose of 2500 larvae were given a secondary dose of the same size and the distribution compared with similarly aged primary infections. As the rats in this experiment developed a high degree of resistance Experiment 7 was performed, in which rats were given extremely large secondary doses to see if the resistance could be broken down. In Experiment 8 both the size of the primary and secondary infections was varied.

The non-intestinal stage of this worm's parasitic life can be bye-passed in a rat by transferring to it worms that have finished this stage in donor rats. In Experiment 9 late lung stages were transferred and in Experiment 10 early intestinal stages were transferred. These two experiments were to test if the distributions in rats immunized and/or challenged by the intestinal stages were similar to that seen in normally infected rats.

It is possible that a worm which is highly immunogenic in adult hitherto worm free rats might be able to survive in the wild state by inducing tolerance in very young rats. Experiment 11 was an attempt to demonstrate this immunological tolerance.

MATERIALS AND METHODS.

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RATS

The rats used in this work were the hooded Wistar strain of tamed Rattus norvegicus. They were obtained by the Moredun Institute from the Rowett Institute, Aberdeen.

WORMS

The strain of Nippostrongylus brasiliensis used in this work was obtained from Dr. P.A.G. Wilson of the Department of Zoology, Edinburgh, who in turn obtained it from Dr. C.A. Hopkins of the Department of Zoology, Glasgow. Dr. Hopkins' strain probably came from the Wellcome Laboratories, London. Several isolations of the worm have been made in America and records do not appear to have been kept. However, it is very probable that all the strains in this country did in fact originate from the United States, although Dudgeon (1922) has reported the worm as occurring in native British wild rats.

CULTURE OF INFECTIVE LARVAE

A very characteristic phenomenon of third stage larvae of N. brasiliensis is the fact that they congregate at the edges and projections of the material on which they are being cultured. In this position they remain more or less quiescent in the sheath of the second stage until they are stimulated into activity by warmth or touch.

This property of the larvae can be made use of when culturing from eggs to the infective stage. Not only can the larvae be stored until sufficient are available without their coming to any apparent harm over a period of a few days, but also if the edges of the material are not contaminated with faeces the larvae can be obtained free of faeces simply by removing the edges from the rest of the culture.

Two main types of culture have been developed: charcoal cultures and filter paper cultures. In the former, used by Lucker (1936) and Leigh (1956) faeces are mixed with moist charcoal and cultured in a closed vessel. The larvae congregate in tufts on the upper projections of the charcoal. It is very difficult to harvest the larvae clear of faecal debris and the charcoal itself tends to disintegrate into a fine dust. The advantage of this method is that the cultures can be stored for several weeks without serious deterioration. However, the disadvantage of contamination makes larvae from charcoal culture undesirable for subcutaneous injection. The latter method relies on the faeces being placed on a filter paper substrate. Yokogawa (1922) had cultured larvae on wet filter paper under about 2 mm. of water. Barakat (1951) described a method in which the filter paper was held clear of the water by a cotton wool pillar. Thus the edges were free of the water and the larvae congregated on them. The whole culture was enclosed in a petri dish to preserve the high humidity. The larvae were harvested simply by dipping the edges but not the faeces contaminated centre, of the paper into warm water.

In this work it became apparent that Barakat's method had a practical drawback in that it was time consuming to set up a number of cultures on cotton wool pads and to ensure that the filter papers were firmly balanced and that they did not tip and touch the lid or the base of the petri dishes; for if this happened the larvae did not stop migrating at the edge of the paper, but continued onto the glass and it was no longer possible to harvest them as clean larvae. If the cotton wool could be replaced by water absorbent flat pads this problem could be easily overcome, allowing for the setting up of a great number of cultures with no failures on account of tipping. The pulp boarding used to make commercial beer mats was found to be an ideal material for this purpose. Fortunately, there was locally a printing works, Scottish Automatic Printing Company, Polton House, Lasswade, Midlothian, which not only made pulp board beer mats for a brewery but also printed numbered discs for various gambling games. The firm was able, at very reasonable rates, to use its pulpboard rejects on the disc stamping machine and produce discs measuring 3.2 cm. diameter by 2 mm. thickness. Two of these discs were used to support each filter paper of 6 cm. diameter. The discs and filter paper were placed into petri dishes. Because of the firmness of the mats enough water could be added to ensure the cultures remained moist for up to ten days without there being any danger of the support of the paper collapsing. Mashed faeces were placed in the centre of the filter paper as in Barakat's method. Most of the larvae

used in this work were cultured by this method.

As a further improvement it was found that filter pads retained moisture better than normal filter papers and in some of the work described these were used to culture the infective larvae.

Wilson (1964) found that this technique of culturing on filter papers in petri dishes did not produce enough larvae for his purposes and he has developed a method in which folded filter paper strips are cultured in plastic sandwich boxes. This method has the advantage over the earlier method of saving much time in the setting up of the cultures.

PREPARATION OF LARVAL SUSPENSIONS

To maintain the culture of Nippostrongylus brasiliensis in laboratory rats it was found to be most convenient to adopt a fourteen-day routine cycle. Infected rats have started to produce eggs in their faeces by the seventh day after the administration of larvae and continue to do so in useful numbers until the tenth day. Infective larvae are present four days after setting up cultures of fresh faeces. Thus it is possible to obtain infective larvae fourteen days after infecting a rat.

The routine adopted was to infect rats on Tuesdays. On the following Monday the rat cage would be cleaned out so that only those faeces pellets passed from the Monday night until the Friday evening were collected. Once or twice each day the fresh pellets were removed and cultures set up. Most eggs were passed in the faeces on the Wednesday and Thursday so that on the following Tuesday

the majority of larvae were five to six days old.

Larvae were harvested by dropping the cut edges of the filter papers into 250 ml. of saline at 37°C in a conical sedimentation beaker, in which they were left for about two hours, the beaker being vibrated occasionally to dislodge any larvae which had settled high up on the sides. Once the larvae had settled most of the supernatant was poured off, leaving a volume of between 20 and 40 mls.

The larvae were resuspended and transferred to 12 ml. centrifuge tubes. The volume of the suspension was further reduced by spinning at 1500 r.p.m. for two minutes at a maximum radius of 14 cm. (Maximum G = 360). The supernatant was discarded and the larvae transferred to a 12 ml. graduated centrifuge tube and the volume made up to 10 ml. The number of larvae present in the suspensions was estimated by the following method:

After vigorous shaking, 1 ml. of suspension was withdrawn into an all glass tuberculin syringe and washed into a 100 ml. graduated flask. The volume of the contents of the flask was then made up to 100 ml. with water, the stopper was fixed and the diluted suspension thoroughly shaken. About 2 ml. was withdrawn with a clean pipette and transferred to the cell of an eel-worm counting slide, the grid of which covers a volume of 1 ml. The number of larvae under the grid were counted under a magnification of X 12.5. This procedure was repeated four times on the diluted suspension. If there were sufficient larvae present a second ml. of the original suspension was taken and treated similarly.

The number of larvae remaining in the suspension was estimated from the formula:

$$W = v \times 100 \times \frac{\sum c}{n}$$

Where W is the total number of larvae remaining, v the volume of the suspension in ml., c the count for each cell and n the number of cells counted. The counts for each cell would be distributed according to the Poisson series, provided the suspension had been properly mixed. Thus as a practical check, proper mixing can be assumed if all the counts lie within less than twice the square root of the mean from the mean value itself.

The volume of the suspension was then adjusted, either by adding saline to increase it, or by centrifuging and removing supernatant to reduce it, in order to obtain 10,000 larvae per ml. The final volume, U being calculated from the formula:

$$U = \frac{W}{10,000}$$

INFECTION

The normal route of natural infection of rats by Nippostrongylus brasiliensis is penetration of the skin by the infective larvae. However, Twohy (1956) has shown that sub-cutaneous injection of the larvae cuts one hour off the time taken for them to reach various stages of development but that there is no other noticeable effect. The main experimental drawback of skin penetration is the lack of certainty whether all the larvae or what proportion of them enter the host, and this does not apply when larvae are injected sub-cutaneously.

To reduce the possibility of larvae being unable to enter the host it is necessary to shave the rat to allow the suspension to be placed directly onto the skin, and to anaesthetise the rat to immobilize it to prevent the suspension from being shaken off before the larvae have begun to penetrate.

In experiments in which it is more important to be able to compare, on a numerical basis, the parasitological experiences of each rat than to know that the path of natural infection had been strictly adhered to, it has been generally accepted as better to dispense with the active penetration of the skin by the larvae and in place to introduce the larvae passively by hypodermic injection. This has the advantage that the rats do not need to be shaved, that the dose can be made up in about 1 ml. instead of the much smaller volume necessary for the former method, that the rats do not need to be kept under prolonged anaesthesia and that the number of larvae known to have entered the host can be far more precisely determined.

If an experienced handler was available it was not necessary to anaesthetize the rats to give them a subcutaneous injection provided the larvae were suspended in isotonic saline. If such help was not available light ether anaesthesia was used. The use of a large jar with a pad of ether-soaked cotton wool was not satisfactory as the relative mixture of ether and air could not be controlled. Rats which had an overdose of ether developed respiratory distress due to the increased secretion of bronchial mucus. This probably lowered the resistance of the lungs to the arrival of the larvae, for several routinely infected rats, which

had been coughing after the anaesthetic died 24 to 48 hours later. Once the ether was administered to the jar already mixed with air the level of anaesthesia could be finely controlled and only one in six hundred and fifty rats died from ether-induced pneumonia. Before the change the losses had been seven out of forty three at risk.

The dose of larvae given to rats to maintain the culture of the worm species was based on the results of Hunter and Leigh (1961a). Although Hunter stated that the LD 50 of male and female rats differed his figures show an average LD 50 of 41 larvae per gram for both sexes. Larvae were given at the rate of 39 larvae per gram up to a maximum of 10,000 larvae. Rats under 150 grams were not used. These dose levels have been found in over 300 stock rats to result in few deaths and good returns of eggs.

MAINTAINANCE OF INFECTED RATS.

After infection each rat was kept in a separate cage. The diet was a standard rat and mouse feed, which had been developed at the Microbiological Research Establishment, Porton, compounded into nuts measuring approximately 1 cm. diameter x 2 cm. length.

Food and water, given in drop-bottles, were available in excess of appetite. A layer of sawdust ½ cm. deep was provided as bedding.

At first the cages were of aluminium or zinc alloy and measured 30 cm. x 12 cm. x 12 cm. high. These were not entirely satisfactory on the grounds that the rats were in too restricted a space.

Later it became possible to design a larger cage, and this was

substituted for the alloy box. The base of the cage was an oblong polythene domestic basin. This was covered by a removable lid of ½" mesh 'Weldmesh' welded wire mesh (the apertures measured 11 mm. square), see Figure 1. The lid was folded so as to clip over the edges of the basin, and to provide a recess for holding the feed nuts and a water bottle. The recess for the feed nuts had three sides lined with sheet zinc and this provided a shaded compartment for the rat. (One of these cages, plus its place in a rack cost 15/-, whilst commercial cages were being offered at from 35/- including rack space).

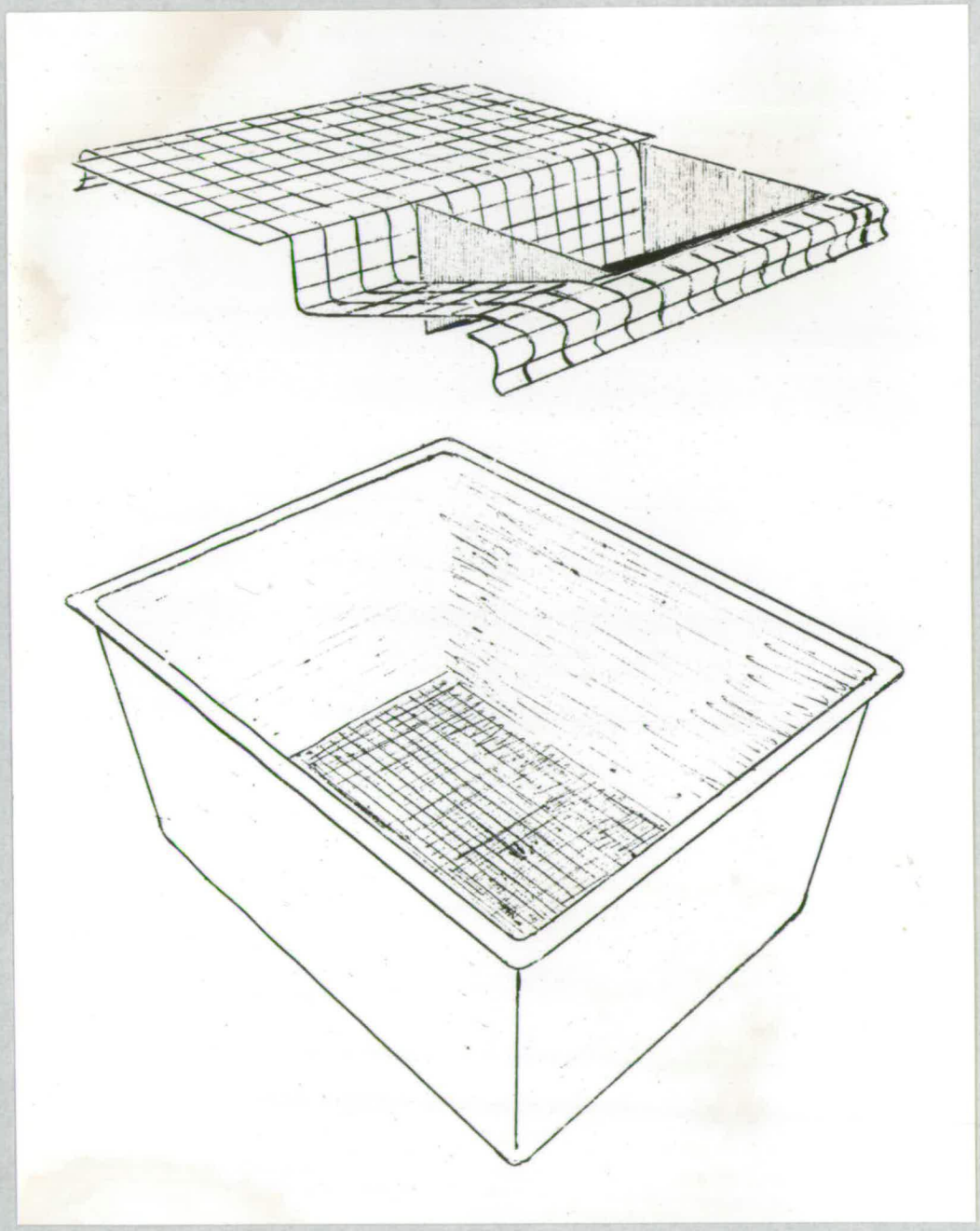
The cages were cleaned out twice each week as routine, and every day when the faeces output of the previous twenty four hours was needed.

IDENTIFICATION OF RATS.

To insure recognition in the event of any accidental mixing of the animals each rat was marked. The system used was to punch up to three holes in each ear; the positions being front, middle and back. The rats could then be recognised according to whether or not any position had been punched. As there were six possible positions the number of combinations involving at least one punch was $(2^6 - 1)$ i.e. 63. It was never necessary to be able to distinguish more than 64 animals at any one time.

While being punched, the rats were under light ether anaesthesia, and the punch used was a chicken toe punch making a hole of about 1 mm. diameter.

FIGURE 1.



Cage, measuring 33 cm. long, 26 cm. wide and 14 cm. deep, designed to hold one rat.

EGG COUNTING

It was found not to be worth while to set up cultures of Nippostrongylus brasiliensis eggs if there were less than 10,000 eggs per gram of faeces.

The best method for estimating the number of eggs in faeces is dilution - flotation. This method, the best known example of which is the McMaster method of Gordon and Whitlock (1939) involves a known weight of faeces being suspended in a known volume of diluent. The specific gravity of the diluent is such that the eggs can float but do not become damaged, in which event they would sink again. The number of eggs in an aliquot sample of the suspension provides an indication of the number of eggs in the original sample of faeces.

Experiment 1 was performed to determine if any of six different solutions had any significant advantages over the others. The optimal solution would be the one in which most eggs floated and in which least damage was done to the eggs on standing.

EXPERIMENT 1

"DETERMINATION OF THE OPTIMAL SOLUTION FOR DILUTION-FLOTATION COUNTS OF NIPPOSTRONGYLUS BRASILIENSIS EGGS IN RAT FAECES"

Experimental Plan

Ten gm. of faeces were collected from an 180 gm. rat which had been infected 7 days earlier with 7,000 larvae. The pellets were steeped in water for some minutes and then mixed into a semi-fluid mass. Twenty four small samples were taken and weighed, the mean

weight of these samples being 0.25 grams.

Two hundred ml. of each of the following solutions were made up to the required specific gravity, which was measured using a floating hydrometer:

Solution	A	NaCl.	S.G.	1.16
"	B	"	"	1.18
"	C	"	"	1.20
"	D	$ZnSO_4$	"	1.20
"	E	"	"	1.25
"	F	"	"	1.30

To find the solution in which most eggs would float, 30 ml. of each solution was mixed with each of three faeces samples and from each suspension eight chambers of McMaster slides were filled. The eggs floating to beneath the grids of these chambers were counted within ten minutes. (Counts on solution F were soon abandoned as it had become obvious that this solution was very unsuitable). The counts were corrected to a standard faeces weight of .25 grams.

To find the solution in which the eggs were least damaged on standing, 30 ml. of each solution was mixed with one faeces sample. The eggs floating in four McMaster chambers were counted within ten minutes and again after three hours (Solution F was not included in this part of the experiment).

Table 1 shows the plan of this experiment.

Results

The results from faeces samples i, ii and iii, corrected to a standard weight of faeces, are given in Table 2. The low number of eggs seen in solution F indicates that this concentration of

TABLE 1.

Plan of Experiment 1.

Solution	Faeces samples		McMaster chambers counted	
			within 10 minutes	after 3 hours
A (Na Cl S.G. 1.16)	i	.32 gm.	8	-
	ii	.21 "	8	-
	iii	.21 "	8	-
	iv	.34 "	4	4
B (Na Cl S.G. 1.18)	i	.24 "	8	-
	ii	.28 "	8	-
	iii	.20 "	8	-
	iv	.23 "	4	4
C (Na Cl S.G. 1.20)	i	.21 "	8	-
	ii	.18 "	8	-
	iii	.29 "	8	-
	iv	.44 "	4	4
D ($ZnSO_4$ S.G. 1.20)	i	.26 "	8	-
	ii	.15 "	8	-
	iii	.14 "	8	-
	iv	.31 "	4	4
E ($ZnSO_4$ S.G. 1.25)	i	.16 "	8	-
	ii	.23 "	8	-
	iii	.28 "	8	-
	iv	.23 "	4	4
F ($ZnSO_4$ S.G. 1.30	i	.21 "	8	-
	ii	.24 "	8	-
	iii	.28 "	abandoned	-
	iv	.26 "	"	abandoned

TABLE 2.

Counts, corrected to a standard faeces weight of .25 grams,

obtained from faeces samples i, ii and iii of solutions A to F.

(Experiment 1)

Samples		Cells								Sample Totals	Treatment Totals	Overall Total
A	i	44.06	53.44	42.19	45.94	50.00	58.75	49.06	48.75	392.19		
	ii	43.81	49.52	37.14	41.90	57.14	45.71	52.38	39.05	366.65		
	iii	48.57	33.33	41.90	22.86	40.95	30.48	30.48	46.67	295.24	1054.08	
B	i	42.92	53.33	44.58	44.58	46.67	45.42	47.92	49.58	375.00		
	ii	48.57	50.71	47.14	45.71	60.00	46.43	45.71	45.00	389.27		
	iii	54.00	44.00	49.00	51.00	62.00	57.00	57.00	57.00	431.00	1195.27	
C	i	52.38	51.43	65.24	70.00	60.95	70.95	58.57	50.48	480.00		
	ii	45.56	46.67	50.00	41.11	50.00	53.33	58.89	55.56	401.12		
	iii	53.10	62.07	63.45	68.97	57.93	55.17	53.10	72.41	486.20	1367.32	
D	i	41.15	55.77	39.23	41.15	47.31	37.69	39.23	40.00	341.53		
	ii	53.33	46.67	44.00	46.67	57.33	44.00	64.00	76.00	432.00		
	iii	38.57	47.14	44.29	31.43	47.14	32.86	48.57	51.43	341.43	1114.96	
E	i	50.00	38.13	63.75	61.25	58.13	43.13	51.88	53.13	419.40		
	ii	33.91	41.74	38.26	31.30	41.74	41.74	46.96	47.83	323.48		
	iii	40.00	32.86	34.29	44.29	48.00	50.00	34.29	42.14	317.87	1060.75	5792.38
F	i	5.24	4.29	1.43	6.19	3.33	6.67	1.91	4.29	33.35		
	ii	8.33	7.50	5.00	9.17	4.17	4.17	5.00	3.33	46.67		

TABLE 3.

The data from faeces-sample iv of solutions A to E.

(Experiment 1)

Solution	Counts of eggs in each chamber		Proportional change
	10 minutes	3 hours	
A (Na Cl S.G. 1.16)	82	77	- .061
	72	71	- .014
	98	93	- .051
	77	76	- .013
Mean			- .035
B (NaCl S.G. 1.18)	65	54	- .169
	71	65	- .085
	82	73	- .110
	77	71	- .078
Mean			- .111
C (NaCl S.G. 1.20)	106	95	- .104
	125	113	- .096
	131	115	- .122
	134	115	- .142
Mean			- .116
D ($ZnSO_4$ S.G. 1.20)	58	46	- .207
	57	49	- .140
	71	66	- .070
	85	83	- .024
Mean			- .110
E ($ZnSO_4$ S.G. 1.25)	41	38	- .073
	37	34	- .081
	31	34	+ .097
	56	53	- .054
Mean			- .028

the salt was rapidly destroying the eggs. Solution F was not considered in the statistical treatment of the data. An analysis of variance was done on the data from solutions A to E to determine if a significantly greater number of eggs were floating in solution C.

The results obtained from faeces sample iv of solutions A to E are given in Table 3. As the purpose is to compare the relative change over three hours, these counts have not had to be corrected to a standard faeces sample weight. An analysis of variance was also done on the proportional changes of each pair of counts to determine if Solution C caused significantly more damage than the others. The method used was essentially that of Snedecor (see Snedecor, 1956).

If r is the number of McMaster chambers counted in each of s samples, from each of t treatments, then the total number of chambers counted is rst; and if the number of eggs counted in a chamber is represented by X; in a sample by x_s and in a treatment by x_t, then:

the correction factor C will be $(\sum X)^2/rst$

the mean chamber count $\bar{X}$ will be $\sum X/rst$

the mean sample count $\bar{X}_s$ will be $\sum X/st$

the mean treatment count $\bar{X}_t$ will be $\sum X/t$

and the sum of squares, S.of S, of the deviations from the various will be:

S.of S.	between chambers, $\sum x^2$, will be:-	$\sum X^2 - C$	(rst-1)	D.of F.
"	between samples, $\sum x_s^2$, will be:-	$\sum X_s^2/r - C$	(st-1)	"
"	between treatments, $\sum x_t^2$, will be:-	$\sum X_t^2/rs - C$	(t-1)	"
"	between chambers within same samples:-	$\sum x^2 - \sum x_s^2$	st(r-1)	"
"	between samples within same treatments:-	$\sum x_s^2 - \sum x_t^2$	t(s-1)	"

TABLE 4.

Estimation and analysis of variance of data from samples i, ii and iii of samples A to E. (Experiment 1)

Estimation of statistics

$\sum X = 5792.38$	rst = 120	C = 279597.22
$\bar{X} = 48.27$	$\bar{X}_s = 386.15$	$\bar{X}_t = 1158.48$
$\sum X^2 = 290494.86$	$\sum X_s^2 = 2282434.90$	$\sum X_t^2 = 6777645.37$
$\sum x^2 = 10897.64$	$\sum x_s^2 = 5707.14$	$\sum x_t^2 = 2804.67$
$\sum x^2 - \sum x_s^2 = 5190.50$		$\sum x_s^2 - \sum x_t^2 = 2902.47$

Analysis of variance

Source of variation	S.of S.	D.of F.	Mean Square	Components
Between treatments	2804.67	4	701.17	$\sigma^2 + 8\sigma_s^2 + 24\sigma_t^2$
Samples in treatments	5190.50	10	290.25	$\sigma^2 + 8\sigma_s^2$
Chambers in samples	2902.47	105	49.43	σ^2
TOTAL	10897.64	119	91.58	$\sigma^2 + \sigma_s^2 + \sigma_t^2$

$\sigma^2 = 49.43$ $\sigma_s^2 = 30.10$ $\sigma_t^2 = 17.12$

Tests of significance

1. Is σ^2 within the expected limits of a Poisson distribution?

$\chi^2 (n = 1) = \sigma^2/\bar{x} = 1.027$ $(P > .25)$ Answer YES

2. Was the mixing of the faeces perfect? i.e. does $\sigma_s^2 = 0$?

$F(10.105) = \dfrac{\sigma^2 + 8\sigma_s^2}{\sigma^2} = 5.87$ $(P < .001)$ Answer NO

3. Are there no significant differences between the treatments? i.e. does $\sigma_t^2 = 0$?

$F(4,10) = \dfrac{\sigma^2 + 8\sigma_s^2 + 24\sigma_t^2}{\sigma^2 + 8\sigma_s^2} = 2.42$ $(P > .05)$ Answer YES

TABLE 5.

Estimation and analysis of varianceoof data from faeces sample iv of solutions A to E. (Experiment 1)

Estimation of statistics

$\sum X = 1.597$ $n = 20$ $C = .127520$

$\bar{X} = .080$ $\bar{x}_t = .319$

$\sum X^2 = .207877$ $\sum x^2 = .080357$

$\sum X_t^2 = .636783$ $\sum x_t^2 = .031676$

$\sum x^2 - \sum x_t^2 = .048681$

Analysis of variance

Source of variation	S.of S.	D.of F.	Mean Square	Components
Between treatments	.031676	4	.007919	$\sigma^2 + 4\sigma_t^2$
Within treatments	.048681	15	.003245	σ^2
TOTAL	.080357	19	.004229	$\sigma^2 + \sigma_t^2$

$\sigma^2 = .003245$ $\sigma_t^2 = .001169$

Tests of significance

1. Are there any significant differences between the treatments? i.e. does $\sigma_t^2 = 0$.

$F(4,15) = \dfrac{\sigma^2 + 4\sigma_t^2}{\sigma^2} = 2.44$ $(P>.05)$ answer NO

None of the five solutions, A to E, with Specific Gravities ranging from 1.16 to 1.25 were proved to be significantly better for the purpose of floating eggs than the others. The failure to obtain complete mixing of the faeces may have masked differences between the treatments. However, of these five solutions, NaCl at a Specific Gravity 1.20 is the most convenient to set up as this is saturated common salt at room temperatures. This experiment has shown, at least, that there are no serious dis-advantages to using saturated salt as the flotation medium.

EGG COUNTING TECHNIQUE

The technique for counting eggs was standardized as follows:

Fresh faeces pellets, weighing about .5 gm. are collected from the rat. The pellets are accurately weighed, w.

The pellets are broken up in 15 ml. of saturated NaCl solution S.G. 1.20.

Chambers of McMaster slides are filled with the faeces suspension; the volume under the grid of a chamber being .15 ml.

The number of eggs, C, under the grids are counted.

The number of chambers, N, counted is 4, unless at least 50 eggs have already been counted in less than 4 chambers.

The number of eggs (in thousands) per gram of faeces is found by the formula:

$$\frac{E.p.gm.}{1000} = \frac{C}{10 \cdot W \cdot N}$$

As there need be no significant error in determining W or recording N, the error in the method lies only in C. If mixing is complete C is dependent on the Poisson series and its standard error is $\sqrt{C}$. When 50 eggs have been counted the standard error is 15%.

Estimation of the number of eggs passed by a rat in a twenty-four hour period does not provide an estimate of the number of eggs laid by the population of parasites in the rat during the same period, as no account has been taken of the transit time of the eggs from the small intestine to the anus.

The ideal method to determine the number of eggs passed in twenty-four hours would be to collect all the faeces passed and to treat them as one sample. However, the surface of rat pellets does not remain moist for more than an hour or two after being passed. Eggs of N. brasiliensis can be seen to collapse by being left to dry under the microscope, and such collapsed eggs do not reform on being reimmersed in water. Therefore, to use the twenty four hour collection of faeces would mean that some of the eggs had already been destroyed. The daily output of eggs was, therefore, calculated by multiplying the weight of faeces passed in the day by the egg count found in a fresh pellet of faeces.

All the pellets were collected at the same time each day and were stood for five minutes in water, about 5 mm. deep. This allowed the pellets to soak up water to correct for that lost by drying. The

pellets were then weighed.

Whenever possible the egg counts were made on pellets taken from the rectum of the rat. When this was not possible the pellet that looked as if it was the one most recently passed was selected. To take a pellet from the rat's rectum the animal is picked up by the right hand across the back, with the thumb and fingers in front of and behind the forelegs. If there is a pellet in the rectum outside the pelvic canal it can be felt with the thumb and forefinger of the left hand, by placing these on the skin on either side of the anus and gently pushing inwards and squeezing together. The pellet can be felt as a hard mass between the finger tip and thumb. The pellet is withdrawn by pushing the finger and thumb beyond the inner end of the pellet and then squeezing through the skin and at the same time drawing the left hand back, so that the pellet is forced outwards into the anus. Once the anus is dilated by the pellet the rat usually finishes the process itself, but if it does not, continued drawing back of the left hand with the forefinger and thumb pressing together anterior to the pellet is sufficient to force the pellet completely through the anus.

The most accurate estimate of the number of eggs passed is to use the running average of the egg counts done at the beginning and end of the period in question.

An alternative is to use the sum of half the previous day's faeces output with half of the following day's faeces output, the egg count then representing the egg concentration half way through the period.

To estimate the number of eggs passed by the worm population it

is necessary to have an estimate of the time taken for the eggs to pass from the small intestine to the anus. This was determined in Experiment 2.

EXPERIMENT 2.

THE ESTIMATION OF THE TRANSIT TIME OF FOOD FROM THE MOUTH TO THE ANUS OF RATS.

Experimental plan

Two rats were fed with a stained food for a short period. The faeces were collected using an apparatus which recorded the time of passing. The amount of stained material in each pellet was estimated and expressed as a percentage of the total amount recovered. The time taken for 50% of the material to pass was determined.

Special methods

The food was prepared from a standard diet by boiling some nuts in a 0.5% solution of Saffranin for ten minutes. After drying it was recompounded into nuts by baking with flour and water. The stained food was available to the rats for one hour, after which it was replaced by the normal diet.

The faeces of the rats was collected by placing the animal in a cage with a large mesh wire bottom. This cage was placed over a large plastic funnel, diameter of spout 1.5 cm., which in turn was placed 5 cm. inside the circumference of a horizontal hard board disc 125 cm. diameter. This disc was on a turntable rotating through an angle of 360° in 24 hours. Thus the faeces pellets were falling onto a table moving 16 cms. per hour. The pellets did not bounce or roll, except

those landing on their ends fell onto their sides. Thus the position of a pellet on the table provided an accurate indication of the time it was passed by the rat. The faeces were collected from the rats over a period of five days after feeding the labelled food. The faeces were suspended in water and after mixing aliquot samples were taken.

The number of stained particles in the samples was counted under a magnification of X 50.

At the same time Dr. B.S.W. Smith of the Biochemistry Department at Moredun Institute performed a similar experiment on three rats, to which he gave 2 ml. of a solution containing ^{51}Cr, administered orally, and then measured the amount of radioactivity in each pellet of faeces.

Results

The percentages of the total number of stained particles that had been passed by given times are given in Table 6 as are the results obtained by Dr. Smith.

The presence of fibre in the diet appears to have a very important effect on the rate of passage of material through the alimentary tract. Rats 4 and 5 were both on fibreless diets and 50% of the labelled material did not pass until after 37 and 18 hours respectively and 100% had not passed by 100 hours after dosing. The other three rats all had fibre in the diet. More evidence is needed to determine whether the 50% transit time of 7 hours in Rat 3 is due to simple variation between rats or to the labelled material being in solution and not, as in Rats 1 and 2, its being attached to the large fibrous material.

TABLE 6.

Cumulative percentage recovery of stained fibre and ^{51}Cr from faeces at intervals after administration.

(Experiment 2)

Time of passing. Hours after dosing	Stained fibre in feed M.R. Brambell		^{51}Cr by oesophageal tube B.S.W. Smith		
	Rat 1	Rat 2	Rat 3 (Normal diet)	Rat 4 (No fibre)	Rat 5 (No fibre NoMg.)
4	0	0	0	0	0
6	0	0	27.2	0	0
8	15.7	0	66.5	0	0
10	38.4	13.8	81.2	0	0
12	46.7	28.8	86.4	0	3.0
14	60.7	40.8	92.4	24.7	23.0
16	72.9	67.2	94.5	24.7	42.6
18	90.0	69.7	96.5	24.7	50.1
20	96.5	78.5	98.1	33.3	50.1
24	99.6	80.5	99.4	33.3	68.9
28	100.0	86.5	99.7	33.3	68.9
32		93.0	99.9	46.0	81.5
36		96.2	100.0	48.7	85.1
40		98.7		58.5	94.2
48		99.1		62.3	94.2
56		99.5		72.6	95.8
64		100.0		78.5	96.7
72				78.5	97.5
80				84.9	98.5
88				88.7	98.7
96				90.2	98.9
104				94.0	100.0
112				95.0	
120				96.3	
50% passed by approx.	12 hrs.	15 hrs.	7 hrs.	37 hrs.	18 hrs.

However, it can be taken from these results that 12 hours is a better figure to take as 50% transit time than 24 hours. If it is assumed that most of the hold up of material is in the large intestine and that the stomach does not hold ingesta up for more than an hour or two, then 12 hours can be taken as an approximate time for half the eggs laid by the worms in the jejunum to pass. It can also be assumed that very few eggs take longer than 24 hours to pass.

On these assumptions it is possible to get an estimate of the egg laying activity of the worm population twelve hours previously. The faeces passed between, say, midnight and sampling at midday, contain eggs laid before those on which the count was made, and faeces passed between sampling and the following midnight contain eggs laid after those counted. Thus to multiply the weight of faeces passed during a calendar day by the eggs per gram estimated at midday provides an estimate of the number of eggs laid by the worm population from midday of the previous day to midday of the same day. As rats were infected nearer to midday than midnight the midday - midday twenty four hours is a more useful period to measure as it is a whole number of days after infection. However, this requires a knowledge of the amount of faeces passed during the twelve hours after sampling, but this was impossible to determine on the day the rat was killed. To make the counts on all days comparable it was assumed that the weight of faeces passed during the period from midnight to midnight was the same as that passed from the previous midday to the intervening midday.

KILLING OF RATS.

The quickest methods of killing rats generally involve breaking or damaging the central nervous system. This cannot be done in a non-premedicated animal without the haphazard stimulation of many nerves and thus considerable muscular activity for some moments after death. Muscular activity whether of the small intestine itself or as violent spasms of the diaphragm and abdominal wall might easily cause more violent movements of the small intestinal contents than is met with in a normal life. This would make it difficult to be certain that the worms had not been moved and would invalidate any results obtained concerning the position of the worms within the small intestine.

Muscular contraction at death can be prevented by using curare-like drugs, a practice not much liked, or by slow induction of anaesthesia, preferably using a volatile anaesthetic. Careful induction of anaesthesia using an ether-air mixture is not difficult and narcotic excitement can be easily avoided. This method was adopted in this work. Once anaesthetized the animals were transferred from the ether-air inducing chamber to a jar containing a pad soaked in ether, the rats being separated from this by a dry cotton wool pad placed on top of the soaked one.

Rats when alarmed or excited show no hesitation in urination and it is a measure of the success of this method of killing that as often as not the dead rats were found to have full bladders. There were no serosal petechiae or echymoses and prior to death no convulsions were observed. Death usually occurred between ten or fifteen minutes after induction had been achieved.

POST MORTEM EXAMINATION OF INFECTED RATS.

Once the animal was dead it was laid on its back. The abdomen and thoracic cage were opened by two incisions, made with blunt ended scissors, up either side from the midline of the os pubis. These cuts followed a line high up on both flanks and the ribs were cut at the same time as the skin. The skin flap removed included the site of injection, which was usually difficult to locate unless it had become secondarily infected.

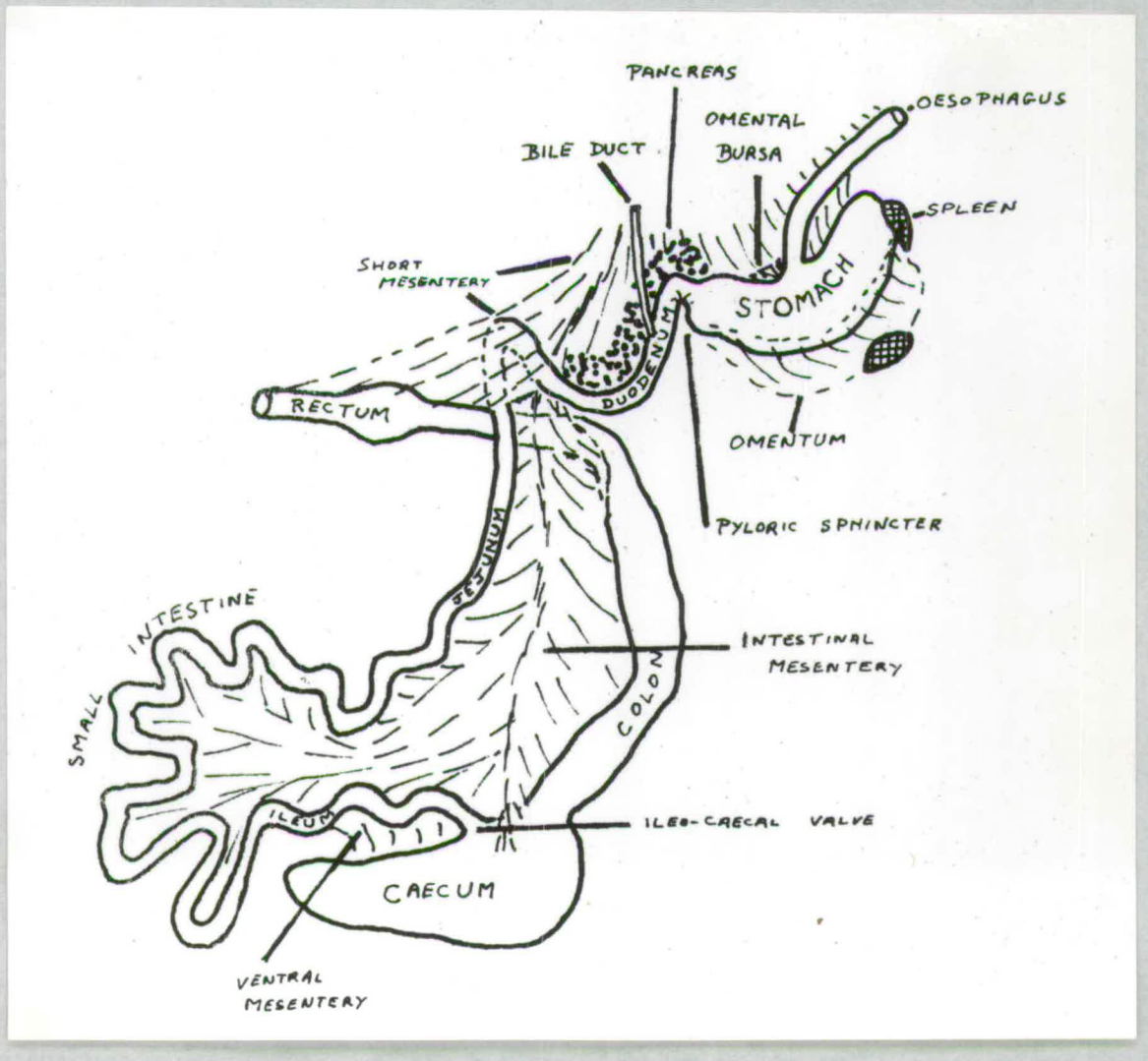
The intestines were removed with the stomach by cutting the rectum, the mesentery at their root and then the oesophagus. The spleen and pancreas came away with the alimentary tract but the liver was left in the animal. The lungs were removed with the heart by cutting along the septum and then through the oesophagus and trachea at the base of the neck.

The lungs, which were not always taken, were separated from the heart and thoracic oesophagus by teasing.

The small intestine was separated from the other viscera by very carefully pulling the mesentery so that it tore at the point where it joined the intestine. Provided the bile duct was recognised and cut, this procedure was not difficult. Once the small intestine was free of mesentery it was separated from the remaining viscera by cutting through the caecum round the ileo-caecal valve and across the pyloric region of the stomach immediately next to the pyloric sphincter.

Figure 2 shows in semi diagramatic form the relative position of the main anatomical features of the small intestine. The organ begins at the pyloric sphincter and ends at the ileo caecal valve.

FIGURE 2.



Diagram, to show relation of small intestine to other parts of Alimentary canal.

Near to its anterior* end the bile duct opens at the sphincter of Oddi.. Along the length of the small intestine are masses of lymphoid tissue which can easily be seen, these are Peyer's patches. The mesenteric attachment has a few features, the duodenum is supported in the mesentery of the colon and at the posterior* end of the duodenum the dorsal part of this mesentery is very short and can be described with a reasonable amount of precision. The rest of the mesentery is almost uniform. The blood supply to the intestine passes through the mesentery.

In order to describe the position of a worm, etc. within the small intestine a satisfactory anatomical reference point would have to be taken from amongst the features just described. It would have to recur in the same position relative to both ends of the organ in each rat and it should be as near the half way point as possible, in order to minimise the effect of experimental error in measurements on the calculation of its relative position. Clearly there is no suitable structure, as is shown below:

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* The terms 'anterior' and 'posterior' are used in the zoological sense of the head being 'anterior' and the tail 'posterior'. They are not synonymus with ventral and dorsal as in human anatomy.

Position of Bile Duct

In an adult rat the bile duct opens 4 - 5 cms. from the pyloric sphincter. The length of the whole of the small intestine is 100 - 120 cms. Thus a very small error in the measurement from the pylorus could very seriously upset the proportion of the lengths of the small intestine anterior and posterior to the opening.

Position of Short Mesentery

The short mesentery is not a structure of the small intestine itself but of a separate, although closely applied, organ. The assumption has been made, therefore, if this structure is used as a reference point, that the same part of the small intestine is always closest to it. However, this organ is only 9 - 10 cms. from the pyloric sphincter and, to a lesser degree, has the same drawback as the bile duct.

Peyers Patches

These are precise structures in the intestinal wall. They do not, however, occur in a pattern which can be recognized in individual rats, though evidence will be presented to show that there is a discernable pattern in the distribution of these structures. There are usually about 20 of them in young adult rats, but this number is likely to fall as the rat ages. It is not possible to select any particular patch and use it as a reference point.

Meckels Diverticulum

This vestige of the junction of the embryonic gut and the

yolk sac stalk is expected to occur somewhere in the region of the junction of the ileum and jejunum. It is well known in man (Arey, 1937) but in this work a structure which might be Meckel's diverticulum has only been seen in one rat (Figure 3). This structure occurred almost half way along the small intestine, and would, had it been a more frequent occurrence, have been a highly suitable reference point.

Blood Supply

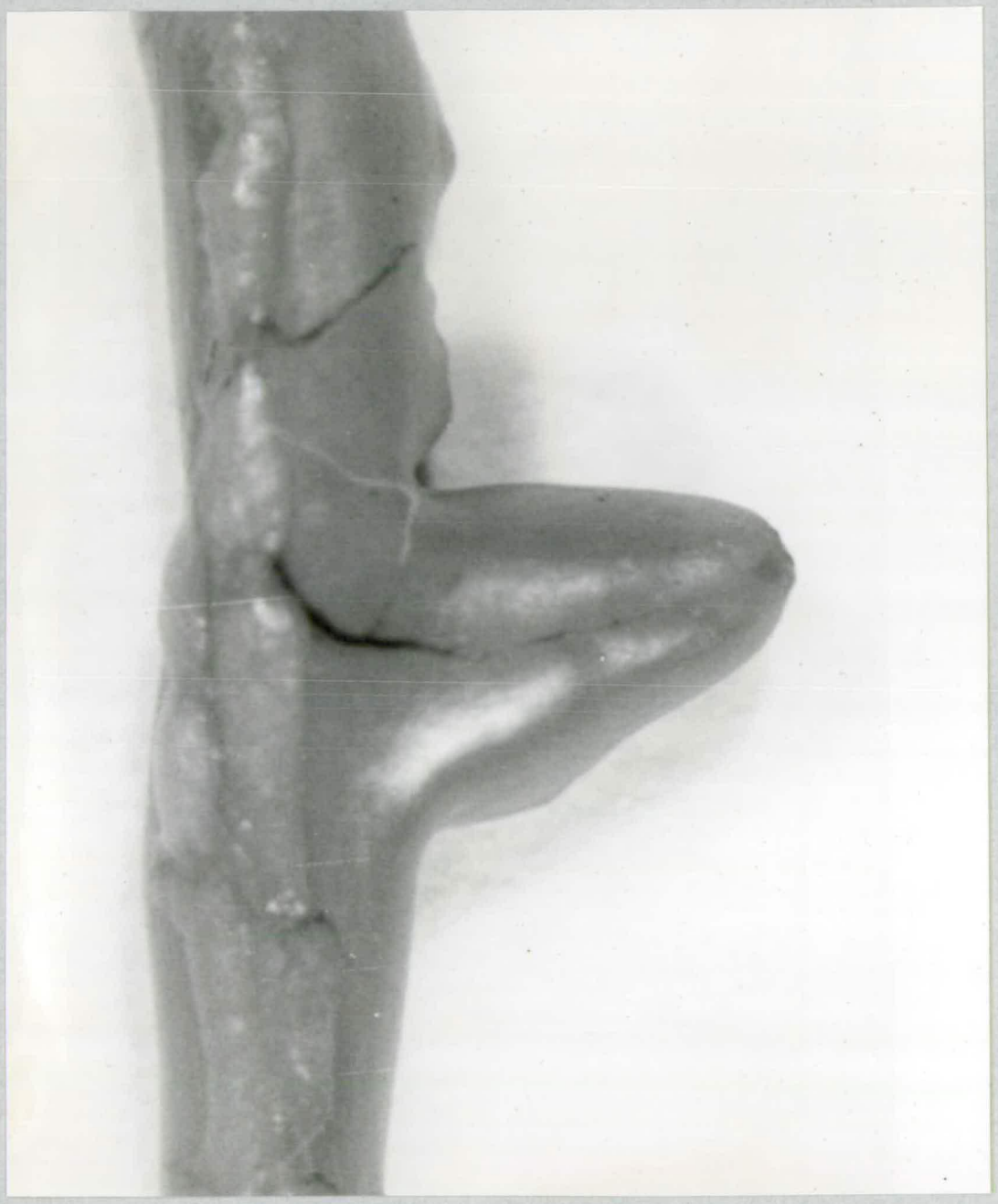
Figure 4 shows drawings of the blood supply to the small intestine in two rats. It is clear that this feature is not suitable for providing reference points.

As there are no suitable points along the small intestine the only remaining way of fixing the position of any point in the organ is to find its relative position direct from the two ends, while the intestine is placed under standard conditions.

In this work it was thought that it would be useless to attempt to fix a position with any accuracy unless the intestine was in a standardized state of contraction. It is known (Clark, , 1952) that adrenaline in low concentrations causes the muscles of the small intestine to relax. A totally relaxed small intestine can be regarded as being in a standard state of (zero) contraction. In order to measure the distances from the two ends of the relaxed intestine it is necessary to have it straight and this requires a certain amount of tension. Small changes in tension have a marked effect upon the length of the intestine and it was therefore necessary to use a standardized tension when straightening the organ.

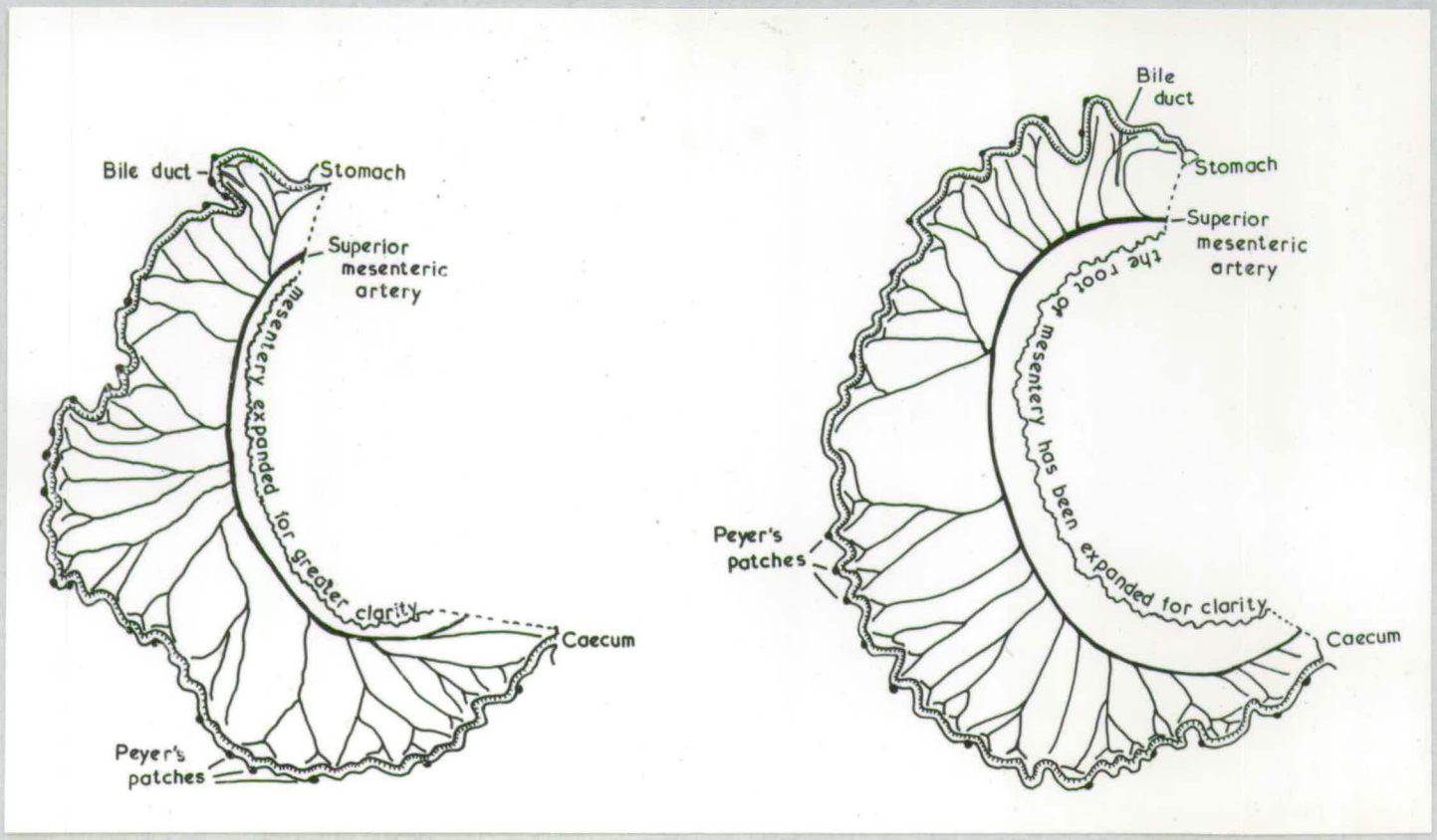
To relax the small intestine and apply the standard tension a

FIGURE 3.



Photograph of diverticulum, possibly Meckel's diverticulum, seen in Section 8 of one rat. Magnification: X 6

FIGURE 4.



Drawings of two samples of small intestine to demonstrate the unsuitability of the blood supply as an aid to the subdivision of the organ.

special apparatus was developed. In essence it was a bath 150 cms. long in which the adrenaline saline could be maintained at 37°C. Inside the bath was the subsidiary apparatus with which to apply the tension.

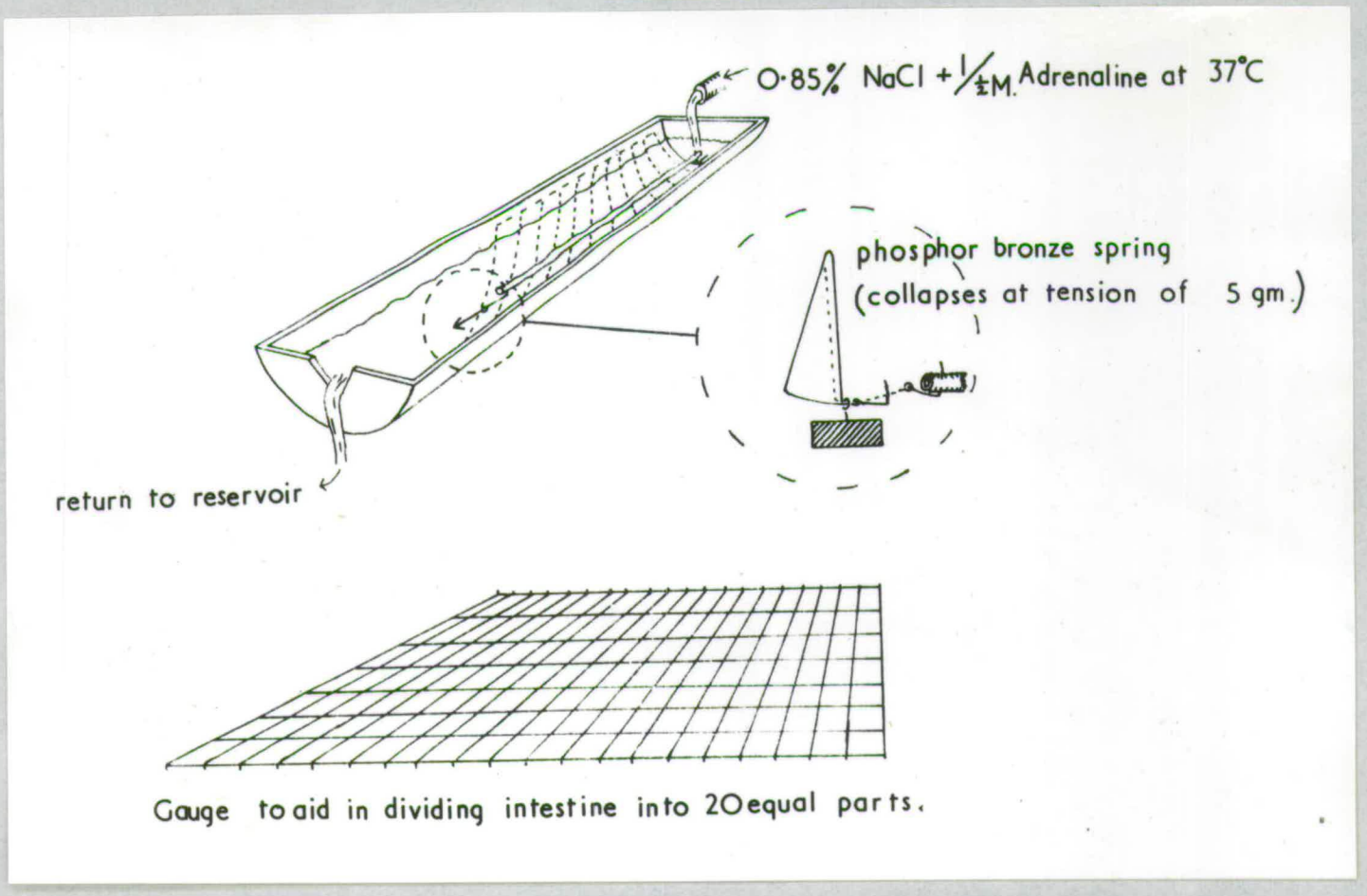
The bath was made from plastic rainwater guttering which was both light and readily available at a suitable length. The two ends were blocked, and at one a V-shaped overflow was provided. The bath was connected to a thermostatically controlled circulating pump and 0.85% saline containing 1/4 Million Adrenaline, was passed into one end and recollected from the other (Figure 5).

The tension applied was 5 grams. This was applied by hooking the part of the stomach still attached to the anterior end of the small intestine to a fixed hook and the part of caecum still attached to the posterior end to a hook on a collapsible spring. This spring was made of phosphor-bronze wire and its collapsing stress was tested at intervals (see Figure 5).

Drawn onto the floor of the bath was a grid which enabled relative positions on intestines of different lengths to be seen at a glance. The usual number of subdivisions made was twenty and the grid was marked out to show twenty equal subdivisions of intestines ranging in length from 80 cms. to 160 cms. This grid is also shown in Figure 5.

[In the course of the work it was convenient to have a concise and distinct name for this relaxing bath. Any adequate technical term would have been lengthy and a name with classical relevance was sought. The Etruscans used more elaborate and complicated systems of divination than did the

FIGURE 5.



Haruspicator: the apparatus in which the small intestine of rats can be divided into proportional parts under standard conditions.

Roman augurs. One of the methods they were reputed to have learnt from no less a person than Jupiter's grandson, Tagus, was to examine the entrails of slaughtered animals. The men who did this were known as the 'haruspices' and by the time of the Roman Empire these had organised themselves into a professional 'collegium haruspicorum' of sixty members. The college was never a state priesthood but rather an august body of salaried expert advisers. What finer footsteps to follow? And what could be more useful in aiding the examination of entrails by a modern haruspex than a Haruspicator. The lily of the gutter has been truly gilded.]

Because of the absence of suitable 'landmarks' along the length of the small intestine the only tests of the method for dividing the intestine are firstly to be sure that the same intestine behaves consistently under the treatment, and secondly to see if the results obtained on the distribution of the worm species being examined are in any way reproducible.

Experiment 3 was performed to test whether the same intestine behaved consistently under the treatment, and opportunity was taken to show that it behaved inconsistently under other treatments.

EXPERIMENT 3.

TO TEST IF THE SMALL INTESTINE OF RATS BEHAVES IN A CONSISTENT FASHION WHEN REPEATEDLY PLACED INTO THE 'HARUSPICATOR'

Experimental Plan

This was a composite experiment designed to show:

(i) that the same small intestine when replaced at intervals into the Haruspicator relaxed in a consistent manner.

and that other treatments gave rise to inconsistent results.

(ii) to find how the amount of error obtained varied at positions along the small intestine.

Part (i) was performed on each of three fresh specimens of rat small intestine. Part (ii) was performed on four specimens of small intestine.

Special Methods

In Part (i) the intestines were first placed in the Haruspicator and an entomological pin was fixed at the position half way along the length of the organ. The intestines were then subjected in sequence to the treatments summarised below.

Haruspicator	1/500,000 Adrenaline in 0.58% NaCl at 37 °C, under tension of 5 gms.
Haruspicator - Tension	as above but no tension applied.
Saline	as Haruspicator but no adrenaline
Air	held in air and gently laid onto a wet bench for measuring.
Air and Tension	held in air and pulled straight by hand before laying on bench.
Air and Dry Bench	as in Air but laid onto a dry bench.
Cold Water	one minute in cold water before measuring.
4 °C	five minutes in refrigerator before measuring on wet bench.
Formalin	measured on wet bench after fixing in 4% Formaldehyde saline.

In Part (ii) four specimens of rat intestine were placed in turn into the Haruspicator. Each was straightened under the standard conditions

and marked with five pins placed at equal intervals, i.e. 1/6, 2/6, 3/6, 4/6 and 5/6 along the length from the pyloric sphincter. Tension was then relaxed and after about one minute, this time was not measured accurately, tension was reapplied. This was repeated ten times on each intestine and the relative positions of the five markers on each occasion were measured. The Haruspicator is marked out in centimetres and the small intestine lies about one centimetre above the scale. Because of parallax it is not very practicable to measure with confidence to nearer than half a centimetre. If the scale was more refined and adjustable so that it could be brought exactly alongside the intestine the accuracy of measurement could be improved. However, this would involve the risk of the intestine touching the scale and not being free to find its own position. As it is, the method's accuracy lies to the nearest half centimetre.

Results

The results of Part (i) are given in Table 7, and the results obtained from Part (ii) are given in Table 8.

As measurements were only made to the nearest half centimetre it was not always possible for a point along the intestine to be accurately measured. Thus if an intestine that was originally 110 cms. long had its half way point marked at 55 cms., and if at a subsequent measurement its length was found to have increased to 110.5 cms. the position of the half way marker would not be recorded as 55.25 cms. but either as 55.0 or 55.5 cms. Thus in the six comparisons of treatment in the Haruspicator in part (i) all the half-way markers were as close as

TABLE 7.

Results of Part (i) of Experiment 3.

Small Intestine	a Pyloric Sphincter to Mark (cms)	b Total length (cms)	c (a/b)x 100 (%)	d (b/2)- a (cms)	e (20d/b)x 100 (%)	Treatment
1	58	116	50	0 (by definition)	-	Haruspicator
	57½	114½	50.2	- ¼	-4.4	Air
	55	110½	49.8	¼	4.5	Cold Water
	54	108½	49.8	½	4.6	Saline
	50	101	49.5	1	9.9	Cold Water
	57½	115½	49.8	¼	4.3	Air
	59	118	50	0	0	Air and Tension
	48	98	49.0	1	20.4	4°C
	59	121½	48.6	1¾	28.8	Air
	62	128	48.4	2	31.3	Air and Tension
	60	120	50	0	0	Haruspicator
2	60½	121	50	0 (by definition)	-	Haruspicator
	59½	118	50.4	-½	-8.5	Air
	58½	117	50	0	0	4°C
	47½	97½	48.7	1½	25.6	Haruspicator-Tension
	60½	121	50	0	0	Haruspicator
	53½	106½	50.2	-¼	-4.7	Cold Water
	60½	121½	49.8	¼	4.1	Haruspicator
	50½	103	49.0	1	19.4	Formalin
3	65	130	50	0 (by definition)	-	Haruspicator
	51	101½	50.2	-¼	-4.9	4°C
	65	130	50	0	0	Haruspicator
	57	117	48.7	1½	+25.6	Cold Water
	65	130	50	0	0	Haruspicator
	63	124	50.8	-1	-16.1	Air
	68	135½	50.2	-¼	- 3.7	Air and Tension
	68	133½	50.9	-1½	-18.7	Air and Dry Bench
	66	132	50	0	0	Haruspicator
	53½	108	49.5	+½	9.3	Formalin

TABLE 8.0

The results obtained from part (ii) of Experiment 3.

The measured positions of markers originally placed at intervals of one sixth of the length of rat small intestine after each time the intestine had been withdrawn and reimmersed into the 'Haruspicator'.

Intestine	Initial length	Length on remeasuring intestine	Deviations of markers from their theoretical positions, expressed as % of total length.				
			1	2	3	4	5
4	114	114	0	0	+.4	0	0
		114	0	0	0	0	0
		114	0	0	0	0	0
		114	0	0	0	0	0
		114.5	-.1	-.1	-.2	-.3	+.1
		115	-.2	-.3	0	-.2	-.3
		115	-.2	-.3	0	-.2	-.3
		115	-.2	+.2	0	-.2	+.2
		115	-.2	-.3	0	-.2	+.2
		115.5	-.2	0	+.2	0	-.2
5	132	132	0	0	0	-.4	0
		132	0	0	0	-.4	0
		132	0	0	0	0	0
		13?	0	0	0	-.4	0
		133	-.2	-.2	-.4	-.5	-.2
		133.5	-.2	0	-.2	-.4	-.1
		133.5	-.2	0	-.2	-.4	-.1
		134	-.3	-.1	0	-.3	-.5
		134	-.3	-.1	0	-.3	-.5
		135	0	0	-.4	-.8	-.7
6	123	123	0	0	0	0	0
		123	0	0	0	0	0
		123	0	0	0	0	-.4
		123	0	0	0	0	-.4
		124	-.2	-.2	0	-.2	-.2
		125	+.1	-.1	0	-.3	-.1
		125	+.1	-.1	0	-.3	-.1
		126	0	0	0	0	0
		126	0	0	0	0	0
		130	-.2	+.2	0	-.2	-.2

7	117	117	0	0	0	0	0
		117	0	0	0	0	0
		117	0	0	0	0	0
		117	0	0	0	0	0
		119	+.1	-.1	0	-.3	-.5
		119	+.1	-.1	0	-.3	-.5
		120	0	0	0	0	0
		120	0	0	0	0	0
		121	-.2	+.2	-.4	-.6	-.2
		121.5	-.2	0	-.2	-.4	-.6
		$\sum x$	-.25	-.14	-.14	-.70	-.56
		$\sum x^2$	.71	.54	.84	3.08	2.68
		s^2	.018	.014	.022	.079	.069
		s	.13	.12	.15	.28	.26

it was possible to measure to their theoretical position. Meanwhile in the other treatments the marker was only sometimes in its expected position, and the results were not consistent. This consistency of the results obtained through using the Haruspicator was borne out in part (ii). In this part it can be seen that the majority of deviations from the expected position are negative. This was because as the intestine slowly lengthened with repeated handling the increase in length was less at the intermediate points, thus the whole intestine could lengthen from 110 to 110.5 cms. but none of the intermediate markers would have moved sufficiently for their position not to be measured as being the same as before. Nevertheless, even after repeated reimmersion in the Haruspicator when the intestines were beginning to become longer through stretching the markers were very close to their theoretical position. The standard deviation of each of the markers was about .2% of the whole length, i.e. about 4% of the length of a 1/20th section of the intestine.

This method of subdivision of the small intestine relies on measuring one dimension and then dividing this into the appropriate number of sections. Ultimately the distribution is a function of three dimensional geometry. In an organ like the small intestine two of the dimensions are so small and comparatively invariable that they can be ignored. In this respect the small intestine is an easier organ to use than others, although the few attempts at describing the distribution of a worm have been made with organs which have a more or less standard outline when laid out in two

dimensional form (Sommerville, 1956, 1963). Indeed even when describing the distribution of Brunner's glands in the human duodenum Landeboe-Christenson (1944) expressed his results in relation to the two-dimensional outline of the organ. However, two dimensional plotting of the distribution can only occur where the major indentations and prominences of the outline and the principal subsidiary structures all occur in the same relative position in every specimen. Clearly this is not possible in the small intestine and the method of 'haruspication' has provided a way of expressing the distribution within a single specimen. For evidence that the method allows comparisons between specimens to be made, the results obtained from the worm distributions, especially in Experiment 4, must be examined.

RECOVERY OF WORMS.

Once the small intestine has been relaxed in the Haruspicator it is possible to measure the positions of the arbitarily chosen twenty subdivisions of the organ. These have to be marked as they cannot be cut in situ because once the first cut is made the tension is released so that the positions for further cuts are lost. The method of marking was to transfix the intestine at the boundaries of the subdivisions with entomological pins. This had the added advantage that the pins prevented any movement of contents from one section to another during the subsequent handling. As soon as the nineteen pins were in place the intestine was lifted out of the bath and placed on a white enamel tray.

The subdivisions were cut off from each other by running the

blades of scissors along the posterior side of the pins. Any spillage of contents was easily seen against the white background and it proved to be very easy to keep the subdivisions and their contents quite separate from each other.

The subdivisions were transferred to open Petri dishes and any spillage was pipetted from off the enamel tray and placed into the dish. The subdivisions were opened by cutting with scissors along the line of the mesenteric attachment and laid out in the dish with the mucosa uppermost. The contents and mucosa was then scraped from the muscular layers with spatulas. When all the mucosa had been removed the muscular layers were lifted out with forceps and placed onto pieces of flat glass. At all stages of this process the instruments and the muscular layers were washed to prevent any worms being removed from the dish. The muscular layers were examined under a dissecting microscope at a magnification x 25.0 to see if any worms were still attached. If so, these were picked off with a dissecting needle.

A count of the number of Peyers patches in the subdivision was also taken at this stage. These organs are on the antimesenteric side of the intestine, hence the opening of the sections by cutting along the mesenteric side.

The material left in the petri dish was made up of numerous lumps of mucosa and intestinal contents and before the worms could be picked out this material had to be broken down to a moderately even suspension. It was found that pumping the material in and out of an all glass 20 ml. syringe with a nozzle measuring 1 mm. internal diameter did not damage fresh unfixed worms. This action very effectively broke up the

mucosa and clumps of digesta, releasing the worms into the suspension.

The material was then transferred to rectangular 'Perspex' searching trays marked longitudinally with lines 6 mm. apart. Provided the material was only about 2 mm. deep all the worms could easily be seen and picked out on a dissecting needle or in a pipette. The interruptions to the search caused by picking out the worms often resulted in the direction the tray was being moved being forgotten. To overcome this hazard the trays were marked with arrows between the lines.

It was considered to be more satisfactory to separate the worms from the other debris before attempting to differentiate and count them.

Once all the worms had been recovered, and this was ascertained by doing a second search of the material, they were fixed in a Glycerol, Ethanol, Formalin mixture and stored.

These processes could be interrupted at any stage after the subdivisions had been separated on the enamel tray, by transferring the material to bijou specimen bottles (glass with metal screw-on tops - capacity about 8 ml.) and placing in a deep freeze cabinet at -20°C for indefinite periods. This did not cause any visible damage to the worms but did kill them (though one worm was still alive after being frozen in water for fourteen days).

DIFFERENTIATION OF WORMS.

Worms of the species Nippostrongylus brasiliensis reach the small intestine as 4th stage larvae. There is very rapid and marked growth at this stage until it reaches the period of the last moult. After

moulting the fifth stage larvae grow even more markedly to become adults.

The newly arrived 4th stage larvae are generally coiled by the time they are examined. They are small and their sex cannot easily be determined. At the time of the last moult it is not easy to distinguish larvae that are moulting from those that are about to moult on one hand and from those that have just moulted on the other. Further, it was felt that if the method of breaking up the mucosal bris was going to alter any stage of the worm it would alter the moulting stages. Consequently, the larvae that are in the period around moulting have not been differentiated. Such larvae could easily be sexed and for the purposes of this work were included with adults. It is possible to distinguish fecund adults from non-fecund ones on the basis of the presence or absence of eggs and sperm in the two sexes. It is felt that the only satisfactory non-subjective way to distinguish the various stages of immaturity between entering the last moult and the adult stage is to measure the worms with the aid of a camera lucida and the material has been kept for this purpose.

Figure 6 gives a diagrammatic representation of the various stages of this worm's life cycle. It is not possible to give the time scale as well as this depends on the state of resistance of the host.

FIGURE 6.

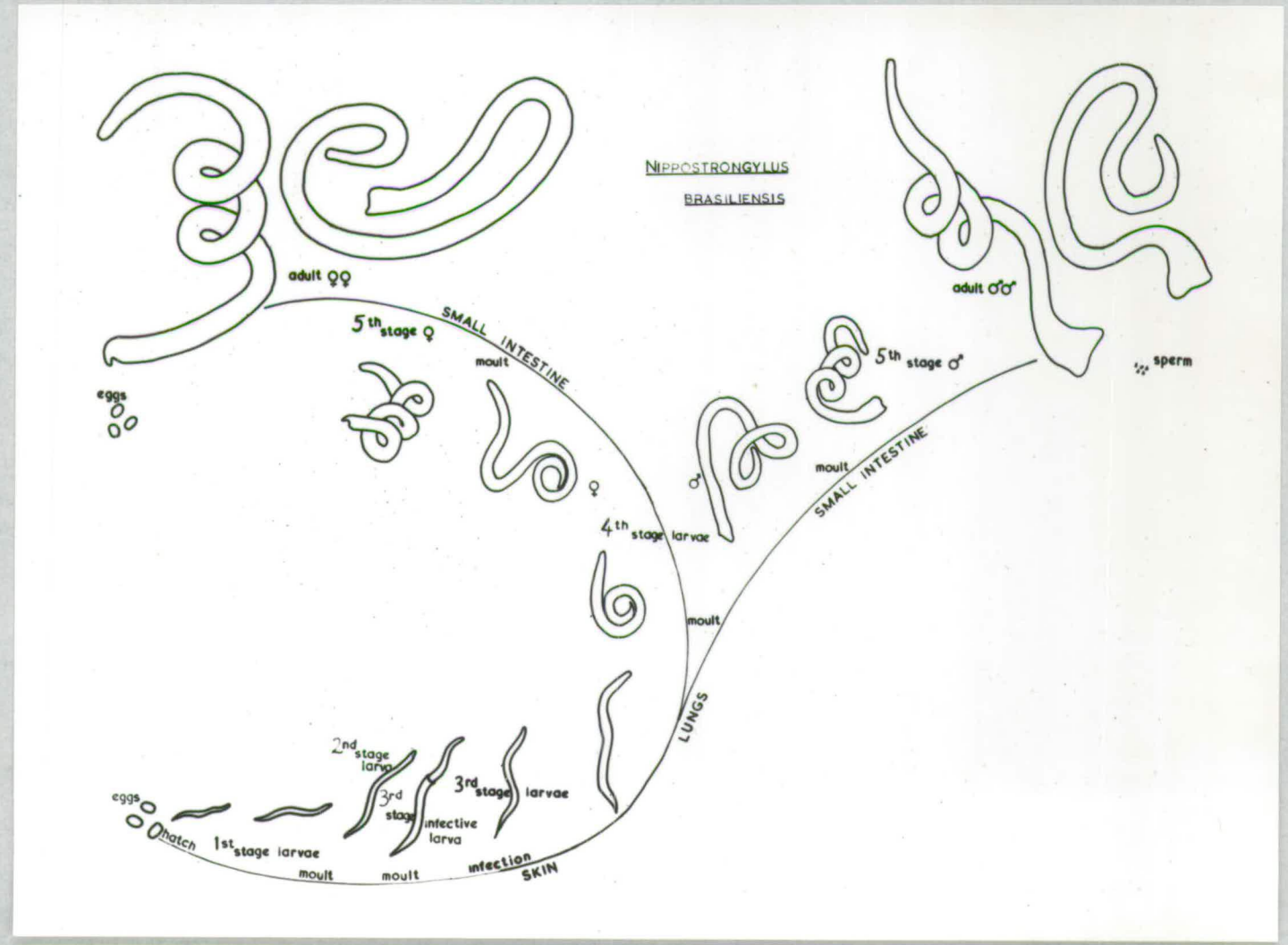


Diagram to show the relative sizes of the developmental stages of Nippostrongylus brasiliensis. Magnification: X 25

EXPERIMENTAL.

EXPERIMENTAL

DISTRIBUTION OF PRIMARY INFECTIONS.

EXPERIMENT 4.

THE DISTRIBUTION OF NIPPOSTRONGYLUS BRASILIENSIS IN THE SMALL INTESTINE OF RATS ON SUCCESSIVE DAYS AFTER THE START OF A PRIMARY INFECTION BY 1800 LARVAE.

Experimental Plan

Sixty rats, 30 males and 30 females, were used. At the start (Day 0) the rats were 120 days old and the weights of the males were 221 ± 14 gm. and of the females 190 ± 12 gm. Two rats of each sex were kept as uninfected controls and the remaining 56 animals were given 1 ml. of a suspension containing 1800 infective larvae of N. brasiliensis per ml. on Day 0. During the experiment the daily output of faeces was collected and egg counts were made on the last pellet produced before the cages were cleaned. The number of eggs passed per day was estimated for each rat.

One infected animal from each six was selected at random and killed on each day after infection, from Day 1 to Day 27, the remaining two animals being killed on Day 30. A control animal was killed on each of Days 0, 10, 20 and 30. The worms in each of the twenty sections of the small intestine of each rat were counted and classified according to sex.

Results

Table 10 Summarised data derived from egg counts.
Table 11 Worms recovered from each section on each day.
Table 12 Principal parameters of worm distribution in each rat.
Table 13 Sex distribution of worms.
Table 14 Fitting of distribution data to Normal, Binomial and Poisson series.

TABLE 9.

Plan of Experiment 4.

Day	Control Egg Counts	Infected Egg Counts	Control Rats Killed	Infected Rats Killed
0 (Rats infected)	4	56	1	
1	3	56		1♂ 1♀
2	3	54		1 " 1 "
3	3	52		1 " 1 "
4	3	50	1	1 " 1 "
5	3	48		1 " 1 "
6	3	46		1 " 1 "
7	3	44		1 " 1 "
8	3	42		1 " 1 "
9	3	40		1 " 1 "
10	3	38	1	1 " 1 "
11	2	36		1 " 1 "
12	2	34		1 " 1 "
13	2	32		1 " 1 "
14	2	30		1 " 1 "
15	2	28		1 " 1 "
16	2	26		1 " 1 "
17	2	24		1 " 1 "
18	2	22		1 " 1 "
19	2	20		1 " 1 "
20	2	18	1	1 " 1 "
21	1	16		1 " 1 "
22	1	14		1 " 1 "
23	1	12		1 " 1 "
24	1	10		1 " 1 "
25	1	8		1 " 1 "
26	1	6		1 " 1 "
27	1	4		1 " 1 "
28	1	2		-
29	1	2		-
30	1	2	1	1 " 1 "

Table 15 Sex of host and worm distribution.

Figure 7 Worms recovered and eggs passed each day.
Figure 8 Histograms of worm distribution.
Figure 9 Sections containing more than 5% of worms recovered.
Figure 10 Worm numbers in Section 1 compared with those in Sections 5-7.
Figure 11 Males per 100 adult worms (from all Sections) on various days.
Figure 12 Males per 100 adult worms (Sections 1-4) on various days.
Figure 13 Males per 100 adult worms in various sections.

The number of eggs passed per day by a female worm is an estimate based on the assumption that all female worms in a rat are equally active; an assumption which is very difficult to justify. However, the figures given must give some indication of the relative activity of the female worms at various times after infection. In the absence of accurate knowledge on the transit time for eggs laid in the upper part of the small intestine to reach the rectum not very much confidence can be put on the time relationship given between the numbers of female worms present and the numbers of eggs passed. A change of a few hours in the assumed transit time can make it appear at the one extreme that the rate of egg laying has fallen sharply before the numbers of female worms began to diminish, to at the other extreme that the female worms are still laying at almost full production while their ranks are being reduced, presumably by the rat's reaction.

The amount of data collected from the twenty sections of each of the fifty-six infected rats was so large that it has had to be presented in a summarised form. Individual variation was great, but it can be seen that sufficient rats were used to discern a definite pattern of the distribution throughout the course of the infection. Figure 9 is not

TABLE 10.

Mean number of eggs passed by a rat on each day, the mean number of female worms recovered from each rat on each day (running average of 2 days). Mean number of eggs passed per day by a female worm.* (Experiment 4).

Day	Infected Rats Surviving	E.p.d/1000 per rat	♀ worms per rat	E.p.d. per ♀ worm
0	56	0	0	0
1	56	0	0	0
2	54	0	84	0
3	52	0	280	0
4	50	0	371	0
5	48	23.2	458	50
6	46	134.4	455	300
7	44	144.8	421	340
8	42	239.5	449	530
9	40	287.7	441	650
10	38	334.6	498	670
11	36	268.4	501	540
12	34	117.5	447	260
13	32	77.3	243	320
14	30	31.4	100	310
15	28	6.4	62	100
16	26	.3	19	20
17	24	0	40	0
18	22	0	32	0
19	20	0	10	0
20	18	0	1	0
21	16	0	3	0
22	14	0	2	0
23	12	0	2	0
24	10	0	2	0
25	8	0	1	0
26	6	0	1	0
27	4	0	2	0
28	2	0	2	0
29	2	0	2	0
30	2	0	2	0

* As it has been assumed that eggs being passed in the faeces had been laid by the worms 12 hours before, the number of female worms present 12 hours earlier has been taken as the average of the previous and the present day.

TABLE 11.

Total number of worms recovered from twenty sections of the small intestines of pairs of rats killed on successive days after infection.

(Experiment 4).

Days after Infection	Sections 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Male worms	Fem. worms	4th stg. lar.	To wo
1			2	2	10	20	13	5	2		4	5	3	3		1	2						72	
2			13	132	262	316	199	84	103	2	36	23	1	4	1						196	337	643	1
3	1	5	15	97	293	407	370	217	106	56	32	20	33	15	9	7		1	2	1	659	783	245	1
4		15	18	241	353	359	181	93	23	22	18	18	22	7	5	1					640	702	34	1
5			14	249	519	642	330	147	113	26	3	4	2								913	1130	6	2
6			3	38	269	514	261	49	56	51	3	1			2						556	690	1	1
7	43	24	149	247	613	302	255	94	37	56	18		2	[illegible]	2		5				853	994		1
8	85	28	25	24	336	476	298	192	19	9								3		3	695	801	2	1
9	83	53	92	145	424	480	187	209	81	44	16	25	7		12		2		2	5	904	963		1
10	39	6	35	38	167	598	610	116	73	31	22	19	10	15	52	68	61	34			967	1027		1
11	40	49	29	142	461	441	320	142	115	99	39	14	28	7	15	5	24	9	6	4	1014	975		1
12	85	56	60	20	237	457	189	245	139	214	52	16	4	25	22	24	7	5	14		1059	812		1
13		3	1	7	104	107	187	95	13	27	7	6		10	7		1				417	158		
14	24	5	7	6	12	31	[illegible]	70	95	16	8	6		3	19	22		5	2	6	301	242		
15	4	12	14	15	5	2	2						2			2	2	2			58	4		
16	12	9	3	9	20	[illegible]	23	10	15	13	11	8	1		15				58	2	163	72		
17	31	114	37	30	16	10	29	13	3	2	8	34	22	35	10	7			3	5	319	89	1	
18	8	65	62	53	53	20	15	35	23	13	2	3			5		2				321	38		
19	1	1																			1	1		
20			1																			1		
21	36	14	14	3	2																60	9		
22	2								1												3			
23		3	[illegible]	6																	3	6		
24	4		2																		4	2		
25	1	5																			3	3		
26	2				1																1	2		
27	6	3	5	5		3	1						3								22	4		
30	20	34	4																		54	4		
Male worms	371	381	359	741	1911	2278	1761	906	539	334	138	97	61	65	65	50	37	37	39	16	10186			
Fem. worms	156	120	231	652	2001	2697	1752	846	416	312	112	93	73	51	111	[illegible]82	67	22	46	9		9849		
4th st. lar		3	15	116	245	236	163	64	62	35	29	12	6	8		5	2		2	1			1004	
Total worms	527	504	605	1509	4157	5211	3676	1816	1017	681	279	202	140	124	176	137	106	59	87	26				21

TABLE 12.

The Mean and Mode positions and the standard deviations of the worm distributions expressed in terms of twentieth sections of the small intestine and the total number of worms recovered from each rat. (Experiment 4).

Day killed	Male Rats					Female Rats				
	Rat	Mean	Mode	S.D.	Total	Rat	Mean	Mode	S.D.	Total
1	1	6.7	5.5	3.1	47	2	8.2	6.5	3.5	25
2	3	5.9	4.5	2.0	695	4	6.0	5.5	1.7	481
3	5	6.4	5.5	2.2	812	6	6.5	5.5	2.3	875
4	7	5.1	4.5	2.0	968	8	6.5	5.5	2.0	408
5	9	5.0	4.5	1.5	1073	10	6.1	5.5	1.2	976
6	11	5.5	4.5	1.2	416	12	6.0	5.5	1.5	831
7	13	4.4	3.5	2.2	842	14	5.5	4.5	1.6	1005
8	15	5.1	5.5	2.5	706	16	5.7	5.5	1.2	792
9	17	5.3	5.5	2.1	818	18	5.6	5.5	2.7	1049
10	19	7.4	6.5	3.9	987	20	6.9	5.5	3.1	1007
11	21	5.3	4.5	2.5	885	22	6.9	5.5	3.0	1104
12	23	6.9	5.5	2.6	1212	24	6.2	5.5	4.1	659
13	25	6.2	4.5	2.5	187	26	6.8	6.5	1.7	388
14	27	3.7	1.5	1.5	13	28	7.8	6.5	5.7	530
15	29	2.7	2.5	1.4	29	30	6.0	3.5	5.7	33
16	31.	2.3	0.5	2.9	22	32	10.6	5.5	5.7	213
17	33	6.0	1.5	5.1	360	34	6.2	1.5	5.7	49
18	35	4.5	2.5	2.8	345	36	5.1	1.5	5.3	14
19	37	-	-	-	0	38	1.0	1.0	0.7	2
20	39	-	-	-	0	40	2.5	2.5	0	1
21	41	1.3	0.5	1.0	64	42	2.7	1.5	1.6	5
22	43	3.2	0.5	4.6	3	44	-	-	-	0
23	45	-	-	-	0	46	2.8	3.5	1.0	9
24	47	-	-	-	0	48	1.2	0.5	1.0	6
25	49	1.3	1.5	0.2	4	50	1.5	1.5	0	2
26	51	1.8	0.5	2.3	3	52	-	-	-	0
27	53	3.8	0.5	3.7	25	54	2.5	2.5	0	1
30	55	1.1	1.5	0.2	37	56	1.4	1.5	0.6	21

TABLE 13.

Sex incidence of worms at various times and in various sections.

(Experiment 4).

Days after Infection				Sections			
Day	♂worms	Adults	♂%	Section	♂worms	Adults	♂%
2	196	533	36.8	1	371	527	70.4
3	659	1442	45.7	2	381	501	76.0
4	640	1342	47.7	3	359	590	60.8
5	913	2043	44.7	4	741	1393	53.2
6	556	1246	44.6	5	1911	3912	48.8
7	853	1847	46.2	6	2278	4975	45.8
8	695	1496	46.5	7	1761	3513	50.1
9	904	1867	48.4	8	906	1752	51.7
10	967	1994	48.5	9	539	955	56.4
11	1014	1989	51.0	10	334	646	51.7
12	1059	1871	56.6	11			
13	417	575	72.5	12	296	574	51.6
14	301	543	55.4	13			
15				14			
16	540	705	76.6	15			
17				16			
18				17	309	697	44.3
19				18			
20	472	542	87.1	19			
21				20			
22							
23				Whole experiment			
24							
25				♂worms	Adults	♂%	
26							
27				10186	20035	50.8	
30							

TABLE 14.

Values of k- and g- statistics calculated according to Snedecor (1956, p.200) and the fitting of these to the values expected in Normal, Binomial and Poisson distributions.

(Experiment 4).

Value of k statistics	Estimates of	Expected values calculated from data for		
		Normal	Binomial	Poisson
k_1 = 6.4	Mean*	6.4	6.4	6.4
k_2 = 3.6	Variance	3.6	4.4	6.4
k_3 =10.8	3rd Cumulant	0	1.6	6.4
k_4 =57.0	4th "	0	-1.3	6.4
$g_1 = \frac{k_3}{k_2\sqrt{k_2}}$ = +1.58 (p<.01)			$g_2 = \frac{k_4}{k_2^2}$ = +4.40 (p<.01)	

* Worms in Section 1 have been given a position value of 1.0 and not 0.5, etc.

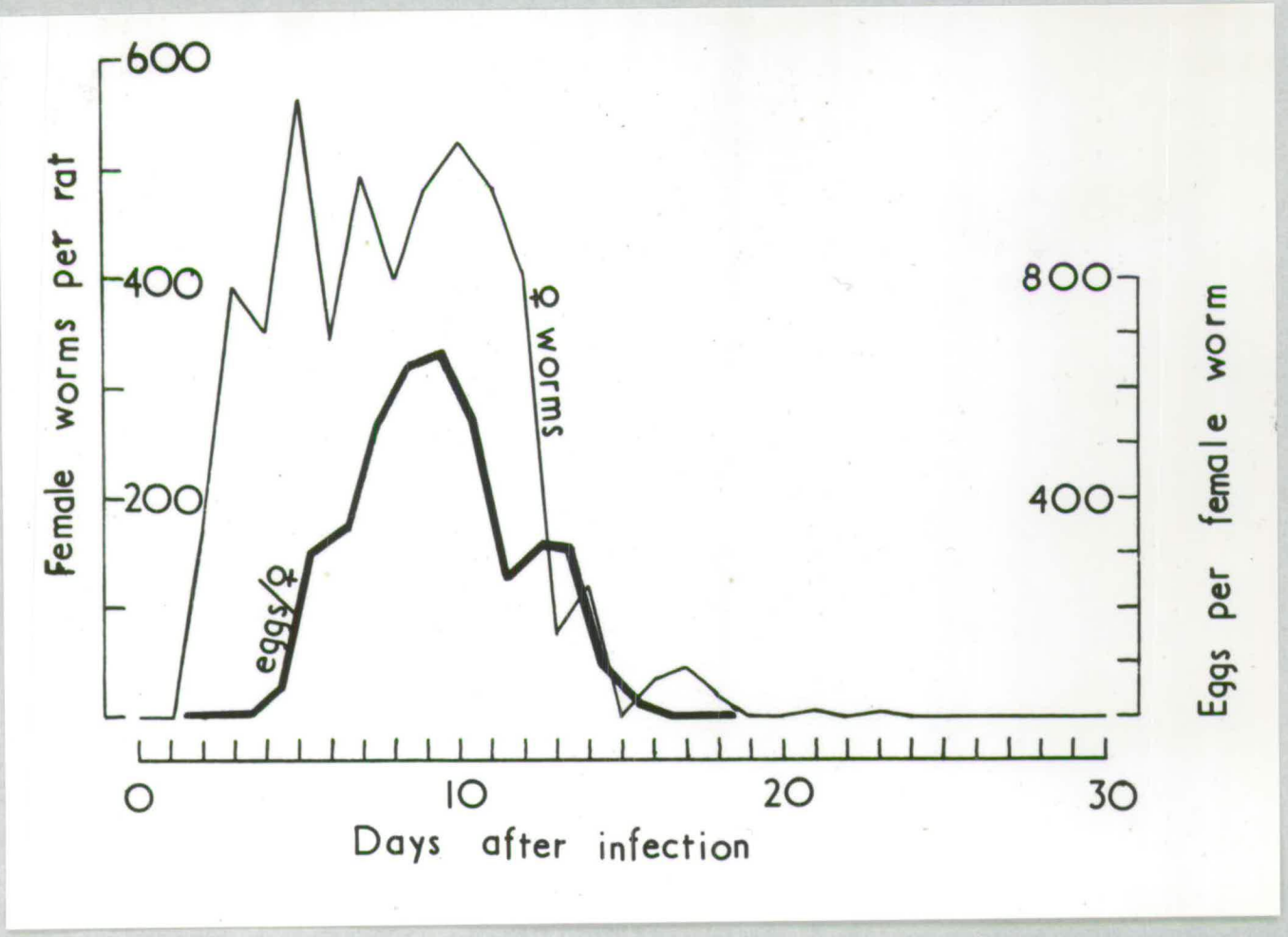
The k- and g- statistics calculated according to Snedecor (1946) p.176, except k_1 value has been decoded (Code X = 6). Expected values have been calculated from data according to Fisher (1954) p.75, 1st table.

TABLE 15.

Worms recovered during experiment from each sex of host (Experiment 4).

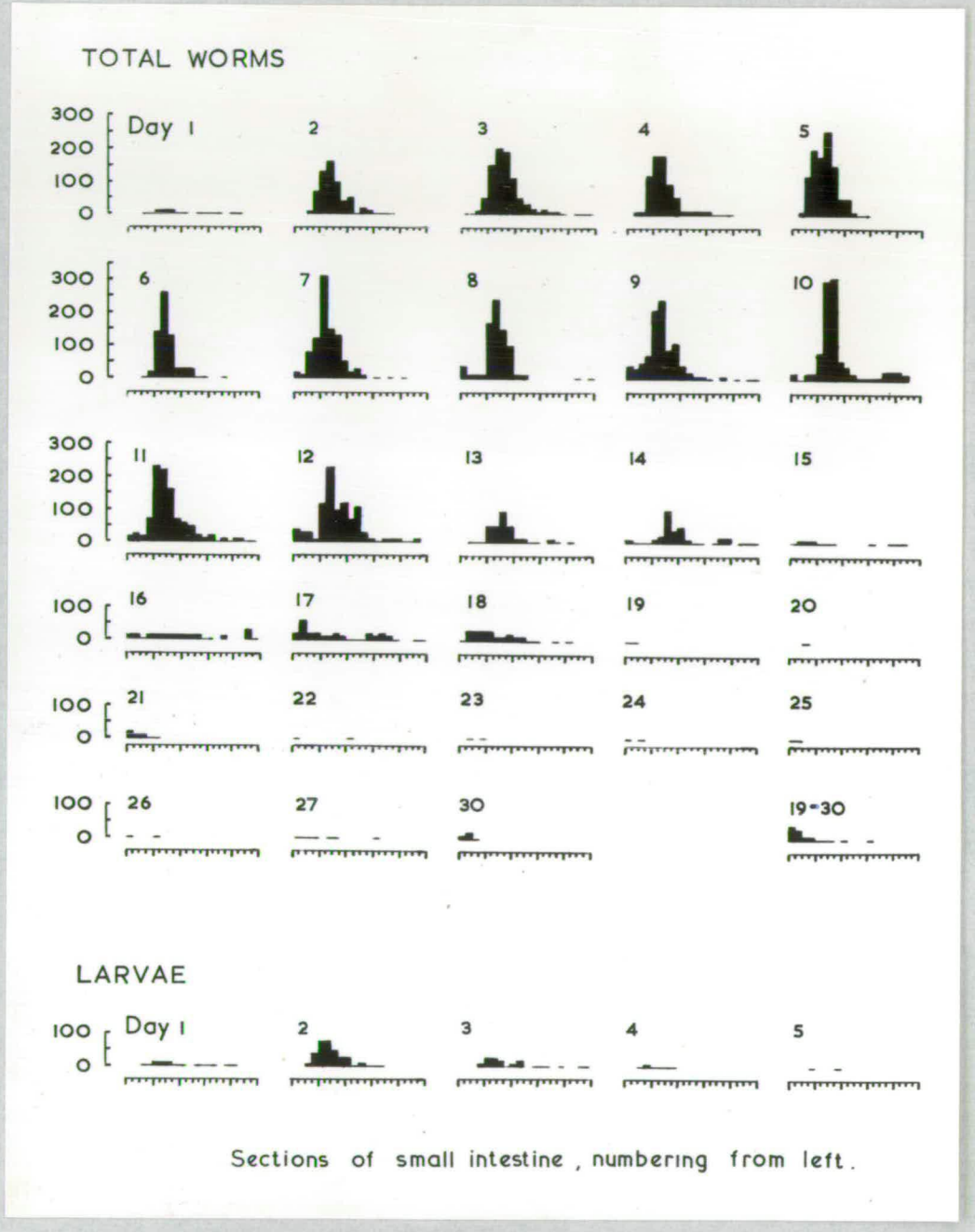
Sections of small intestine	Days 1-6			Days 7-12			Days 13-18			Days 19-24			Days 25-30			Days 1-30		
	Male Rats	Fem. Rats	Total	Male Rats	Fem. Rats	Total	Male Rats	Fem. Rats	Total	Male Rats	Fem. Rats	Total	Male Rats	Fem. Rats	Total	Male Rats	Fem. Rats	Total
1	0	1	1	233	142	375	48	31	79	38	5	43	23	6	29	342	185	527
2	16	4	20	115	101	216	182	26	208	11	7	18	28	14	42	352	152	504
3	44	21	65	244	146	390	104	20	124	14	3	17	5	4	9	411	194	605
4	632	127	759	513	103	616	94	26	120	3	6	9	5	0	5	1247	262	1509
5	1150	556	1706	1004	1234	2238	138	72	210	0	2	2	1	0	1	2293	1864	4157
6	979	1279	2258	1033	1721	2754	49	147	196	0	0	0	3	0	3	2064	3147	5211
7	552	802	1354	943	916	1859	103	359	462	0	0	0	1	0	1	1599	2077	3676
8	272	323	595	591	407	998	47	176	223	0	0	0	0	0	0	910	906	1816
9	176	227	403	188	276	464	30	119	149	1	0	1	0	0	0	395	622	1017
10	59	98	157	289	164	453	17	54	71	0	0	0	0	0	0	365	316	681
11	29	67	96	68	79	147	17	19	36	0	0	0	0	0	0	114	165	279
12	34	37	71	33	41	74	46	11	57	0	0	0	0	0	0	113	89	202
13	34	27	61	9	42	51	22	3	25	0	0	0	3	0	3	68	72	140
14	15	14	29	13	34	47	33	15	48	0	0	0	0	0	0	61	63	124
15	13	4	17	39	64	103	14	42	56	0	0	0	0	0	0	66	110	176
16	1	8	9	32	65	97	2	29	31	0	0	0	0	0	0	35	102	137
17	2	0	2	50	49	99	3	2	5	0	0	0	0	0	0	55	51	106
18	0	1	1	35	16	51	0	7	7	0	0	0	0	0	0	35	24	59
19	2	0	2	14	8	22	3	60	63	0	0	0	0	0	0	19	68	87
20	1	0	1	4	8	12	4	9	13	0	0	0	0	0	0	9	17	26
Total	4011	3596	7607	5450	5616	11066	956	1227	2183	67	23	90	69	24	93	10553	10486	21039
Mean	5.5	6.2	5.9	5.9	6.1	6.0	5.3	7.8	6.7	1.3	2.2	1.6	2.2	1.4	2.0	5.6	6.3	6.0
Mode	4.5	5.5	5.5	5.5	5.5	5.5	1.5	6.5	6.5	0.5	1.5	0.5	1.5	1.5	1.5	4.5	5.5	5.5

FIGURE 7.



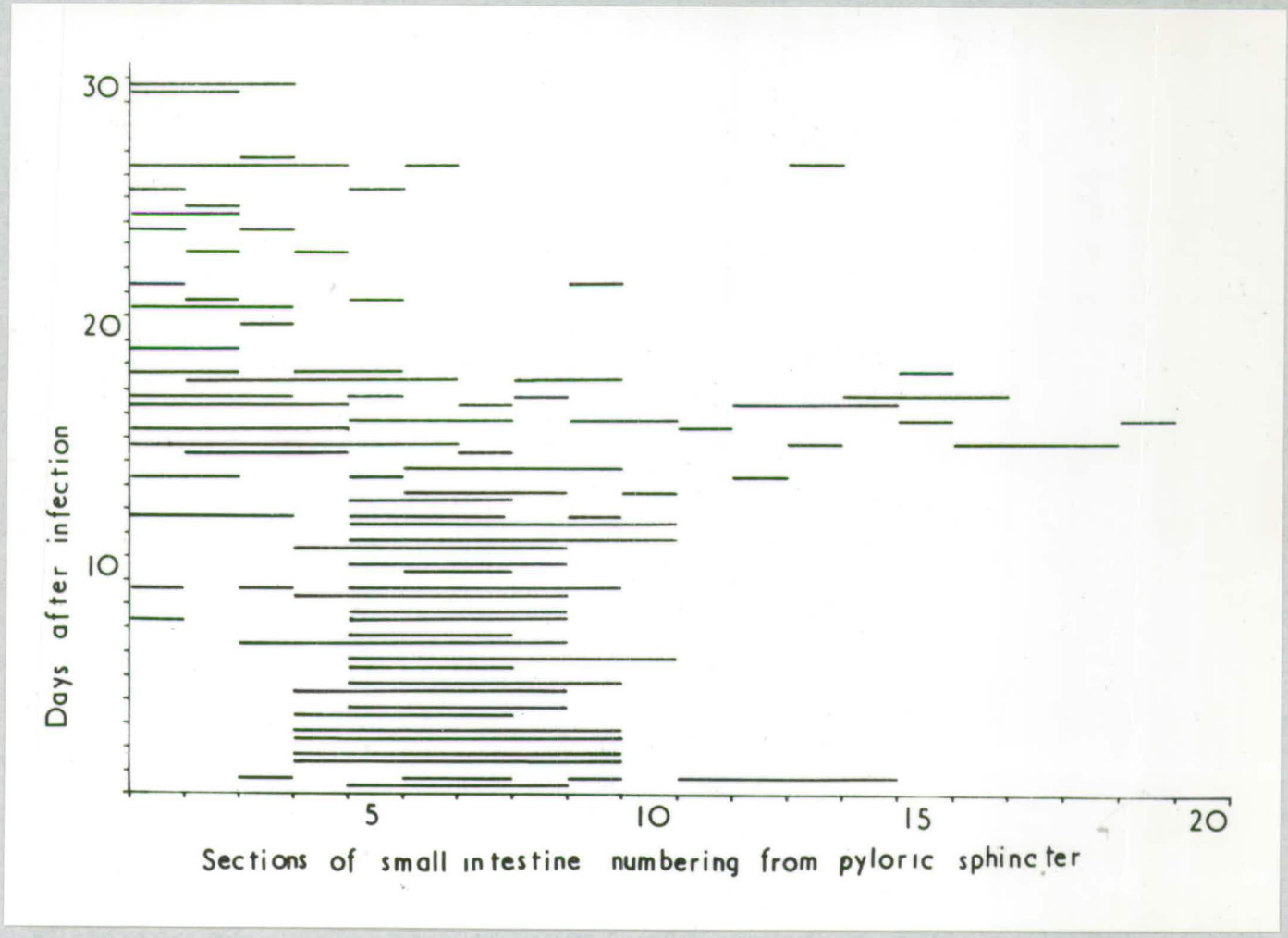
The numbers of female worms recovered per rat and the rate of egg laying by female worms on each day after infection.
(Experiment 4).

FIGURE 8.



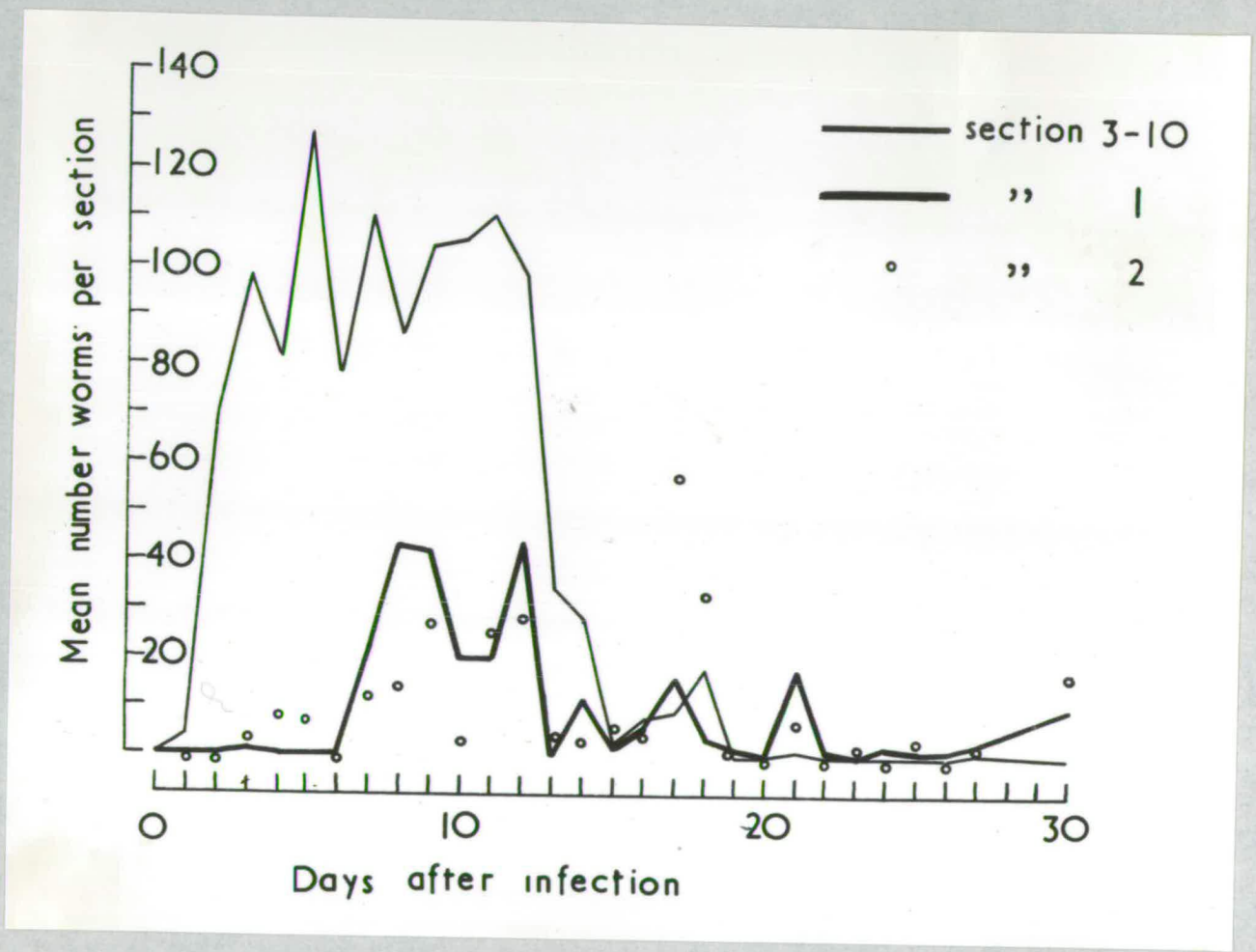
Histograms showing the number of worms in each section of the small intestine on each day after infection. (Experiment 4).

FIGURE 9.



Sections which contained more than 5% of the total number of worms recovered from the small intestine of each rat on experiment. Each horizontal line represents one rat. (Experiment 4).

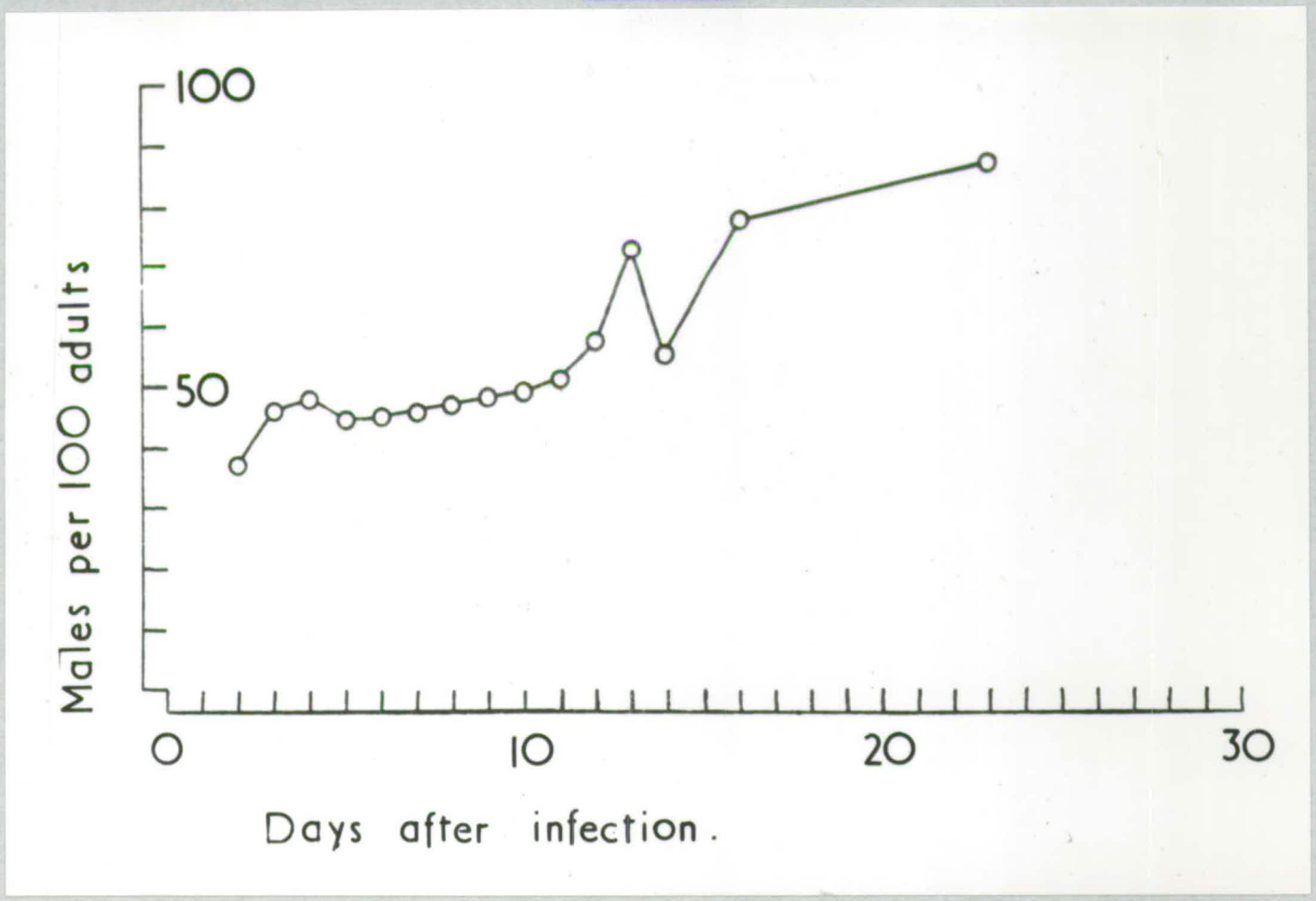
FIGURE 10.



The numbers of worms recovered from Sections 1 and 2 on each day after infection compared with the mean number recovered from Sections 3-10.

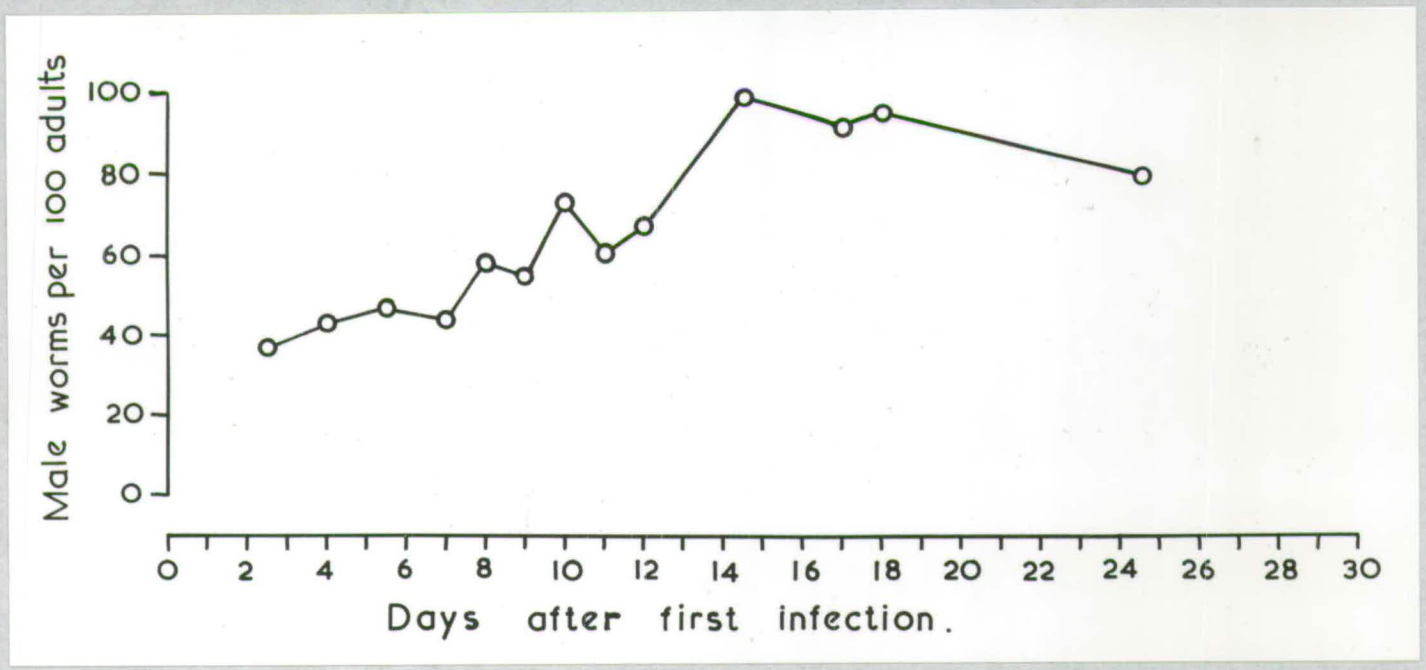
(Experiment 4).

FIGURE 11.



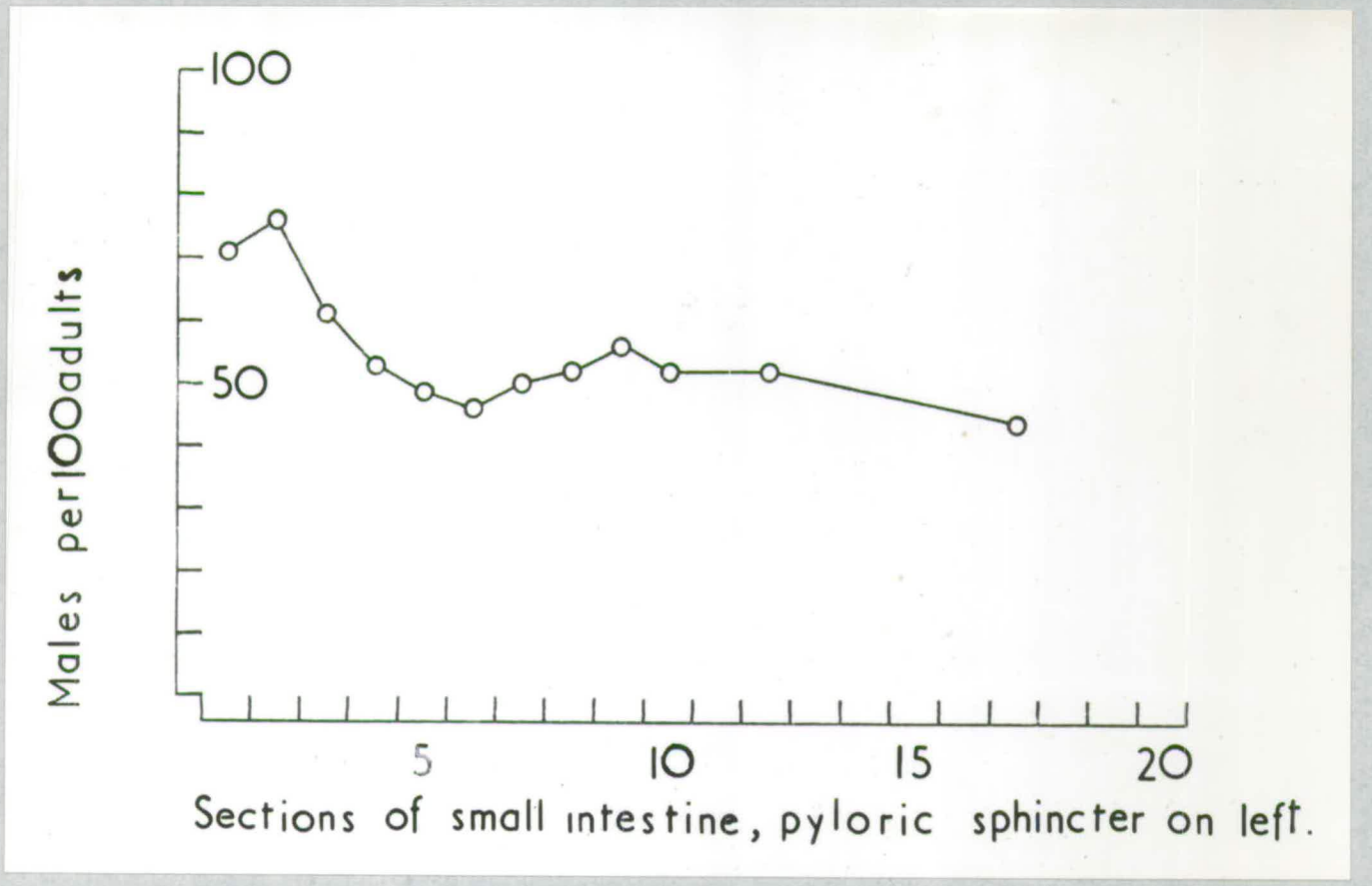
The percentage of male worms amongst adults recovered from the whole of the small intestine on each day of the experiment. Each plot based on at least 500 adult worms. (Experiment 4).

FIGURE 12.



The percentage of male worms amongst adults recovered from Sections 1-4 of the small intestine on each day of the experiment. Each plot based on at least 100 adult worms. (Experiment 4).

FIGURE 13.



The percentage of male worms amongst adults recovered from each section of the small intestine over the whole experiment. Each plot based on at least 500 adult worms. (Experiment 4).

an acceptable form of presenting all the data collected, but is a demonstration of how the pattern of the distribution changes during the infection. This form of presentation in which 5% of the worms present in each rat are recorded has the double advantage of removing differences in total numbers and the stragglers, both of which factors are troublesome in making comparisons between days.

It can be seen that the data of worm distributions can be divided conveniently into four phases mostly of six days each. Phase 1, from Days 1-6, is the period in which the larvae arrive in the small intestine and settle principally in Sections 5, 6 and 7. The second phase from Days 7-12 is the one in which the infestation is at its height. The rate of egg laying is at its highest and the size of the population undiminished. However, although the majority of worms are still in Sections 5, 6 and 7 some worms were being recovered in this period from Sections 1 and 2. Phase III, from Days 13-18 is the period in which the population is markedly reduced, and one in which a redistribution of the survivors takes place. Phase IV consists of the last twelve days of the experiment and presumably longer. In this period almost all of the worms have moved out of Sections 5, 6 and 7, and those that had attached in the more posterior sections have also gone. Only worms attached in the anterior sections 1, 2 and 3 survive in any numbers. Apart from one worm found in Section 1 on Day 3 no worms had been found in this section until Day 7.

Table 11 and Figure 8 show the distribution of 4th Stage larvae that have not yet begun to moult. Unless larvae have begun to moult before reaching the small intestine, and none were found in this stage in the

lungs, trachea, oesophagus or stomach when these were examined in some rats, then it must be assumed that no more larvae arrived after Day 5 and that the changes in distribution which occurred after this were due to movements of the existing adult population. Further, these larvae settled mainly in the sections in which the majority of adult worms were found, and there is no evidence to support Chandler's (1935) contention that the larvae settle in a more posterior position than that taken up by the adult worms.

Comparison of the numbers of larvae seen with the numbers of adult worms found allows a calculation of the mean maximum time that passes between the larvae reaching the small intestine and entering the phase of the last moult. A total of 1004 larvae were seen. The mean number of adults seen on any day (total for two rats) between Days 2-12 was 1606. Thus, as sampling was done at 24 hour intervals, the larvae spent 1004/1606 parts of 24 hours between reaching the intestine and passing into the final moult, i.e. 15 hours.

The data on the relation of male to female worms has been grouped so that at least 500 adults were present in each calculation. The relative number of males increases as the infection gets older. This means that the females are being removed or leaving earlier than the males. Also the relative number of males is greatest at the anterior end of the small intestine and shows a progressive decline along the organ. It has already been established that after about Day 18 only Sections 1, 2, 3 and 4 can support many worms and the predominance of males towards the end of the experiment might be due to the fact that the females could not parasitise this part. Figure 12 shows the relative number of males

in Sections 1-4 during the experiment and it can be seen that up to Day 9 females are able to parasitize this region in more or less equal numbers to the male worms. The points in this table were calculated from data grouped so that at least 100 adults were present. Comparison of Figures 11 and 12 suggests that the relative increase in the proportion of males as the infection ages is more marked in Sections 1-4 than it is in the small intestine as a whole.

Between Days 1 and 6 the worms in the small intestine are concentrated largely into Sections 4 to 9. Later they become more widely dispersed. The initial site of attachment may be under a simple mathematical influence, which might result in the worms being distributed normally. Fisher (1954) has described the use of k statistics to estimate the first four cumulants (kappa parameters) of a population distribution. The k - statistics were calculated by the method described by Snedecor (1956, p. 200). In Table 14 the values of the k - statistics obtained are compared with the expected values if the worms were distributed according to a Normal, Binomial or Poisson distribution. Also in the table the g - statistics are given to test for kurtosis and skewness of a normal distribution.

The value of k_1 is the mean position of the worms and is 5.863 if it is assumed that all the worms in each section are halfway along its length. k_2 is the variance and has a value of 3.620. It is the k_3 and k_4 statistics which can be used to test the goodness of fit to any of the three types of distribution being tested. In a Normal distribution they would both be zero. They are not zero and the g - statistics indicate that the distribution is both skew with

an excess of worms anterior to the mean position and that it shows positive kurtosis, that is there is an excess of worms in the mode position.

The values of k_3 and k_4 do not fit the Binomial or the Poisson distributions and the conclusion must be drawn that the worms are not distributed according to any simple mathematical principle. In determining the form of binomial the number of possible sections a worm could be in is 20 and the mean position of the worm is 5.9 + .5 (to give the position of a worm in each section as a whole number) = 6.4. As the mean of a binomial has the value of np it follows that p has the value of .32 and q .68. The four k - statistics should have identical values in a Poisson distribution.

Table 15 shows that, on average, the Mean and the Mode of the population in female rats is about 1 section more posterior than in male rats. Female rats tend to be lighter than male rats of the same age and the difference found in the distribution of the population of worms between the two sexes may be in fact related to the weight of the host and not to its sex. The experiment was not designed to answer this point and the data is not particularly suitable for the effect of weight to be examined. However, if weight is the factor involved, then it ought to be possible to compare the difference in weight between pairs of rats of the same sex killed on consecutive days with the differences in the position of the population mean.

As Through the course of experiments still to be described, there will are be many such pairs of rats. The correlation of the weight of the rat with the mean position of the worms has been deferred to the end of this thesis (see page 145).

EXPERIMENT 5.

THE EFFECT OF DIFFERENT LEVELS OF INFECTION ON THE DISTRIBUTION OF NIPPOSTRONGYLUS BRASILIENSIS IN THE SMALL INTESTINE OF RATS.

Experimental plan

Forty rats, 20 males and 20 females; aged 200 days at the start of experiment (Day 0) were used. The males weighed 400 $\pm$ 40 gms; and the females 239 $\pm$ 21 gms. Four of the rats were kept as uninfected controls, which were killed on Days 0, 3, 6 and 9. The other thirty-six animals were divided into three groups of 12 rats. The first group was infected with 1000 infective larvae of N. brasiliensis per rat. The second group with 2500 infective larvae and the third with 6250 infective larvae per rat. Four animals from each group were killed on each of Days 3, 6 and 9. The rats were divided between the groups at random within the sexes, the sexes being distributed so that there were two of each sex in each sub group of four. The intestines were treated as in Experiment 4, except that the 20 divisions were each halved and only the worms in the anterior half counted. The estimated population in the small intestine was thus twice the number of worms counted. Prior to death egg counts were made on the faeces and these were expressed as eggs passed per day.

Results

Table 17 Data derived from egg counts
Table 18 Worms recovered from each section in different groups.
Table 19 Principal parameters of the worm distribution in each rat and group.
Table 20 Sex incidence of worms in each section.

Figure 14 Egg count data.
Figure 15 Histograms of worm distribution in each group.
Figure 16 Sections containing more than 5% of worms recovered.
Figure 17 Male worms per 100 adults in each section.

TABLE 16.

Plan of Experiment 5.

Day	Control Rats		Infected Rats					
			12 rats given 1000 larvae		12 rats given 2500 larvae		12 rats given 6250 larvae	
	egg counts	killed	egg counts	killed	egg counts	killed	egg counts	killed
0 (rats infected)	4	1 ♂	12		12		12	
1	3		12		12		12	
2	3		12		12		12	
3	3	1 ♀	12	2♂2♀	12	2♂2♀	12	2♂2♀
4	2		8		8		8	
5	2		8		8		8	
6	2	1 ♂	8	2♂2♀	8	2♂2♀	8	2♂2♀
7	1		4		4		4	
8	1		4		4		4	
9	1	1 ♀	4	2♂2♀	4	2♂2♀	4	2♂2♀

TABLE 17.

Data derived from egg counts.

(Experiment 5)

Thousands of eggs passed per day per rat.										
Days after Infection	0	1	2	3	4	5	6	7	8	9
Survivors in each group	12	12	12	12	8	8	8	4	4	4
1000 larvae	0	0	0	0	0	0	106	200	358	124
2500 "	0	0	0	0	0	0	263	833	830	403
6250 "	0	0	0	0	0	0	665	1159	1249	793

Eggs passed per day per larva given.				
Days after Infection	6	7	8	9
1000 larvae	106	200	358	124
2500 "	105	333	332	161
6250 "	106	185	200	127

Eggs passed per day per female worm.		
Days after Infection	6	9
1000 larvae	602	477
2500 "	594	1049
6250 "	359	680

Control rats: no eggs seen during the experiment.

TABLE 18.

Total number of worms recovered from each section of small intestine from each group on Days 3, 6 and 9 (4 rats to each grouping).

(Experiment 5).

When killed:	3 days			6 days			9 days		
Infected with:	1000	2500	6250	1000	2500	6250	1000	2500	6250
Section 1		1	4		1	25	21	8	9
2	1	2	11	1		15	37	22	23
3	3	10	21	3	15	74	30	17	27
4	2	16	111	8	245	328	31	67	123
5	12	66	442	121	318	1093	108	248	414
6	50	289	704	242	556	1574	236	422	606
7	21	295	890	142	307	1949	192	405	1163
8	16	211	168	59	177	903	100	120	373
9	7	47	97	32	76	608	71	93	517
10	5	8	33	4	13	282	73	25	480
11	1	9	10		6	72	11	6	179
12	1		11			33	6	6	142
13						16	2		32
14						13	1		31
15									12
16		1					1	1	11
17		1					2		8
18							1		8
19									7
20									7
Total	119	956	2502	612	1714	6985	923	1440	4172
% of infective dose	6.0	19.2	20.0	30.6	34.2	55.8	46.2	28.8	33.4

TABLE 19.

The Mean and Mode positions and the standard deviations of the worm distributions, expressed in terms of twentieth sections of the small intestine, and the total number of worms recovered from each rat and group. (Experiment 5).

Day	1000 larvae					2500 larvae					6250 larvae				
	Sex	Mean	Mode	σ	To-tal	Sex	Mean	Mode	σ	To-tal	Sex	Mean	Mode	σ	To-tal
3	M	6.1	6½	1.5	19	M	6.1	6½	1.4	343	M	5.0	5½	1.1	690
	M	6.3	5	1.5	24	M	6.3	6½	1.4	188	M	5.7	5½	1.3	625
	F	6.2	5½	2.2	30	F	7.5	7½	1.3	134	F	6.2	6½	1.2	657
	F	6.0	5½	1.3	46	F	6.1	5½	1.9	291	F	6.9	6½	1.1	530
6	M	5.5	5½	0.9	167	M	4.6	3½	1.3	663	M	5.6	5½	1.4	1521
	M	5.9	5½	1.4	243	M	6.3	5½	1.2	508	M	5.3	5½	1.3	1813
	F	5.9	5½	1.1	129	F	5.7	5½	1.0	281	F	6.5	6½	1.4	1901
	F	6.4	6½	0.9	73	F	6.5	6½	1.3	262	F	7.5	6½	1.8	1750
9	M	4.5	4½	2.3	122	M	5.8	5½	1.1	430	M	7.7	8½	2.6	1044
	M	6.5	5½	2.4	335	M	6.0	6½	1.2	359	M	6.2	6½	1.2	1028
	F	5.9	5½	1.9	329	F	5.5	4½	1.6	371	F	6.1	6½	1.7	1032
	F	7.0	8½	2.3	137	F	6.4	6½	2.3	280	F	8.9	9½	2.5	1068

Statistics of principal groupings.

Grouping	Rats	Mean	Mode	σ	Total	Mean Total	% Take
Male Rats	18	5.8	5½	1.7	10122	562.3	34.6
Female Rats	18	6.8	6½	1.9	9301	516.7	31.8
Killed on Day 3	12	6.0	6½	1.4	3577	298.1	18.3
6	12	6.1	6½	1.6	9311	775.9	47.7
9	12	6.8	6½	2.3	6535	544.6	33.5
Infected with 1000 l.	12	6.0	5½	1.9	1654	137.8	27.6
2500 l.	12	5.9	5½	1.5	4110	342.5	27.4
6250 l.	12	6.5	6½	2.0	13659	1138.3	36.4
All infected Rats	36	6.3	6½	1.9	19423	539.5	33.2

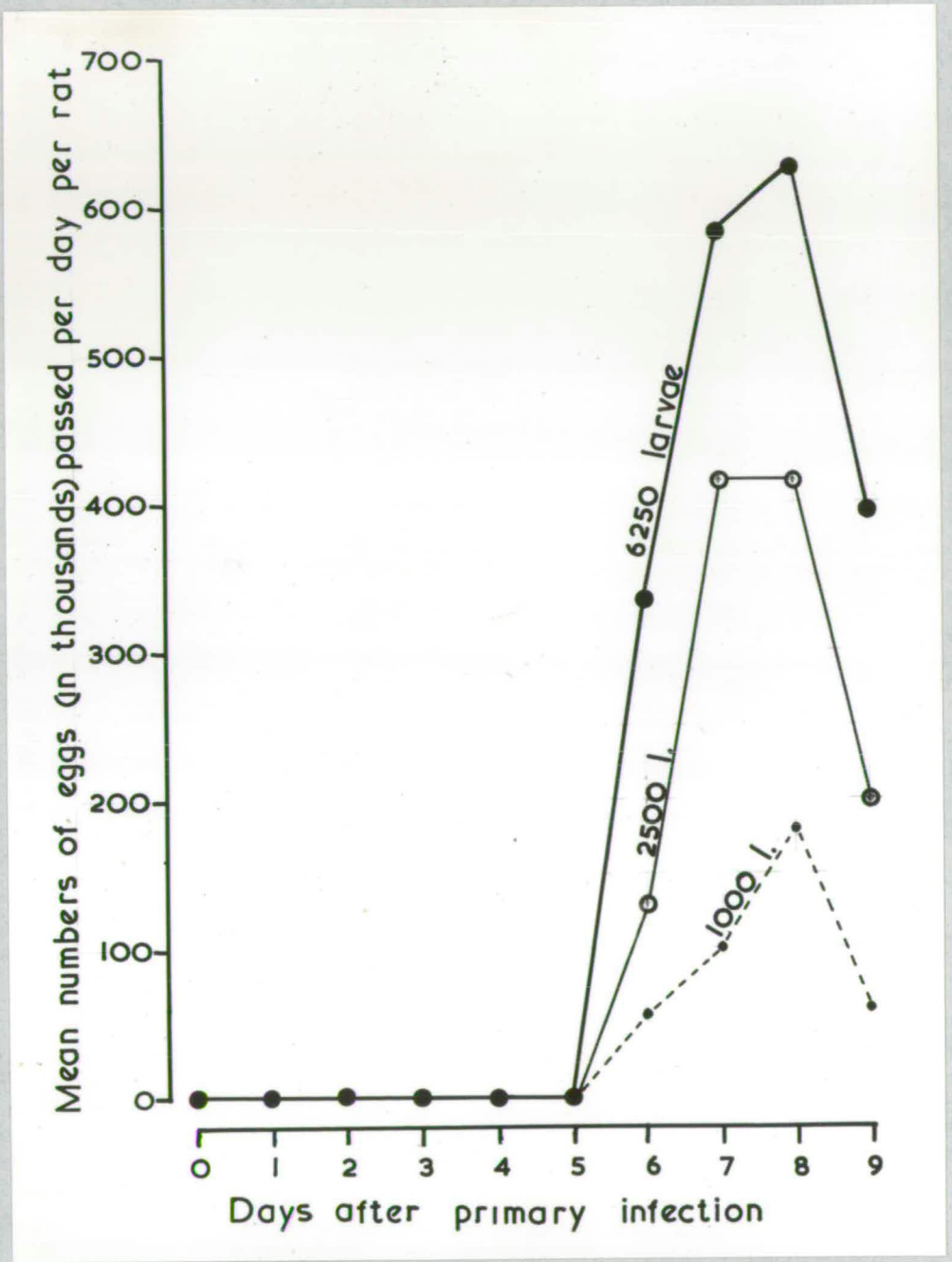
TABLE 20.

Sex incidence of worms in the various sections of the small intestine.

(Experiment 5).

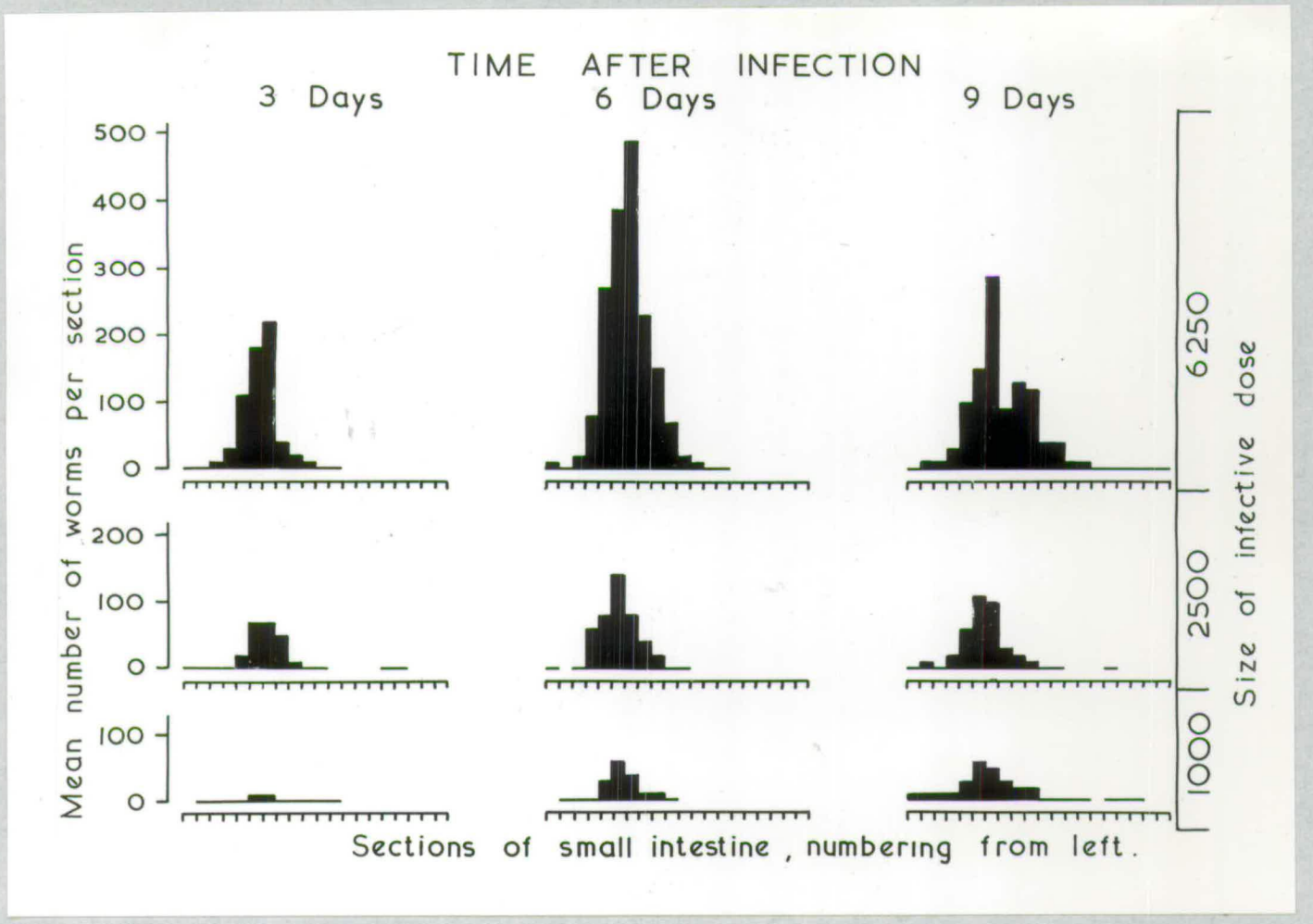
Section	♂ Worms	Adults	♂ %
1	46	64	67.9
2	64	98	
3	125	166	75.3
4	444	792	56.1
5	1196	2292	52.2
6	1633	3628	45.0
7	1723	4137	41.6
8	792	1726	45.9
9	599	1390	43.1
10	288	876	32.9
11	123	271	45.4
12	104	187	55.6
13	34	50	
14	34	45	
15	7	12	
16	6	13	67.3
17	5	10	
18	6	9	
19	5	7	
20	6	7	
All Sections	7240	15780	45.9

FIGURE 1A.



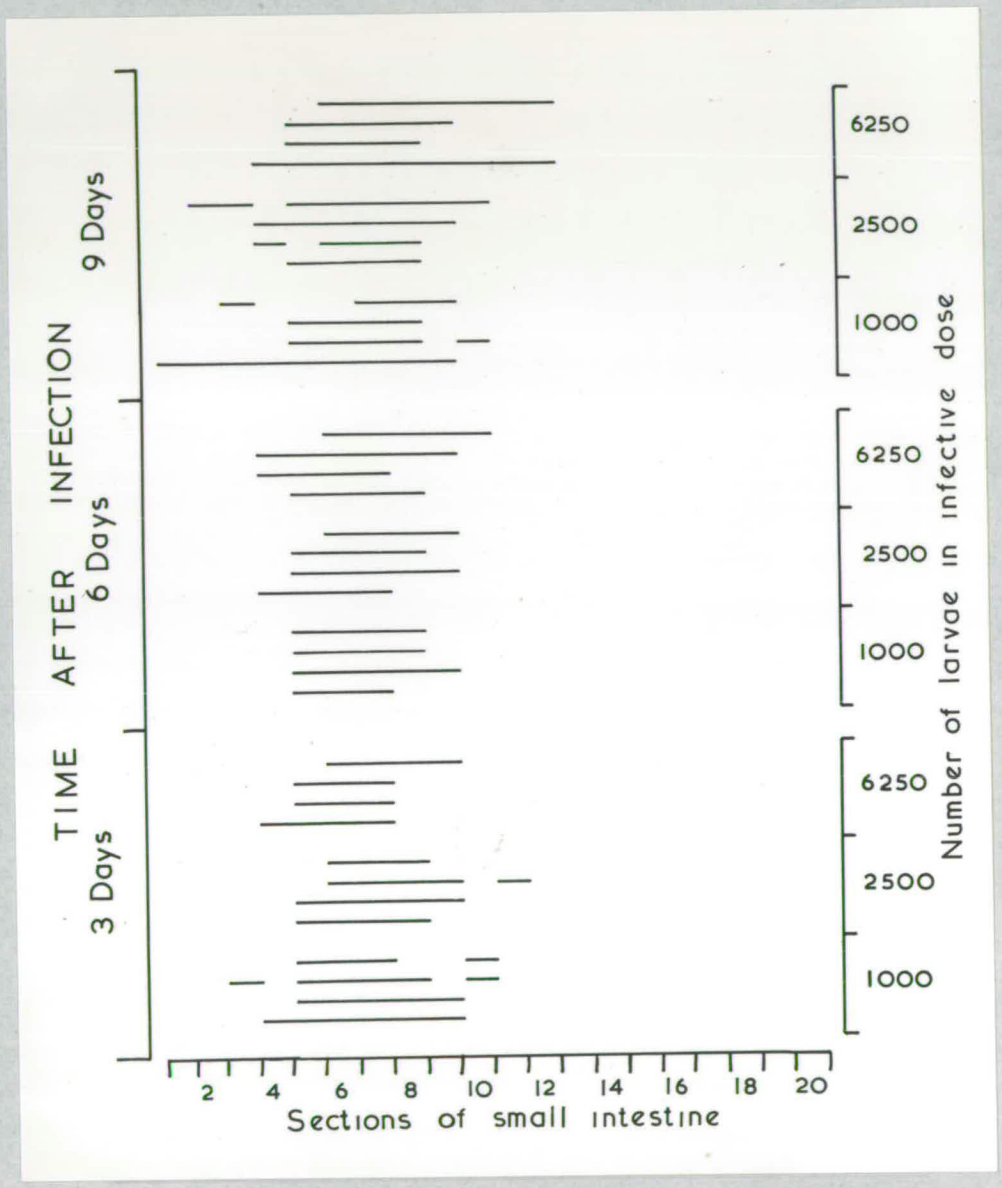
The mean numbers of eggs passed per day by rats in each of the three infected groups (Experiment 5).

FIGURE 15.



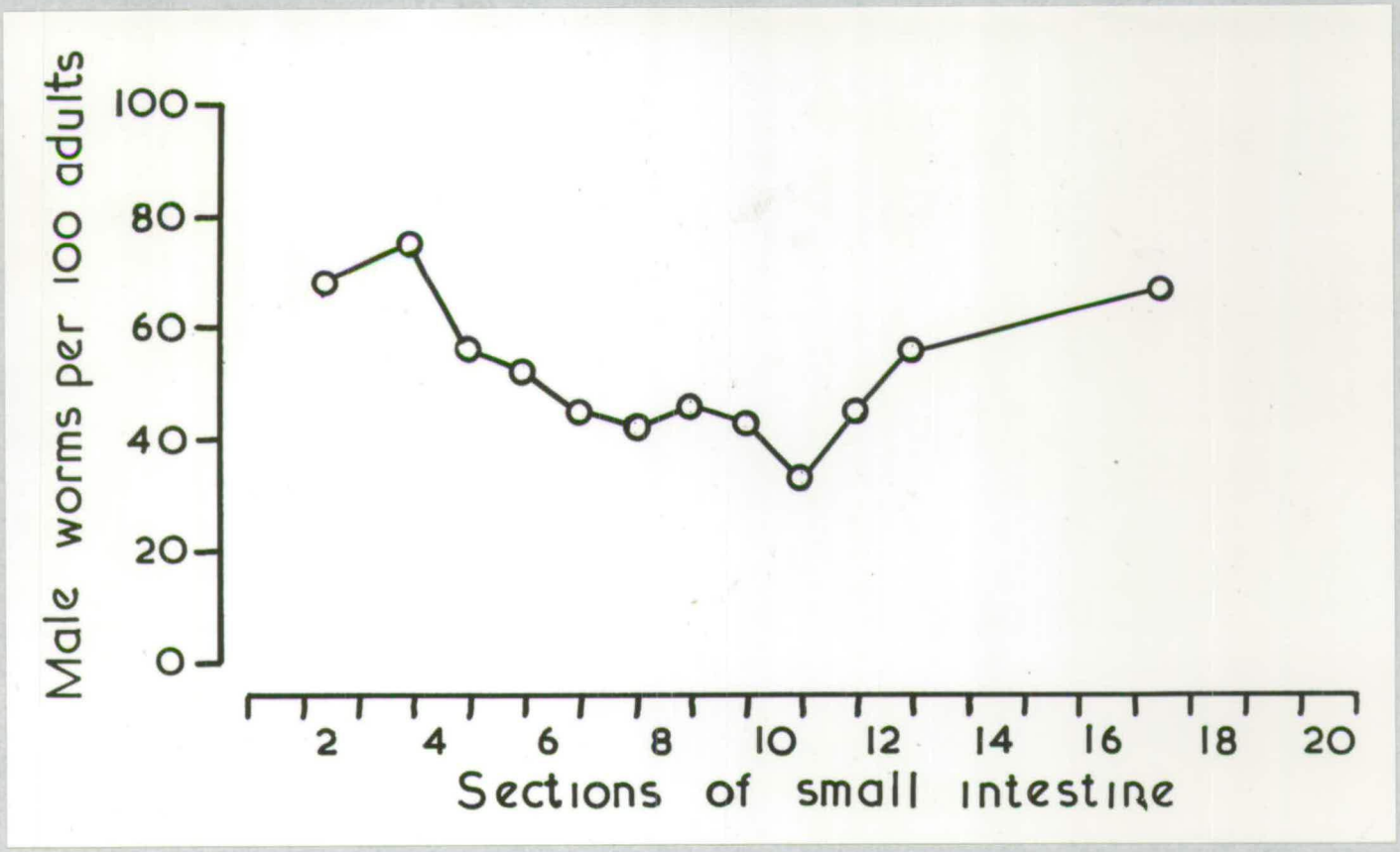
Histograms showing the mean number of worms recovered from each small intestine section in each group of infected rats.
(Experiment 5).

FIGURE 16.



Sections which contained more than 5% of the total number of worms recovered from the small intestine of each rat. Each horizontal line represents one rat.
(Experiment 5).

FIGURE 17.



The percentage of male worms amongst adults recovered from each section of the small intestine over the whole experiment. Each plot represents at least 100 adult worms. (Experiment 5).

The number of eggs laid by a female worm are not strictly comparable with the estimates in Experiment 4 because the numbers of female worms present on consecutive days are not known. No conclusions can be drawn on the effect of dose on the egg laying, there not being enough data and the method being too involved. However, it does appear that healthy female worms at the height of the infection lay between 500 and 1000 eggs per day. This result is in agreement with that found in Experiment 4 of 670 eggs per day.

The distribution in female rats is, as in Experiment 4, centred further from the pyloric end of the small intestine than in male rats. Further consideration of this point is given on page 145.

The population showed little change between Days 3 and 6. The mean positions of worms are almost identical, the mode positions are identical and the scatter of worms, as shown by the standard deviation is similar. By Day 9, however, there is evidence that the distribution had begun to change. The mean position has moved back almost a whole section, though most worms are still found in the same section as before.

In the range of the doses given there is no evidence of saturation of any part of the small intestine. If this had happened the mode position would become fully occupied and worms would colonise the neighbouring sections in relatively greater numbers. This would show itself in an increase in the variance of the worm distribution. The variance in the three dosing groups is so close that this plateau-phenomenon can be discounted. However, there is a suggestion that in the highest dose level the worms are found

slightly furtherfrom the pylorus than in the other two groups.

The percentage of the dose recovered in those rats dosed with 1000 larvae was very much lower in Day 3 than in the other two groups. Thissuggests that small doses are delayed in their transit from the skin to the small intestine to a very much greater degree than large doses. This might be because with higher doses of worms relatively more worms will get the benefit of passing along tracts broken down by other worms.

Figure 16 shows the data from this experiment presented in a similar way to that in Figure 9 of Experiment 4. The three dose rates are seen to give similar results on each of the three days. The relative absence of worms from Section 1 is seen in all groups of doses on Day 3, but there are signs that on Day 6 and Day 9 there is an increase in the number of worms found in this section.

The incidence of males in each section agrees with the results obtained from Experiment 4, except in the more posterior sections.

DISTRIBUTION IN SECONDARY INFECTIONS.

EXPERIMENT 6.

THE DISTRIBUTION OF NIPPOSTRONGYLUS BRASILIENSIS IN RATS AFTER TWO DOSES OF 2500 LARVAE GIVEN 18 DAYS APART.

Experimental Plan.

Thirty-four female rats aged 170 days and weighing 251 ± 39 grams on Day 0 were kept in separate cages. They were divided into four groups:

Group 1, 4 rats, uninfected controls.
1 rat killed on each of Days 18, 21, 24 and 27.

Group 2, 10 rats, infected 2500 larvae on Day 0.
1 rat killed on Day 18.
3 rats killed on each of Days 21, 24 and 27.

Group 3, 10 rats, infected 2500 larvae on Day 18.
1 rat killed on Day 18.
3 rats killed on each of Days 21, 24 and 27.

Group 4, 10 rats, infected 2500 larvae on Day 0; 2500 larvae on Day 8.
1 rat killed on Day 18.
3 rats killed on Days 21, 24 and 27.

Egg counts were performed each day.

The small intestine was divided into twenty equally lengthened sections in the Haruspicator. and The number of worms in each section were recovered and counted. The sexes of the worms were recorded on Days 24 and 27.

The inguinal and axillary lymph nodes were collected and disintegrated in a blender. Aliquot samples were taken for examination under a dissecting microscope and the numbers of worms present were estimated.

The lungs of the rats were treated similarly.

Results

Table 22 Data derived from egg counts.
Table 23 Worms recovered from each section and from the lungs and lymph nodes in each group.
Table 24 Parameters of worm distribution in each rat.
Table 25 Sex incidence of worms in each group.
Figure 18 Egg counts of each group.
Figure 19 Histograms of worm distribution in each group.
Figure 20 Sections containing more than 5% of the worms recovered from each rat.
Figure 21 Sex incidence of worms in each group.

The egg counts showed that the female worms in the secondarily infected rats were prevented from becoming fully mature.

The worms recovered from lymph glands were spasmodically distributed amongst the rats. The lungs still contained some larvae 27 days after a primary infection, though most were recovered three days after infection,

TABLE 21.

Plan of Experiment 6.

Day	Group 1 killed	Group 1 egg count	Group 2 killed	Group 2 egg count	Group 3 killed	Group 3 egg count	Group 4 killed	Group 4 egg count
0			(2500 larvae)				(2500 larvae)	
		4		10		10		10
1		4		10		10		10
2		4		10		10		10
3		4		10		10		10
4		4		10		10		10
5		4		10		10		10
6		4		10		10		10
7		4		10		10		10
8		4		10		10		10
9		4		10		10		10
10		4		10		10		10
11		4		10		10		10
12		4		10		10		10
13		4		10		10		10
14		4		10		10		10
15		4		10		10		10
16		4		10		10		10
17		4		10		10		10
18	1					(2500 larvae)		(2500 larvae)
	1	4	1	10	1	10	1	10
19		3		9		9		9
20		3		9		9		9
21	1	3	3	9	3	9	3	9
22		2		6		6		6
23		2		6		6		6
24	1	2	3	6	3	6	3	6
25		1		3		3		3
26		1		3		3		3
27	1	1	3	3	3	3	3	3

TABLE 22.

Mean number of eggs (in thousands) passed per day per rat in each group.

(Experiment 6).

Day	Infected:	Group 1	Group 2	Group 3	Group 4
	Day 0:	-	+	-	+
	Day 18:	-	-	+	+
0		0	0	0	0
1		0	0	0	0
2		0	0	0	0
3		0	0	0	0
4		0	0	0	0
5		0	0	0	0
6		0	10	0	20
7		0	250	0	200
8		0	360	0	170
9		0	340	0	490
10		0	320	0	360
11		0	350	0	330
12		0	220	0	140
13		0	60	0	100
14		0	80	0	0
15		0	10	0	0
16		0	0	0	0
17		0	0	0	0
18		0	0	0	0
19		0	0	0	0
20		0	0	0	0
21		0	0	0	0
22		0	0	0	0
23		0	0	0	0
24		0	0	10	0
25		0	0	110	0
26		0	0	430	0
27		0	0	390	0

TABLE 23.

The number of worms recovered from each section in each grouping of rats.

(Experiment 6).

Day:	Group 2			Group 3			Group 4		
	21	24	27	21	24	27	21	24	27
Section 1	1		2			13	2	7	1
2	1	1	5	2	3	9	5	2	1
3		1	2	7	42	25	18	56	
4	2		2	112	140	209	34	71	4
5		4	1	355	511	409	66	49	3
6		1	1	385	613	486	29	34	2
7		3		240	426	664	18	10	1
8	3	1		56	172	337	20	1	1
9				3	57	67	22		3
10			1	5	16	31	20		1
11		1		1	1	29	12		
12						18	3		
13					1	10	2		1
14					1	18	11	1	
15						12		1	
16						7			
17						14		1	
18				5		19		2	
19						3			
20					1	3	14	1	1
Total:	7	12	14	1171	1984	2383	276	236	19

TABLE 24.

Mean and Mode positions and standard deviation of worm distribution expressed in terms of twentieth sections of the small intestine.

(Experiment 6).

	Day killed	Mean	Mode	Standard Deviation	Total
Group 1.	18	-	-	-	0
	21	-	-	-	0
	24	-	-	-	0
	27	-	-	-	0
Group 2.	18	-	-	-	0
	21	1.0	1	0.7	2
		-	-	-	0
		5.9	7.5	2.2	5
	24	7.0	6.5	2.0	6
		4.5	4.5	-	1
		3.7	4.5	1.6	5
	27	3.5	1.5	3.4	5
		3.1	2.5	1.5	5
		1.8	.5	1.9	4
Group 3.	18	-	-	-	0
	21	5.6	5.5	1.1	441
		5.6	5.5	1.6	422
		4.6	4.5	1.0	308
	24	5.3	4.5	1.5	757
		5.5	5.5	1.3	529
		5.9	5.5	1.2	698
	27	5.8	5.5	1.7	1229
		7.0	4.5	4.2	401
		6.5	6.5	2.0	753
Group 4.	18	-	-	-	0
	21	8.7	4.5	5.4	88
		4.7	4.5	2.1	39
		6.2	4.5	2.9	149
	24	4.1	3.5	2.3	176
		4.1	2.5	4.3	7
		4.1	4.5	2.4	53
	27	10.8	6.5	13.0	3
		5.8	5.5	3.5	12
		4.8	3.5	1.5	4

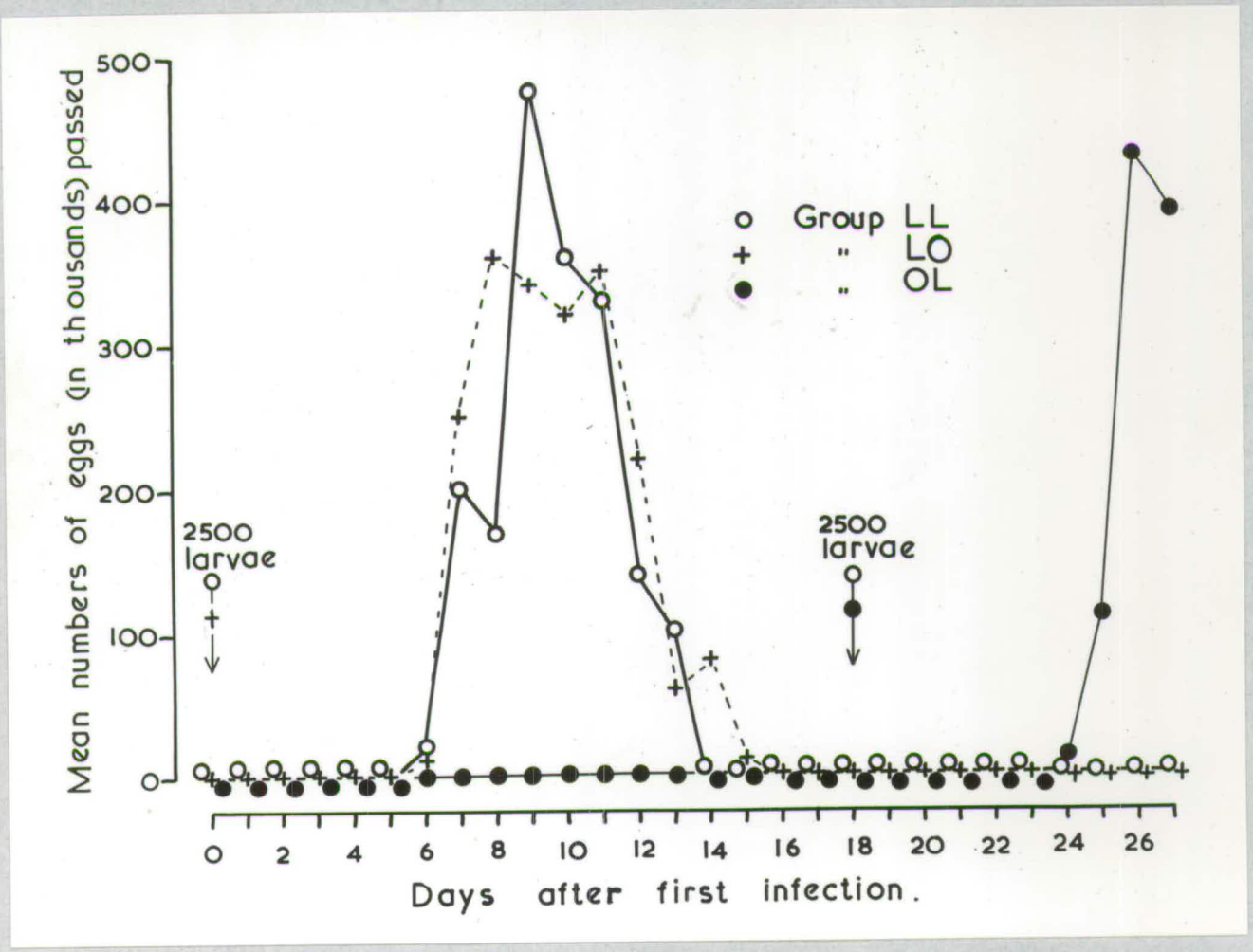
TABLE 25.

The number of male worms per hundred adults in sections of the small intestine of rats in each infected group. (Each entry based on 100 adults wherever possible).

(Experiment 6).

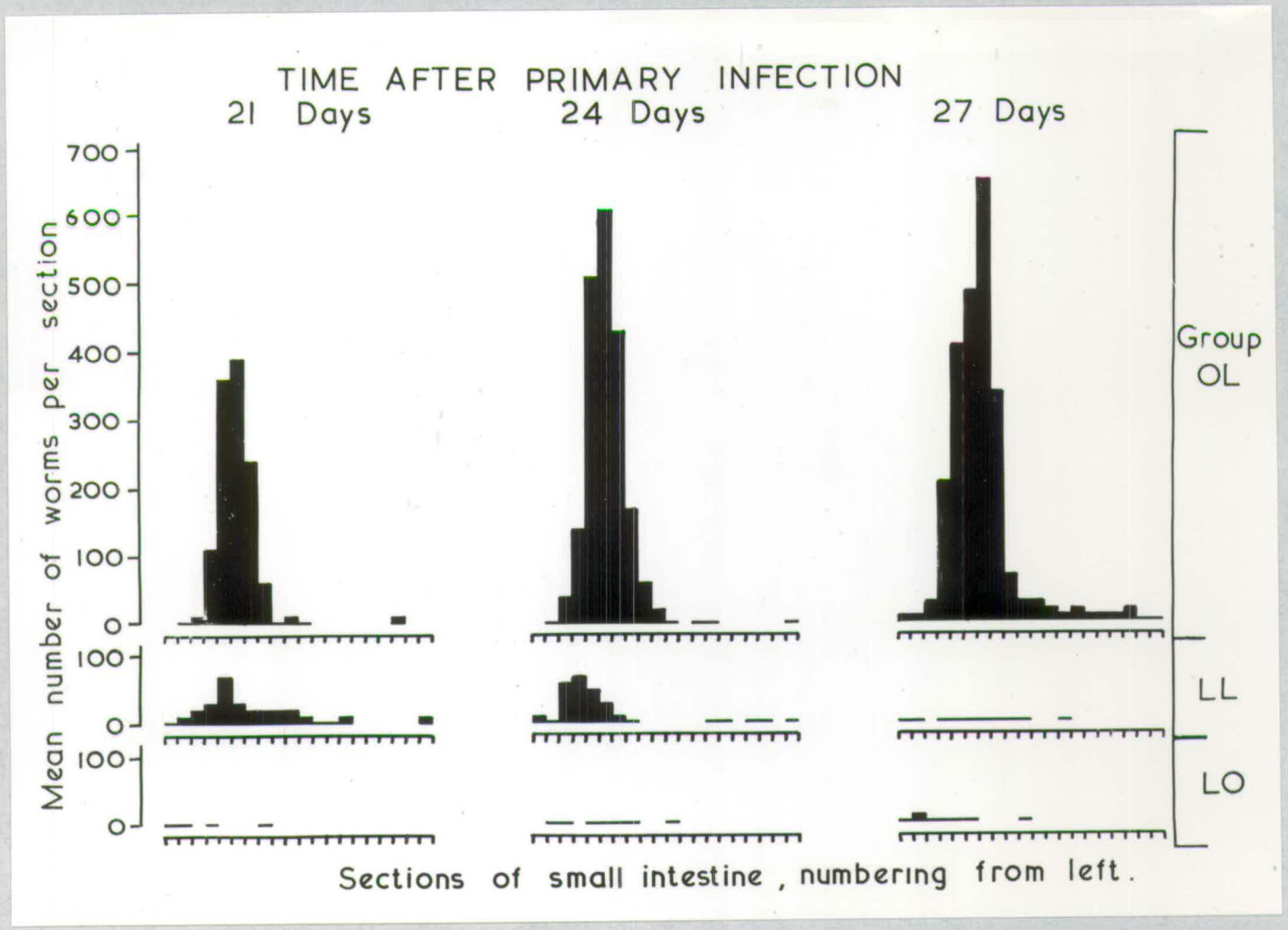
	Group 2	Group 3	Group 4
Section 1			
2		49	
3			31
4			
5		45	
6		43	
7		43	
8		45	
9		47	
10			
11			
12			
13			
14			
15		70	
16			
17			
18			
19			
20			
Total Adults	26	3886	121
Males %	35	45	29

FIGURE 18.



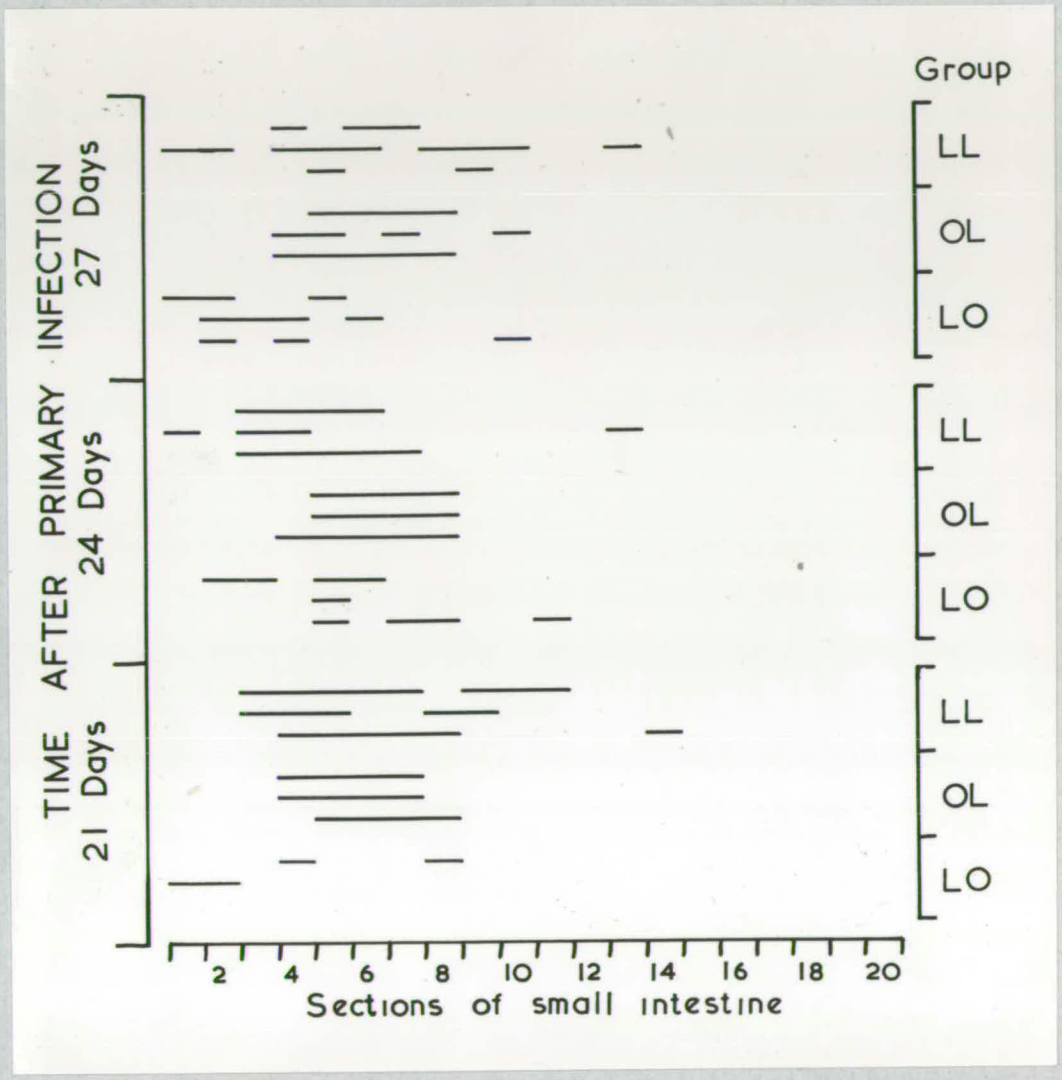
The mean number of eggs passed per day by rats in each of the three infected groups. (Experiment 6).

FIGURE 19.



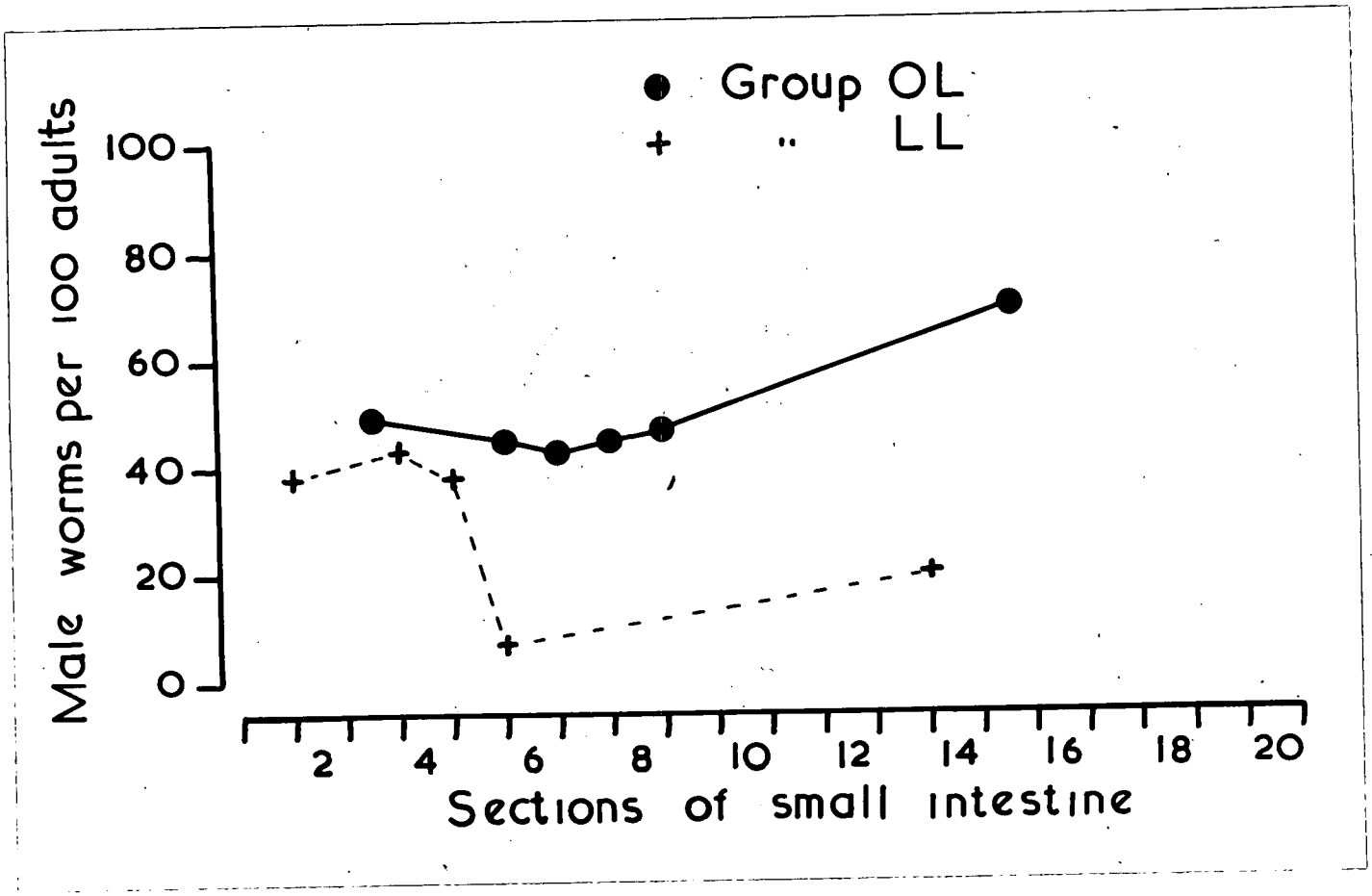
Histograms showing the mean number of worms recovered from each section of the small intestine in each group of infected rats.
(Experiment 6).

FIGURE 20.



Sections which contained more than 5% of the total number of worms recovered from the small intestine of each rat. Each horizontal line represents one rat. (Experiment 6).

FIGURE 21.



The percentage of male worms amongst adults recovered from each section of the small intestine in two groups of infected rats. Each plot in Group LL represents less than 100 worms, each plot in Group OL represents at least 100 worms.
(Experiment 6).

and this applied to secondarily infected rats as well.

The total number of worms recovered from secondarily infected rats was much less than from a primary infection, and the peak recovery had been passed well before Day 9.

Discussion

If it is assumed that the worms' age has no effect on the position it parasitises in the small intestine one would expect that a secondary infection would take up the positions occupied by the remnants of the first infection. Thus in this experiment most of the worms in Group 4 would be expected to be in Sections 1-4. This is not the case, however. Figure 19 shows that most of the worms are in Sections 3-6. This is slightly nearer the pyloric sphincter than the site of most worms in Group 3. The distribution of Group 4 resembles that of Group 3 far more closely than it resembles Group 2. Figure 20 shows a state of affairs in Group 4 more akin to the phase between Days 12-18 in a primary infection than to the phase after Day 18.

The pattern observed in Group 4 was not made up of a group of male worms more or less approximating with the distribution in Group 2 and the posterior tail being largely made up of females; the proportion of the sexes in the anterior half of the small intestine was unchanged from that seen in Group 3 or between Days 6 and 9 in Experiment 4.

EXPERIMENT 7.

THE DISTRIBUTION OF *NIPPOSTRONGYLUS BRASILIENSIS* IN RATS GIVEN VERY LARGE SECONDARY INFECTIONS.

Experimental plan

Eleven rats, which had been given primary infections for the purpose

of obtaining eggs for the maintainance of the stock culture, were given massive secondary infections. This experiment did not include any control animals, it being essentially a 'pilot' experiment.

The Rats were in three groups:

Group 1, consisting of three rats, had been given a primary infection of 8000 larvae, i.e. as their weight averaged 193 grams 42.5 Larvae/gram. The challenge dose was given 40 days later at the rate of 45 larvae/gram. This dose exceeds the lethal dose for rats (Hunter and Leigh 1961a). The animals were killed six days later and the intestine divided into twenty sections.

Group 2, 4 rats weighing 200 grams at the start, were given primary and secondary doses of 40 larvae/gram and 42.5 larvae/gram respectively, these being separated by an interval of 34 days. These rats were killed six days later.

Group 3, were given the same doses as Group 2 but were killed nine days after the second dose of larvae.

Results

Table 27 Principle parameters of worm distribution in each rat.
Table 28 The percentage of male worms amongst adults in each section of the small intestine.

Figure 22 Histograms of worm distribution in selected rats.
Figure 23 Sections containing more than 5% of worms recovered from selected rats.
Figure 24 Male worms per 100 adults in each section.

As only four rats harboured substantial numbers of worms the data from the others has been omitted in Figures 22 and 23.

Rats 1 and 6-11 had been able to throw off almost the whole of the

TABLE 26.

Plan of Experiment 7.

Rat No.	1	2	3	4	5	6	7	8	9	10	11
Sex	♀	♀	♀	♂	♂	♀	♀	♀	♂	♀	♀
Weight at Day 0	185	185	210	205	200	200	195	195	200	190	210
Age at Day 0	72	72	72	88	88	88	88	88	88	88	88
Primary dose of larvae (thousands)	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Days between infective dosing	40	40	40	34	34	34	34	34	34	34	34
Secondary dose of larvae (thousands)	10.5	10.5	10.5	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Days from last dose to killing	6.	6	6	6	6	6	6	9	9	9	9

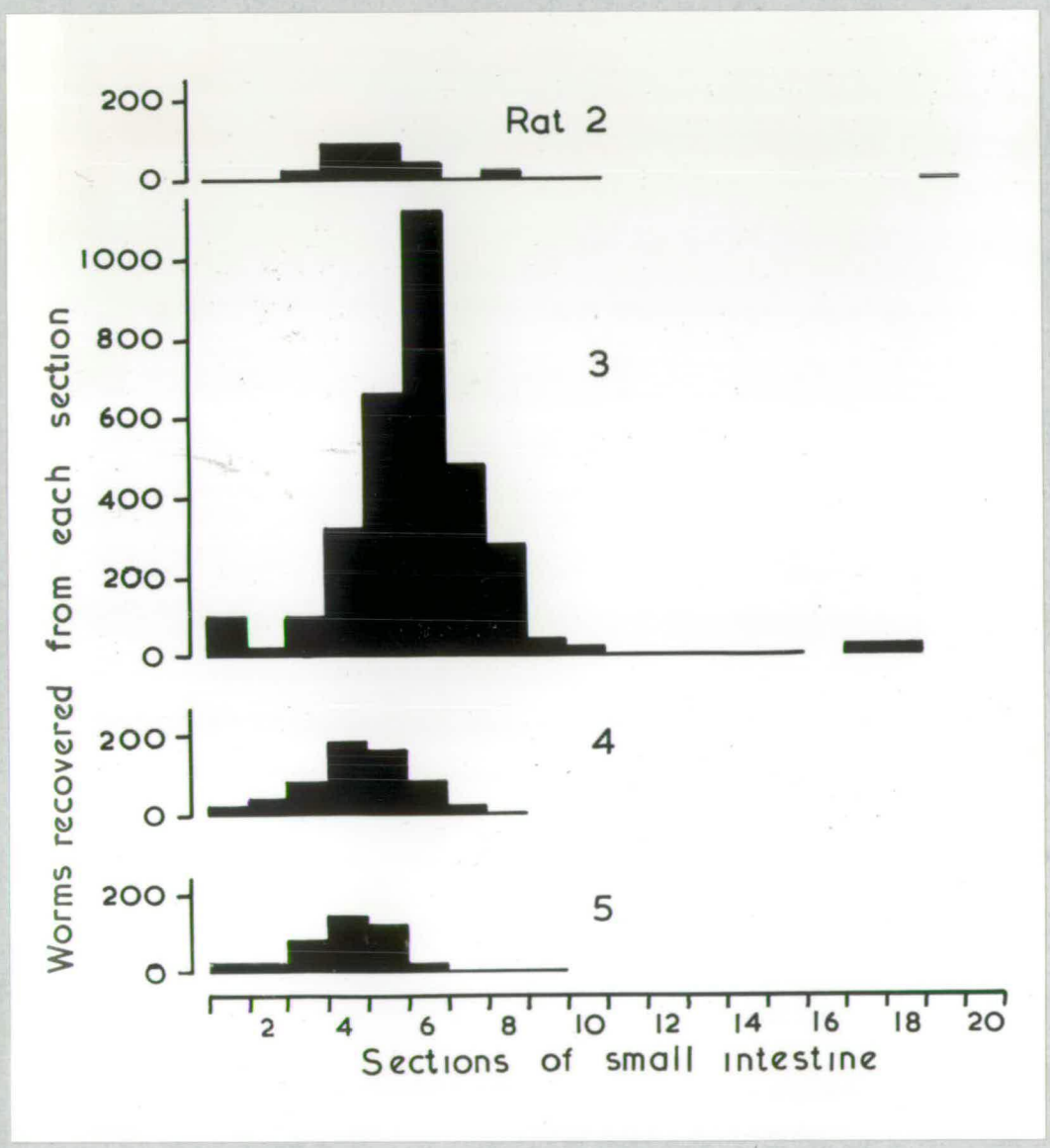
TABLE 28.

The number of male worms in one hundred adults in each section of the small intestine in all the rats.

(Experiment 7).

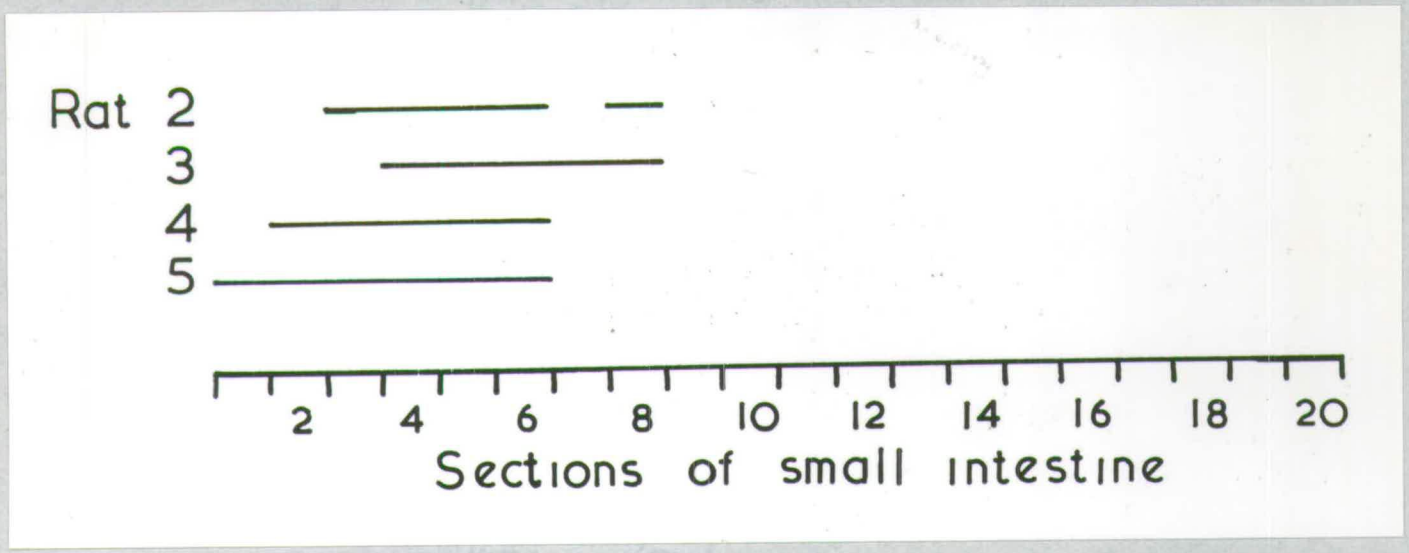
Section	Male worms per 100 adults
1	42
2	39
3	25
4	21
5	25
6	22
7	30
8	33
9	32
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
Total	26

FIGURE 22



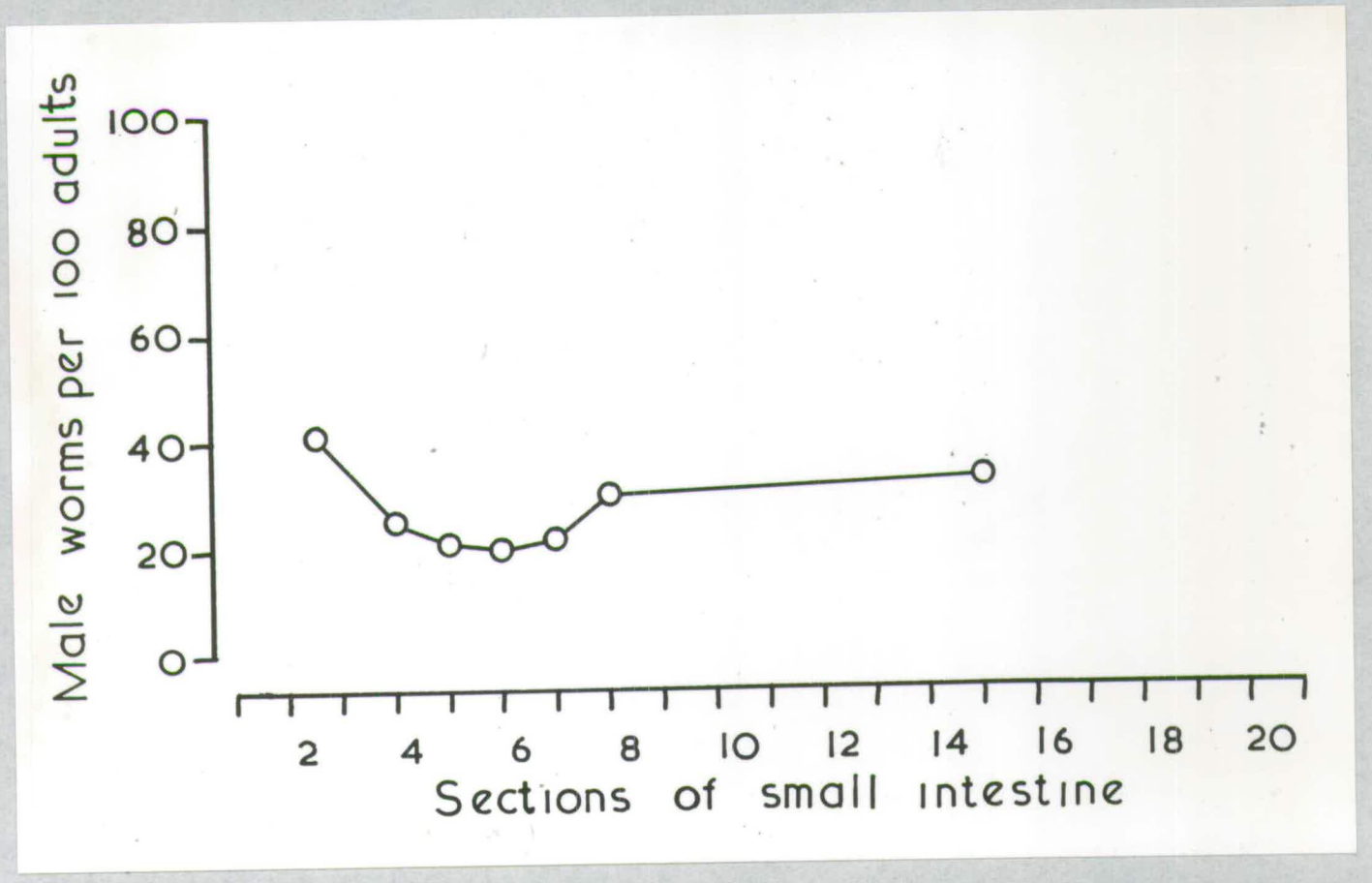
Histograms showing the number of worms recovered from each section of the small intestine of the four most heavily infested rats.
(Experiment 7).

FIGURE 23.



Sections which contained more than 5% of the total number of worms recovered from the small intestine of each of the most heavily infested rats. Each horizontal line represents one rat. (Experiment 7).

FIGURE 24.



The percentage of male worms amongst adults recovered from each section of the small intestine.
Each plot represents at least 100 adult worms.
(Experiment 7).

challenge infection by the time they were killed. Of the other four rats, Rats 2, 4 and 5 behaved in a similar way to Group 4 in Experiment 6. However, Rat 3 reacted in a different way. The worms were distributed almost as if in a primary infection. More than 95% of the total were found in only Sections 4-8. The finding of 110 worms in Section 1 is an interesting feature of this rat, as it occurred six days after infection. These worms were most probably not the remnants of the primary infection because no such numbers were found in other secondarily infected rats. If they were derived from the second infection then they show that Section 1 can support over one hundred worms and that worms under seven days old can parasitize Section 1. (The first worms to parasitize Section 1 in a primary infection were seven days old).

The experiment has shown that even with massive doses of worms most rats are able to throw off secondary infections. It shows that the variation of response to secondary infections is very much greater than to primary infections. It also conclusively shows that even after massive doses the secondary infections do not have similar distribution patterns to those of ageing primary infections.

EXPERIMENT 8.

THE DISTRIBUTION OF NIPPOSTRONGYLUS BRASILIENSIS IN THE SMALL INTESTINE OF RATS AFTER VARIOUS LEVELS OF PRIMARY AND SECONDARY INFECTIONS.

Experimental Plan.

Sixty female rats weighing 259 ± 16 grams and 220 days old were used in the work. On Day 0 some of the rats were infected with 100, 500 or 2500 infective larvae, others receiving no worms at this stage. On Day 14 the experimental rats were given infections of 100, 500, 2500 or

12,500 larvae, the details of the infective doses are given in Table 29. The rats were killed 11 days later and the small intestine was divided into twenty sections in the standard manner.

Eight rats died during the second phase of the experiment and the number which survived is also given in Table 29. One of the rats which died had only had a single dose of 100 larvae. It was found to have had a septicaemia resulting from an infected bite wound, which had already been noticed when the animals were being put into separate cages and which had (mistakenly) been thought not to be infected. The mistake was discovered too late for antibiotic treatment to be of any effect. The other seven animals died of a haemorrahagic and consolidating pneumonia caused by the migration of the infective larvae through the lungs. Six of these had had secondary infections of 12,500 larvae. Such a dose when given as a primary infection would have been 100% fatal to rats of this size. The seventh rat had had only a single dose of 2500 larvae. It is unusual for such a dose to produce a fatal pneumonia in a rat of this size. On Days 8 and 22 the faeces of each rat were examined for eggs and counts made, in order to ensure that the animals were conforming to the pattern seen in Experiment 6.

Results

Table 30 Data derived from egg counts.
Table 31 Worms recovered from each section in each group of rats.
Table 32 The principal parameters of the worm distribution in each rat.

Figure 25 Sections containing more than 5% of the worms recovered in each group.

By Day 25, that is 11 days after the secondary dose of larvae had been given few worms remained in the rats which had had two infections.

TABLE 29.

Plan of Experiment 8.

Animals Dosed

	1st Dose (Day 0): 0	100	500	2500 larvae
2nd Dose: 0	3	3	3	3
(Day 14) 100	4	4		
500	4	4	4	
2500	4	4	4	4
12500		4	4	4

Animals Surviving Day 25

	1st Dose (Day 0): 0	100	500	2500 larvae
2nd Dose: 0	3	3	3	3
(Day 14) 100	3	4		
500	4	4	4	
2500	3	4	4	4
12500		1	4	1

Egg counts performed on Days 8 and 22.

TABLE 30.

Numbers of eggs per gram of faeces in each rat eight days after infection.

Counts within each grouping are arranged in order of magnitude.

(D = already Dead).

(Experiment 8).

Doses		E.p.g. Day 8				E.p.g. Day 22.				Mean Day 8	Mean Day 22
0	0	0	0	0		0	0	0		0	0
0	100	0	0	0	0	0	500	500	1000	0	500
0	500	0	0	0	0	300	700	1300	1500	0	950
0	2500	0	0	0	0	D	33300	8000	13100	0	8130
100	0	400	600	900		D	0	0		630	0
100	100	100	200	700	1200	0	0	0	0	550	0
100	500	0	300	400	800	0	0	0	0	375	0
100	2500	200	300	500	900	0	0	0	400	475	100
100	12500	300	400	700	1000	D	D	D	1500	600	1500
500	0	500	1400	1500		0	0	0		1133	0
500	500	100	700	1100	1100	0	0	0	0	750	0
500	2500	400	800	900	1300	0	0	0	100	850	25
500	12500	600	800	1000	1300	2200	3500	5000	9000	925	4925
2500	0	5200	7600	9800		0	0	0		7530	0
2500	2500	6000	7400	8700	10100	0	0	0	0	8050	0
2500	12500	4200	6900	9100	11000	D	D	0	0	7800	0

TABLE 31.

The number of worms recovered per section per rat from each grouping.
(Experiment 8).

1st dose	0	0	0	0	100	100	100	100	100	500	500	500	500	2500	2500	2500
2nd dose	0	100	500	2500	0	100	500	2500	12500	0	500	2500	12500	0	2500	12500
Rats used	3	3	4	3	3	4	4	4	1	3	4	4	4	3	4	1
Section 1		10	8	45		4	4		4				1	5	2	4
2		10	9	31	3	4	1	1		1			2	3	1	4
3		7	9	53	1	4	2						3		1	
4		14	18	453	9	4	1		2				7			
5		12	58	518	9	3	1		2			1	26	1	1	
6		3	45	184		1	1						15			1
7		1	33	108		1					1		5			
8		3	11	37									1			
9		1	3	18									2			3
10		1	2	23									2			1
11			1	5					1				1			14
12			1	5					1				1			26
13			1	4									1			40
14			1	4												5
15			1	5												11
16			1	4												
17			1	6												14
18			1	3												2
19				2												19
20				3												
Mean Total	0	62	204	1511	22	21	10	1	10	1	1	1	67	9	5	144

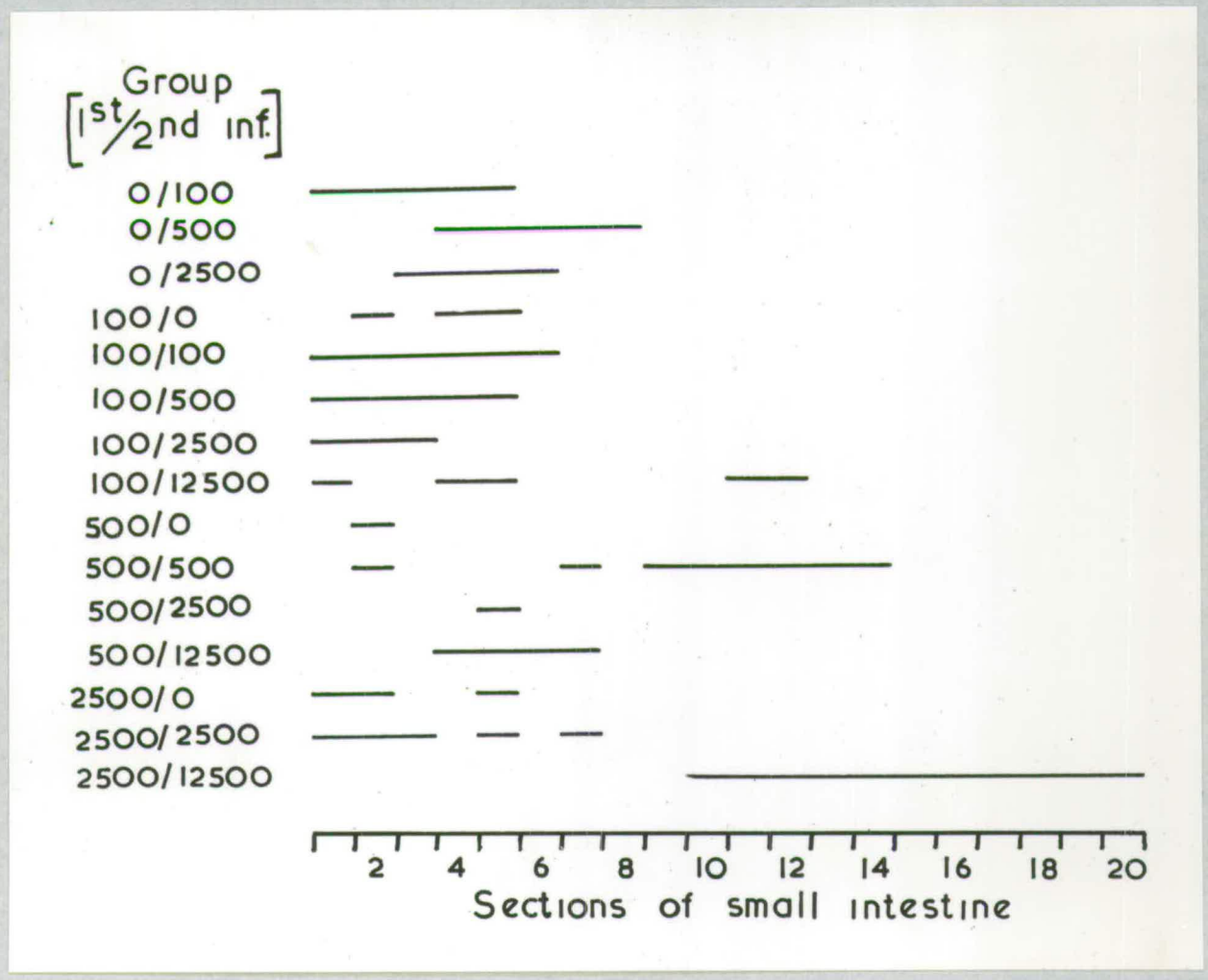
TABLE 32.

The Mean and Mode positions and the Standard Deviations of the worm distributions in the small intestine of each rat.

(Experiment 8).

Rat	Dose rates		Total	Mean	Mode	σ	% recovery
1	0	0	0				
2	0	0	0				
3	0	0	0				
4	0	100	Died				
5	0	100	85	4.0	3	2.6	85
6	0	100	61	3.2	3½	1.2	61
7	0	100	39	2.0	½	1.8	39
8	0	500	146	5.9	5½	2.6	29
9	0	500	179	5.1	4½	3.1	36
10	0	500	374	4.7	4½	2.0	75
11	0	500	103	6.3	5½	2.6	21
12	0	2500	Died				
13	0	2500	2027	4.4	3½	2.1	81
14	0	2500	981	5.4	4½	2.5	30
15	0	2500	1530	4.7	3½	2.5	61
16	100	0	62	3.8	4	1.2	62
17	100	0	0				0
18	100	0	4	2.0	2	.5	4
19	100	100	16	2.5	2½	1.4	16
20	100	100	23	2.7	3	1.6	23
21	100	100	20	1.8	½	1.5	20
22	100	100	19	3.9	3	1.9	19
23	100	500	5	2.7	2	2.2	1
24	100	500	20	1.5	½	1.1	4
25	100	500	6	4.3	2½	4.7	1
26	100	500	1	5.5	5½		< 1
27	100	2500	0				0
28	100	2500	2	1.5	1½	0	< 1
29	100	2500	0				0
30	100	2500	4	1.5	1½	1.2	< 1
31	100	12500	Died				
32	100	12500	Died				
33	100	12500	Died				
34	100	12500	10	4.0	½	4.0	< 1
35	500	0	2	1.5	1½	0	< 1
36	500	0	0				0
37	500	0	2	1.5	1½	0	< 1
38	500	500	0				0
39	500	500	8	9.9	6½	2.6	2
40	500	500	0				0
41	500	500	0				0
42	500	2500	0				0
43	500	2500	2	4.5	4½	0	< 1
44	500	2500	0				0
45	500	2500	0				0
46	500	12500	11	4.7	4½	2.9	< 1
47	500	12500	249	5.3	4½	2.6	2
48	500	12500	0				0
49	500	12500	1	5.5	5½		< 1
50	2500	0	2	.5	½	0	< 1
51	2500	0	25	1.5	½	1.8	1
52	2500	0	0				0
53	2500	2500	12	1.5	1½	.9	< 1
54	2500	2500	3	5.2	4½	1.2	< 1
55	2500	2500	0				0
56	2500	2500	2	.5	½	0	< 1
57	2500	12500	Died				
58	2500	12500	Died				
59	2500	12500	Died				
60	2500	12500	144	14.0	10½	4.4	1

FIGURE 25.



Sections which contained more than 5% of the total number of worms recovered from the small intestine of each group of rats. Each horizontal line represents one group of rats. (Experiment 8).

Table 31 shows that less than 25% of the last dose given were recovered from these rats, whilst in rats with only primary infections up to 85% of the last dose were recovered 11 days later. In the four rats which had had primary and secondary infections of 100 larvae, 16-23% of the last dose were recovered on Day 25. In all the other rats given two infections the number recovered was less than 2%. This suggests that the degree of resistance shown by the rat depends on the size of both the primary and secondary dose; that the higher the primary dose the more resistant the host becomes and that the higher the secondary dose the more likely this resistance is to be elicited. It can be held on this evidence that a primary dose of 100 larvae can render the rat potentially resistant but that this potentiality is only realised on receiving the stimulus of the secondary dose, and that 100 larvae are too few to fully elicit the resisting reactions.

If the evidence of the egg counts is added to this the picture becomes somewhat complicated. All those rats challenged with secondary infections which were the same size as the primary injections showed no eggs in the faeces after eight days.

As the size of the secondary dose increased, relative to the primary dose, eggs were found in increasing numbers in the faeces.

Thus whilst low secondary doses appear not to elicit as effective a reaction against the worms they are also unable to overcome the resistante sufficiently to allow egg laying to take place. Larger secondary doses appear to be able to overcome the resistance to egg laying but in so doing elicit a relatively much greater reaction which more quickly eliminates the worms.

This experiment did not examine the relation of secondary doses that are smaller than the primary doses. The lapse of 11 days after the secondary dose was too long for a study of the distribution of the secondary infection to be made. The size of the egg counts on the eighth day indicated that at that period the secondary infection had become sufficiently well established to reproduce.

TRANSFERRED STAGES

EXPERIMENT 9.

THE DISTRIBUTION OF NIPPOSTRONGYLUS BRASILIENSIS IN THE SMALL INTESTINE OF RATS WHICH HAVE HAD INTESTINAL STAGES OF THE WORM TRANSFERRED TO THEM FROM DONOR RATS AS PRIMARY AND/OR SECONDARY INFECTIONS.

Experimental Plan

Fifty male rats were available for this work. The mean weight was 268 ± 62 gms. and the age on Day 0 was 98 ± 7 days. The animals were divided into two groups, Group 1 consisted of 24 rats which were intended to be donor rats and Group 2 consisting of 25 rats was the experimental group. There was an unusually high variation in weight in this group and in order to make the experimental group as uniform as possible Group 1 was made up entirely of those animals at either end of the range of weights. The weights of Group 2 on Day 0 was 288± 31. gm.

The animals in the experimental group were divided into nine sub-groups (eight containing three animals, one containing two). There were two infecting occasions Day 0-3 and Day 27-30. On each of these each sub-group was given one of three treatments:

(1) normal infective larvae given subcutaneously, on either Day 0 or Day 27;

(2) fourth stage larvae transferred from the small intestine

by stomach tube, on either Day 3 or Day 30;

(3) no worms at all.

During the experiment four animals died from an undiagnosed condition. There appeared to be no ill effects in the remainder. This necessitated a certain amount of rearrangement within the sub groups, as two of the deaths had occurred in Group 2. Table 33 shows the plan of the experiment with the experimental groups in the rearranged order. The rats in Group 1 were divided into two lots, one being infected on Day 0 and killed on Day 3 to provide intestinal stages for transferrence. The second lot were infected on Day 27 and killed on Day 30. The rats in Group 2 were killed on Day 33, that is when the second infection was six days old. The small intestines were removed and divided, using the Haruspicator, into twenty sections.

Special Methods

The method used for transferring the intestinal stages of the worm from one rat to another was as follows: the donor rats were infected subcutaneously on Day 0 and were kept as normally infected rats until Day 3. They were killed under ether and immediately opened. The small intestines were removed and the anterior half was separated. This half was opened in 1% $NaHCO_3$ solution. Any large boluses of digesta were removed. The mucosa was lightly scraped. The bris was placed in 12 ml. centrifuge tubes to allow the worms to sediment. After one washing in bicarbonate they were given further washings in 0.85% NaCl solution. The cleaned worms were resuspended. An aliquot was taken and the number of worms it contained was estimated. The volume of the suspension was then adjusted so that 2 ml. contained 2000 larvae. (On the second occasion

TABLE 33.

Plan of Experiment 9.

GROUP 1	Donor Rats for production of transferred stages.			
	(a) 12 Infected	6000 larvae	Day 0.	Killed Day 3.
	(b) 10 Infected	6000 larvae	Day 27.	Killed Day 30.
GROUP 2	Experimental Rats, all killed on Day 33.			
TT)	3 rats	given	2000 Transferred stages Day 3.	
			1000* Transferred stages Day 30.	
TN)	3 rats	given	2000 Transferred stages Day 3.	
			2000* Normal larvae Day 27.	
TO)	3 rats	given	2000 Transferred stages Day 3 only.	
NT)	3 rats	given	2000 Normal larvae Day 0.	
			1000 Transferred stages Day 30.	
NN)	3 rats (1 died)	given	2000 Normal larvae Day 0.	
			2000 Normal larvae Day 27.	
NO)	3 rats (1 died)	given	2000 Normal larvae Day 0 only.	
OT)	3 rats	given	1000 Transferred stages Day 30 only.	
ON)	3 rats	given	2000 Normal larvae Day 27 only.	
OO)	2 rats	kept as uninfected controls.		

*As it was not known on Day 27 that only enough worms to give each rat 1000 transferred stages would be recovered on Day 30 it had to be assumed that events would be as favourable as on Day 3 when enough 4th stage larvae were recovered to allow for 2000 worms to be transferred to each rat.

there were not enough worms to give each rat 2000 larvae and the suspension was then adjusted so that 2 ml. contained 1000 larvae);

The worms were transferred to the recipient rats by injecting them into a polythene tube placed into the rats oesophagus or stomach. About eight centimetres of 2 mm. bore polythene tubing was attached to the end of a 1.3 mm. bore hypodermic needle fitted to a 2 ml. syringe. Having ensured that the suspension was thoroughly mixed 2 mls. were withdrawn into the syringe. The recipient rat was held by an assistant with its neck as straight as possible. The tube was pushed gently into the mouth and pharynx. Once the rat had swallowed the end of the tube it was pushed until its end was in the region of the lower oesophagus. The worms were injected into the tube. The syringe was detached and 1 ml. of saline drawn in, which was then used to wash the worms remaining in the tube into the oesophagus. The tube was easily withdrawn and the recipient rat returned to its cage.

Results

Table 34 Worms in each section of the small intestine in each grouping.

Table 35 Principal parameters of the distribution of worms in each rat.

Table 36 Mann Whitney and 'F' tests of significance of results obtained from groups 'OT' and 'NT + TT'.

Figure 26 Histograms showing the distribution of worms in each group.

Figure 27 Sections containing more than 5% of the worms recovered from each rat.

Nearly 79% of the worms given by the normal route compared to only 6% of the transferred worms were recovered six days later from the previously uninfected rats. Thus the actual process of transferrence must damage

TABLE 34.

The mean numbers of worms recovered per rat from each section of the small intestine in each group.

(Experiment 9).

Group Rats	'OO' 2	'ON' 3	'OT' 3	'NO' 2	'NN' 2	'NT' 3	'TO' 3	'TN' 3	'TT' 3
Section 1		1			2	4		2	3
2		8			5	1		2	1
3		139		1	17	1		37	1
4		600	1	1	40			147	2
5		459	4		24	1		35	1
6		201	5		67			9	2
7		101	7		5			5	2
8		40	7		5			3	1
9		18	9					2	
10		6	13						1
11		3	4						
12								2	
13		1	3		1				
14									
15			1			1			2
16									
17					1				
18			7						
19									
20								1	
Mean Total	0	1577	61	2	167	8	0	245	16

TABLE 35.

The Mean and Mode positions and the standard deviations of the distributions of worms, as well as the total number of worms recovered from each rat. (Experiment 9).

Group		Mean	Mode	Standard Deviation	Total
1	'OO'				0
					0
2	'ON'	4.2	3.5	1.2	1411
		4.6	4.5	1.5	1637
		4.2	3.5	1.3	1682
3	'OT'	7.5	8.5	1.7	26
		8.3	9.5	2.5	56
		10.4	9.5	3.9	109
4	'NO'	3.5	3.5		1
		2.5	2.5 (Dead)		1
5	'NN'	4.9	5.5	1.7	239
		3.6	3.5 (Dead)	1.2	89
6	'NT'	5.3	4.5	4.5	20
		0.5	0.5	0	9
					0
7	'TO'				0
		1.5	1.5		1
					0
8	'TN'	6.9	3.5	4.1	5
		3.7	3.5	1.7	674
		4.6	4.5	3.0	57
9	'TT'	2.8	2.5	0.6	3
		4.7	0.5	4.2	22
		7.4	6.5	5.5	22

TABLE 36.

Non-parametric Mann-Whitney 'U' test (Seigel, 1956) and parametric 'F' test (Snedecor, 5th ed., 1956) applied to total counts of worms obtained from groups (OT) and (NT+TT).

(Experiment 9).

Data

Group		n	Counts (x)	$\sum X$	$\bar{X}$	$\sum X^2$
(OT)	A	3	26, 56, 109	191	63.7	15693
(NT+TT)	B	6	0, 3, 9, 20, 22,22	76	12.7	1458
Total		9		267	29.7	16151

$C = (\sum X)^2/n = 7921$

Mann-Whitney

Ranking: B; B; B; B; B; B; A; A; A; 'U'=0

p ('U'=0; n_a=6; n_b=3) = .013

Analysis of Variance

Source of variation		S. of S.	D.of F.	Mean Square
Total	$\sum X^2 - C$	6230	8	
Between groups	$\sum X_a^2/n_a + \sum X_b^2/n_b - C$	5202	1	5202
Within Groups		1028	7	149

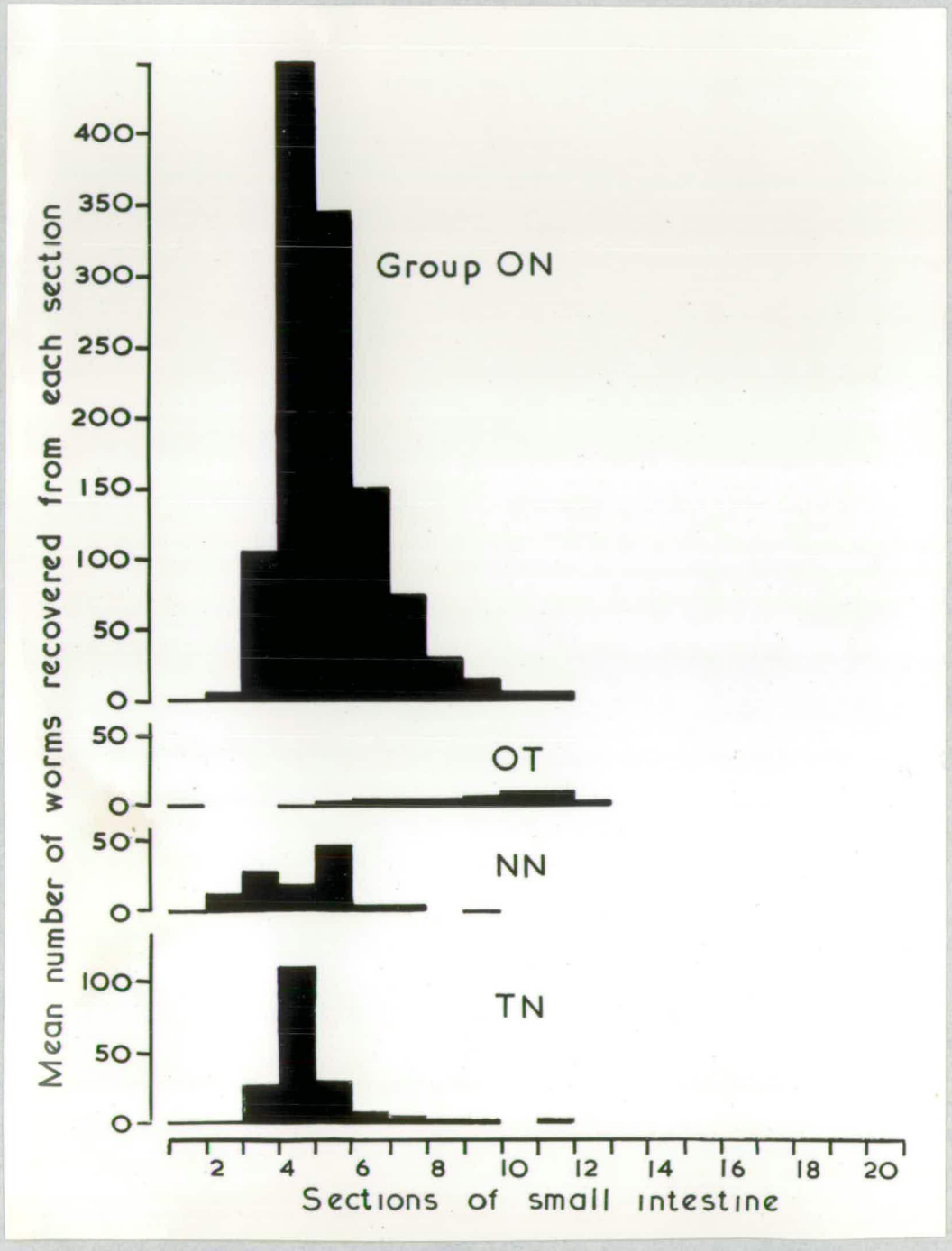
'F' test

Can the variationbbetween groups be solely due to the variation between rats which have received the same treatment?

$F(1 : 7) = 5202/149 = 34.9$ $p < .01$

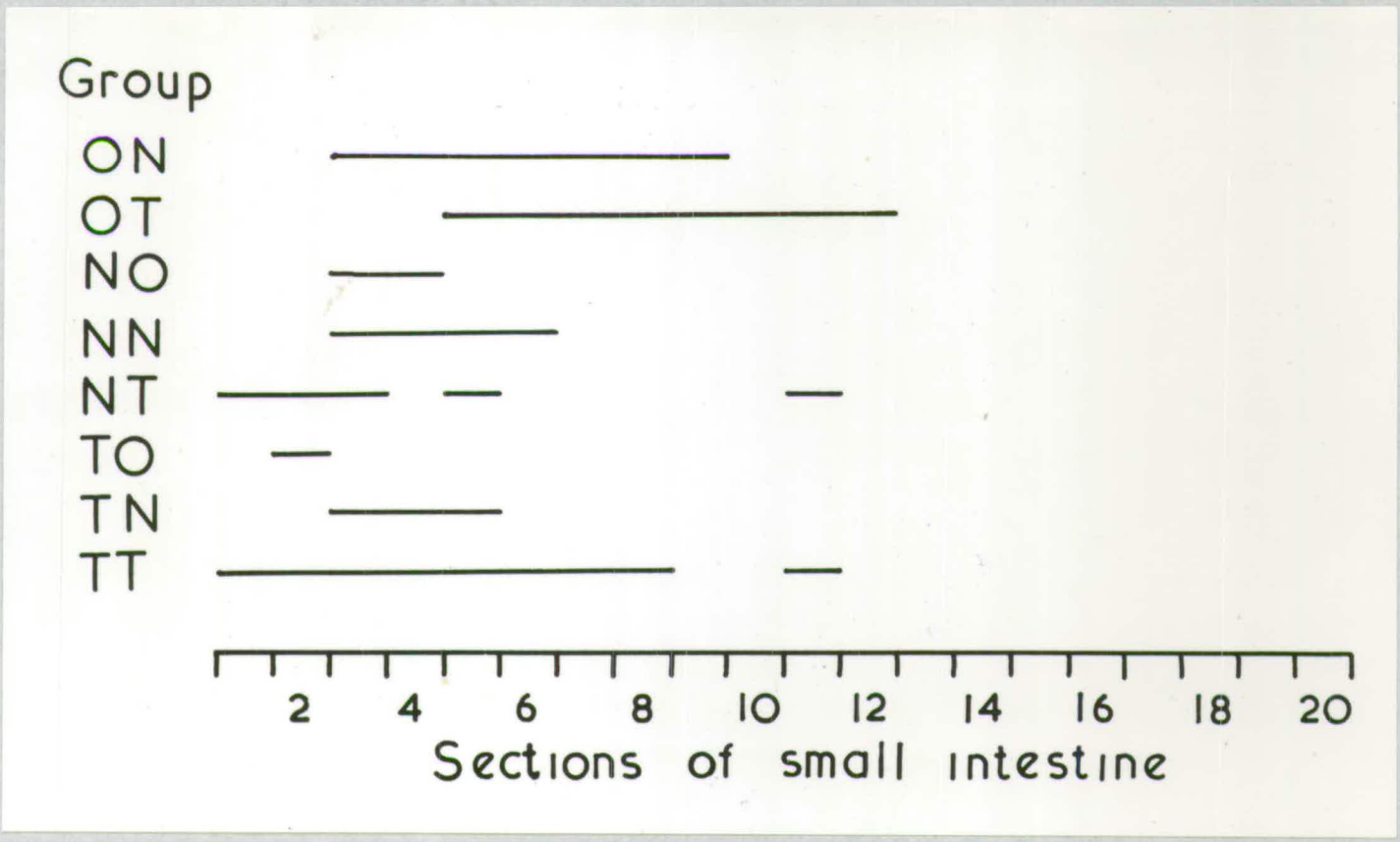
Answer NO

FIGURE 26.



Histograms showing the number of worms recovered from each small-intestinal section in each group of infected rats. (Experiment 9).

FIGURE 27.



Sections which contained more than 5% of the total number of worms recovered from the small intestines in each group of rats. Each horizontal line represents a group of rats. (Experiment 9).

the worms. The distribution of the worms in the 'OT' group when compared with the 'ON' group shows that the transferred worms did not behave normally in the recipient rats. The mean position of the worms in the former group is 9.4 whereas in the normal infected group it is 4.3. This posterior position is most unusual at this stage of a primary infection and was uniform to all three rats in the group. The sex distribution in these rats shows that the effect of transferring is more or less the same in both sexes. The worms were transferred into the stomach and passed from there to the intestine, and it is possible that this was the damaging process. Two alternative methods suggest themselves. One is to subject the rat to surgical interference and introduce the worms directly into the small intestine; the other is to collect the worms from the lungs and give them to the recipient rats by oesophageal tube. It is obviously quite normal for lung stages to pass through the stomach.

The sub groups in this experiment are small. Fifty to Sixty was almost the maximum number of rats that could be handled in one experiment. However, certain very definite inferences can be drawn from the experiment. The 'NO', 'TO' and 'OO' groups have behaved as would have been expected and can be regarded as satisfactory controls. The four groups which had had two infections - 'TT', 'TN', 'NT', 'NN' - show the immunizing effect of the first infection on the second infection. It is known from the 'OT' group that the transferred worms are not in good enough condition to be used as reliable challenge doses, but comparison of the 'TN' and the 'NN' groups shows that they are able to stimulate as good an immunity as the normal worms. Thus the passage of the worms

through the skin and lungs is not an essential prerequisite for stimulating an immunity against further infections of this worm. The evidence from this experiment does not show if the immunity stimulated by the transferred stages operates in the skin and lungs as would the immunity stimulated by normal larvae, but it does show that the immunity acts before the worms are six days old and that it is as good as the immunity resulting from a normal infection. The inference is that this immunity is identical with that induced normally.

The low percentage of worms recovered from the 'OT' group (6.4) makes it difficult to say whether the 2% recovery from the 'NT' and 'TT' groups is significant. The Mann-Whitney non parametric 'U' test shows a probability of less than .012 for these being drawn from the same population. If it is assumed that there is homogeneity of variance the F test when performed on the absolute numbers of worms recovered gives a probability of .01 for the numbers recovered from 'OT' being from the same population as 'NT' or 'TT'. Thus there are grounds for the view that worms which have had no experience of 'immune' skin or lungs can be rejected from the small intestine. This experiment suggests that there are both afferent and efferent immunogenic pathways in the small intestine of rats.

EXPERIMENT 10.

THE DISTRIBUTION OF *NIPPOSTRONGYLUS BRASILIENSIS* IN THE SMALL INTESTINE OF RATS WHICH HAVE HAD LUNG STAGES OF THE WORM TRANSFERRED TO THEM FROM DONOR RATS AS PRIMARY OR SECONDARY INFECTIONS.

Experimental Plan

This experiment was smaller than Experiment 9 and different from it

in that the transferred worms were collected from the lungs and not from the small intestine of the donor rats and in that transferred worms were only given on the second dosing occasion (i.e. the 'TO', 'TT' and 'TN' groups were omitted). This was because not enough rats were available at the time.

Thirty male rats aged 114 days on Day 0 were used. Twelve rats were reserved as donors; the other eighteen being kept for experimental comparisons. These rats were divided into six groups of three each, and given the treatments 'OO', 'NO', 'ON', 'NN', 'NT' and 'OT' respectively. The dosing occasions were separated by an interval of 35 days. The details of dosing are given in Table 37.

Special Method

The twelve donor rats were infected with 6000 larvae on Day 35 and were killed two days later when most larvae would be about to leave the lungs for the intestine. The lungs were removed, cut up with scissors and placed in an Atomix blender, which was run at half speed for 5 minutes, sufficient saline had been added to allow thorough blending to take place. Microscopic examination showed that most of the larvae were apparently undamaged by this process. The larvae were washed in a centrifuge (1000 r.p.m.), concentrated and counted.

13000 lung stages were recovered from a total infection of 72000 larvae, i.e. 18%. 1800 larvae were given to each of the six rats in the 'OT' and 'NT' groups, by oesophageal tube as described in Experiment 9.

The animals were killed on Day 41, six days after the start of the second dosing period.

TABLE 37.

Plan of Experiment 10.

Group I	Donor rats for production of transferred stages.				
	12 rats infected	6000 larvae	Day 35	Killed Day 37	
Group 2	Experimental Rats				Killed Day 41
OO)	3 rats	kept as uninfected controls			
ON)	3 rats	given	2000 Normal larvae on Day 35 only.		
OT)	3 rats	given	1800 Transferred stages on Day 37 only.		
NO)	3 rats	given	2000 Normal larvae on Day 0 only.		
NN)	3 rats	given	2000 Normal larvae on Day 0 2000 Normal larvae on Day 35.		
NT)	3 rats	given	2000 Normal larvae on Day 0 1800 Transferred stages on Day 37.		

Results

Table 38 Worms recovered from each section in each group.
Table 39 Principal parameters of worm distribution in each rat.
Table 40 Mann Whitney and 'F' tests of significance of results obtained from groups 'OT' and 'NT'.

Figure 28 Histograms of worm distributions in each group.
Figure 29 Sections containing more than 5% of the worms recovered from each rat.

The transfer of lung stages by stomach tube was successful in that 9% of the worms were recovered six days later. In normal infections, on the evidence of Experiments 4 and 5, about 50% of the worms can be recovered on Day 6. Although it is not known if the worms that were transferred represent precisely the same worms as would be recovered normally from the small intestine, it must be true that some at least would if left undisturbed have reached the intestine by the sixth day. Thus it can be argued that by this method some 15-18% of the possible take were recovered. Clearly there is need for improvement. Probably the best way of administering the worms would be to feed the donor lungs to the recipient rats, reserving sufficient material to be able to compute the dose in retrospect.

The groups of three rats are obviously on the borderline of significance. However, the results do confirm the finding in Experiment 9 that the intestinal stages are themselves susceptible to ejection by the immune mechanism; that by no 'premedication' of the worm or the host during the migratory stages of the secondary infection is needed and that there is an adequate efferent immunological pathway in the intestine.

TABLE 38.

The mean number of worms recovered from each section of the small intestine in each group.

(Experiment 10).

Group Rats	'OO' 3	'ON' 3	'OT' 3	'NO' 3	'NT' 3	'NN' 3
Section 1				7	7	5
2		10	1	7	1	13
3		91	4	4	3	6
4		145	13	1	2	6
5		123	28		2	4
6		93	37		3	3
7		63	28			3
8		43	26			5
9		16	12			8
10		7	8			4
11		2	2			2
12		1		1		
13						3
14						
15						1
16						1
17						
18						2
19						1
20						1
Mean Total	0	594	159	20	18	68

TABLE 39.

The Mean and Mode position and the standard deviation of the distribution of worms, as well as the total number of worms recovered from the small intestine of each rat.

(Experiment 10).

Group	Mean	Mode	Standard Deviation	Total
'OO'				0
				0
				0
'ON'	5.1	4.5	1.8	821
	4.3	3.5	1.6	465
	4.4	3.5	1.7	491
'OT'	6.7	4.5	1.9	83
	5.9	6.5	1.8	152
	6.0	5.5	1.8	246
'NO'	4.3	4.5	1.7	4
	1.7	1.5	1.9	58
	2.5	2.5	0	2
'NN'	5.6	1.5	3.3	140
	4.6	1.5	4.3	15
	5.2	0.5	3.8	47
'NT'	3.5	3.5	3.6	3
	3.1	0.5	2.2	16
	2.4	0.5	1.8	36

TABLE 40.

Non-parametric Mann-Whitney 'U' test (Seigel, 1956) and parametric 'F' test (Snedecor, 5th ed., 1956) applied to the results obtained from groups 'OT' and 'NT'.

(Experiment 10).

Data

Group	Rats	Totals of worms			$\sum X$	$\bar{X}$	$\sum X^2$
OT (A)	3	83	152	246	481	160.3	90509
NT (B)	3	3	16	36	55	27.3	1561
Total					536		92070

$$C = (\sum X)^2 / n = 47882.7$$

Mann-Whitney 'U' test

Ranking: B B B A A A U = 0

$p\ (U=0;\ n_a = 3;\ n_b = 3) = .05$

Analysis of Variance

Source of variation		S.of S.	D.of F.	Mean Square
Total	$\sum X^2 - C$	44187.3	5	
Between groups	$(\sum X_a^2)/na + (\sum X_b^2)/nb - C$	30246.0	1	30246.0
Within groups		13941.3	4	3485.3

'F' test

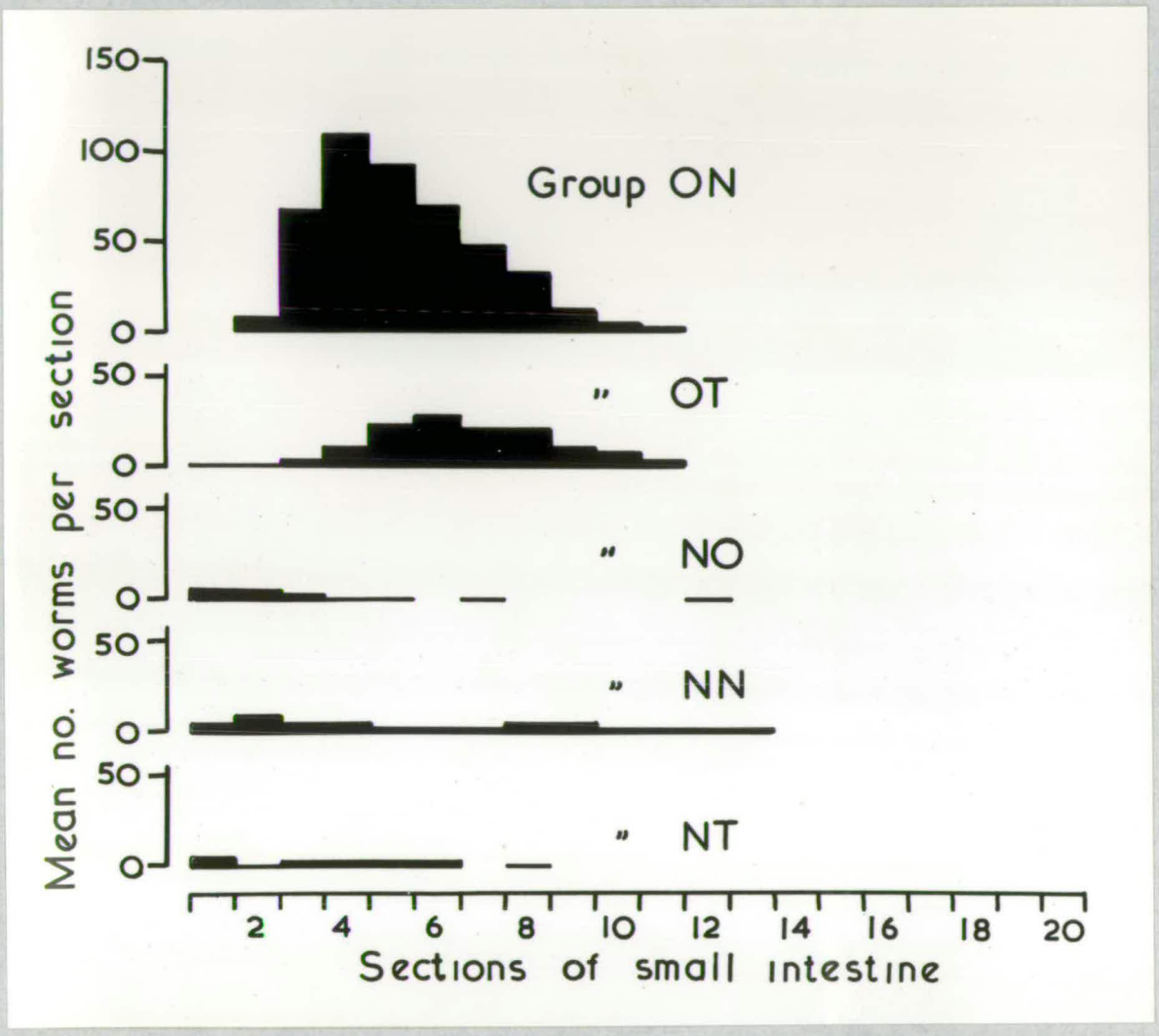
Is the variation between groups due to the variation between rats which have all received the same treatment?

$$F_{(1:4)} = \frac{30246.0}{3485.3} = 8.68 \quad p < .05$$

Answer NO

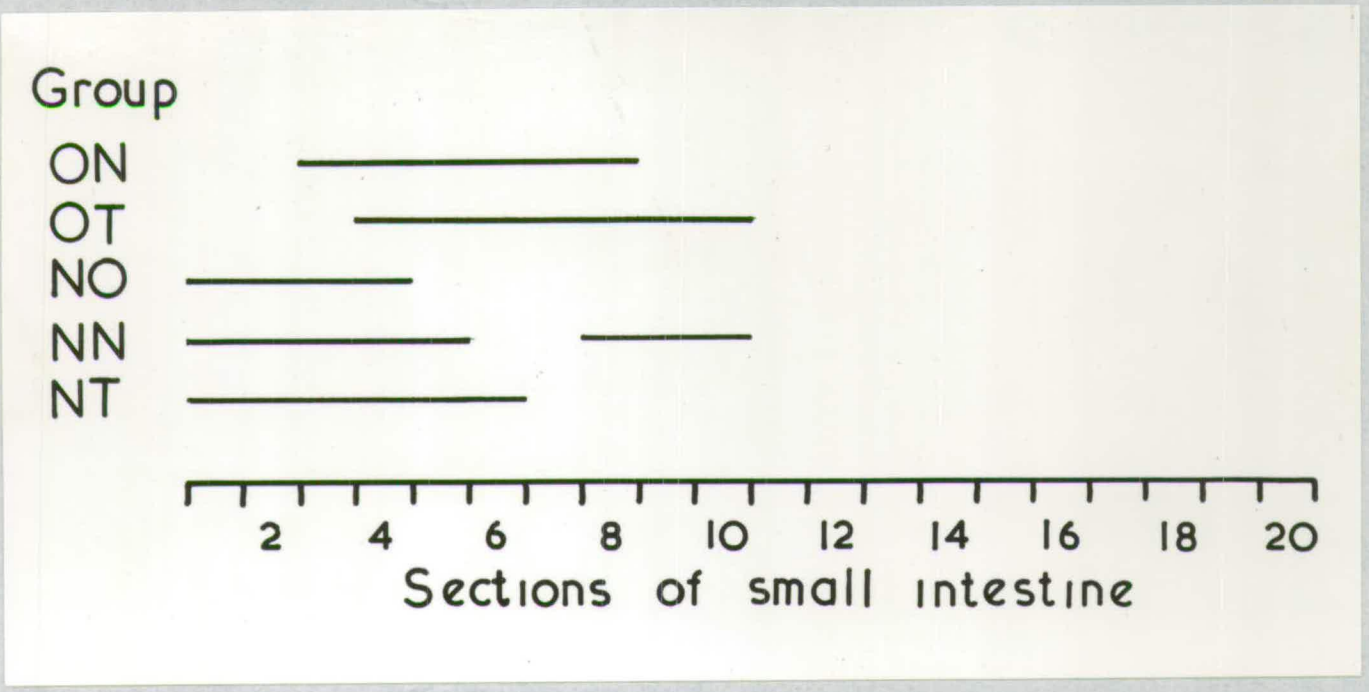
There is therefore a significant difference between the treatments.

FIGURE 28.



Histograms showing the number of worms recovered from each small-intestinal section in each group of infected rats. (Experiment 10).

FIGURE 29.



Sections which contained more than 5% of the total number of worms recovered from the small intestines in each group of rats. Each horizontal line represents one group of rats. (Experiment 10).

EXPERIMENT 11.

AN EXPERIMENT DESIGNED TO SHOW IF IMMUNOLOGICAL TOLERANCE OF NIPPOSTRONGYLUS BRASILIENSIS COULD BE INDUCED IN RATS BY EXPOSING THEM TO INFECTIONS ON THE FIRST DAY OF LIFE

Experimental Plan

If rats are to be shown to be immunologically tolerant of N. brasiliensis they must be given an infection of the worm during the first eight days of life to induce the tolerance. Later in life they must be given a normal 'primary' infection to stimulate an immunity and subsequent to this a 'secondary' infection to test whether or not an immunity has been produced. There seem to be no grounds to assume that in tolerant rats a greater percentage of a 'primary' infection would reach the intestine than in intolerant rats. The proportional increase that might occur could not be very great when it is remembered that between 50 and 80% of a primary infection can be recovered normally. Ewert and Olson (1960) tried to see if mice could be made tolerant to Trichinella spiralis by injecting them at birth with an antigen made from Trichinella larvae; using an increase in the number of worms recovered from a primary infection at six weeks as the criterion of tolerance. In Experiment 11 rats were given a 'tolerating' dose of larvae, an 'immunizing' dose and a 'challenge' dose. Evidence of tolerance would be the recovery of a significantly higher number of worms after the 'challenge' dose in rats which had received the 'tolerating' dose than in those which had not.

In a preliminary experiment half of the rats in each litter had been given the 'tolerating' dose the other half being reserved as controls. However, it was found that cross infection took place, presumably immediately after infection when larvae could be imagined to quite easily

pass through the thin skins of adjacent day-old rats. Because of this whole litters were given the same treatment at one day old, it being hoped that between litter differences would be negligible when compared to differences between treatments.

Six female rats were mated on the same day. The offspring of three of these were infected with about 60 larvae. The larvae were given subcutaneously, in about .1 ml. saline. The mothers were given a light ether-anaesthesia during the handling of the young, which were each dabbed with a spot of ether before being returned to the nest. The ether served to mask any foreign smells on the young. None of the infected rats in two litters died but in the third the mother removed five.

When the rats were six weeks old some of both groups were given an 'immunizing' infection of 300 larvae.

Four weeks later rats were challenged with a further dose of 300 larvae, after which they were killed on the sixth, ninth, twelfth or eighteenth day.

Results

Table 42 Number of worms recovered from each rat.

The challenge dose of larvae was 300. This was probably a mistake. Experiment 8 has shown that 100 larvae in a primary dose can protect rats against a very much higher secondary dose. Had 1000 larvae been used the difference between the numbers of survivors from previous infections and the survivors of the challenge dose would have been much more obvious. In this experiment, quite apart from the rats which had received the 'tolerating' dose, the results from group OOL should have shown more worms

TABLE 41.

Plan of Experiment 11.

Group	Rats	'Tolerating' dose Day 0	'Immunizing' dose Day 42	'Challenge' dose Day 69	Days killed 6	9	12	18
LLL	12	+	+	+	4	4	2	2
LLO	12	+	+		4	4	2	2
LOL	3	+		+	1	1	1	0
LOO	2	+			1	0	1	0
OLL	15		+	+	5	5	3	2
OLO	15		+		5	5	3	2
OOL	3			+	1	1	1	0
OOO	2				1	0	1	0

TABLE 42.

Number of worms recovered from the small intestine of each rat.

(Experiment 11).

Group	Day killed	Worms	Mean	Group	Day killed	Worms	Mean
LLL	6	11		OLL	6	37	
		68				7	
		17				59	
		52				22	
			37			32	31
	9	86			9	5	
		20				85	
		123				18	
		3				125	
			58			19	50
	12	193			12	59	
		25				2	
			109			118	60
	18	10			18	14	
		32				8	11
			21				
LLO	6	86		OLO	6	9	
		101				5	
		14				19	
		22				15	
			56			90	28
	9	59			9	4	
		4				57	
		16				13	
		11				67	
			23			16	31
	12	22			12	48	
		2				49	
			12			8	35
	18	21			18	9	
		0	11			77	43
LOL	6	29		OOL	6	14	
	9	175			9	136	
	12	17	74		12	19	56
LOO	6	3		OOO	6	0	
	12	0	2		12	0	0

than OLO or OLL. The fact that this did not occur is sufficient grounds to regard the experiment as a failure.

DATA COLLECTED FROM RESULTS OF SEVERAL EXPERIMENTS

Effect of weight on distribution of worms as shown in data from several experiments

From experiments 4, 5, 6, 8, 9, and 10 it is possible to assemble 60 pairs of rats with primary infections in which there are no differences in age, sex, history or treatment. The difference in the weights of the two rats in each pair can be compared with the differences in the mean position of the worms, to see if it is the weight of the rat that determines the mean position of the worm population. Table 43 gives this data and also the value of r obtained from it. It can be seen that there is no evidence of significant correlation, although the mean weight difference being nearly 22 grams.

The conclusion drawn is that the differences observed between the distribution of the worms in the two sexes of rat cannot be attributable to the weight difference of the two sexes. These differences must now be assumed to be true sex differences.

Distribution of Peyer's Patches

The number of Peyer's patches in each section was recorded for 106 rats. Some of these had had no worms, some had had one or two infections, and others had had more than two infections. They were drawn from Experiments 6, 7 and 11. Figure 30 shows the distribution of the patches in each sex of rat and Figure 31 shows the distribution in rats which have had one and two infections.

TABLE 43.

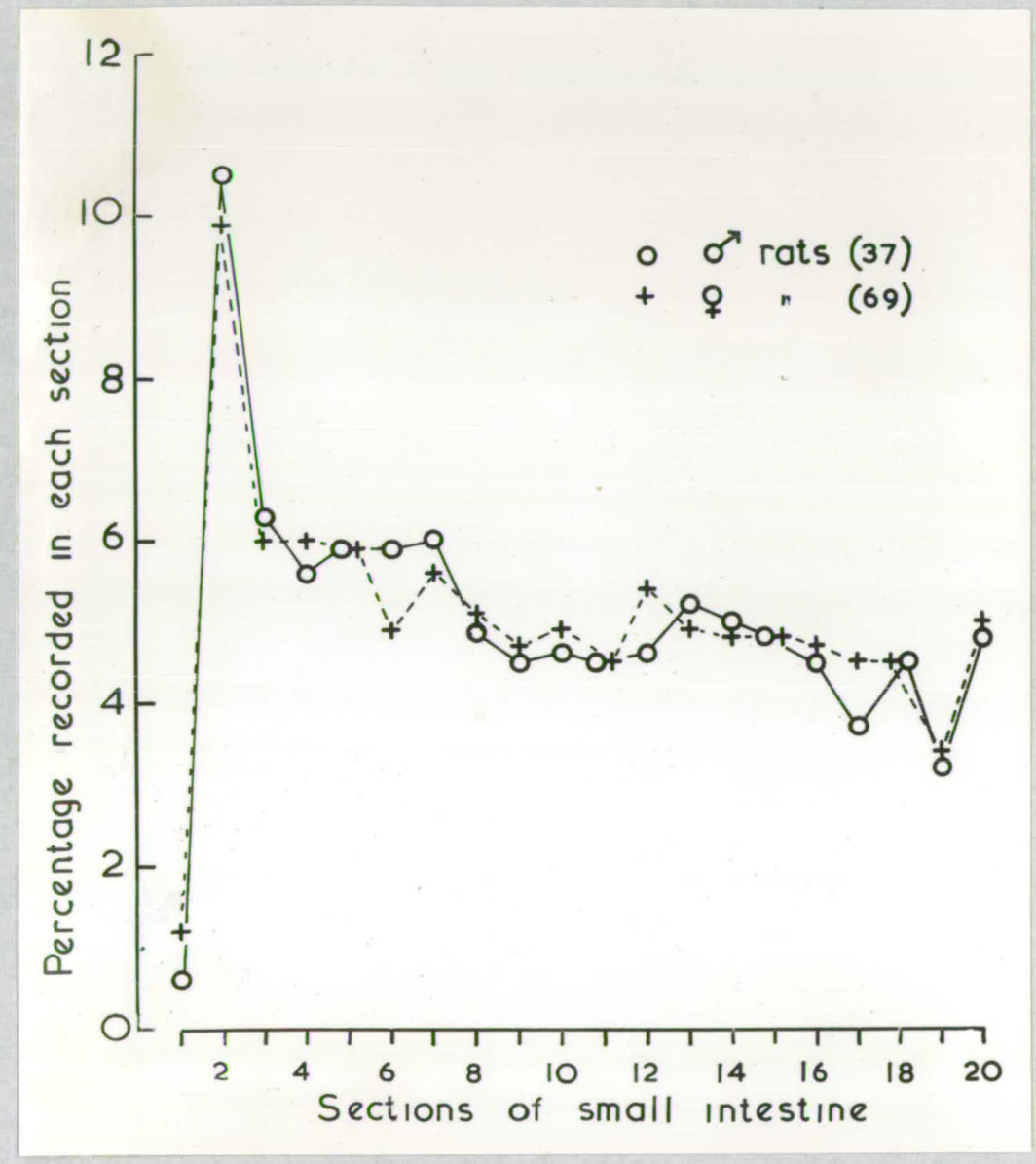
Comparison of the difference in weights (w) and in mean worm positions (m) in rats with primary infections (Diff. w always expressed as positive). Where an odd number of rats in a group the first pairs have always been taken, omitting the last rat.

Exp.	Rat No.	W	M	Rat No.	W	M	Diff W	Diff M	Sex	Exp.	Rat No.	W	M	Rat No.	W	M	Diff W	Diff M	Sex
4	1	244	6.7	3	222	5.9	22	.8	♂	5	9	465	5.0	10	385	5.7	80	-.7	♂
"	5	222	6.4	7	190	5.1	32	1.3	"	"	13	435	5.5	14	365	5.9	70	-.4	"
"	9	217	5.0	11	213	5.5	4	-.5	"	"	17	425	4.6	18	425	6.3	0	-1.7	"
"	13	239	4.4	15	201	5.1	38	-.7	"	"	21	390	5.6	22	400	5.3	10	-.3	"
"	17	218	5.3	19	227	7.4	9	2.1	"	"	25	375	4.5	26	425	6.5	50	2.0	"
"	21	212	5.3	23	215	6.9	3	1.6	"	"	29	450	5.8	30	438	6.0	12	-.2	"
"	25	220	6.2	27	260	3.7	40	-2.5	"	"	33	365	7.7	34	380	6.2	15	-1.5	"
"	29	245	2.7	31	220	2.3	25	4	"	"	3	275	6.2	4	220	6.0	45	.2	♀
"	33	226	6.0	35	202	4.5	24	1.5	"	"	7	225	7.5	8	240	6.1	15	-1.4	"
"	41	217	1.3	43	214	3.2	3	-1.9	"	"	11	230	6.2	12	230	6.9	0	-.7	"
"	49	235	1.3	51	226	1.8	9	-.5	"	"	15	255	5.9	16	245	6.4	10	-.5	"
"	53	229	3.8	55	205	1.1	24	2.7	"	"	19	205	5.7	20	250	6.5	45	.8	"
"	2	188	8.2	4	197	6.0	9	-2.2	♀	"	23	215	6.5	24	195	7.5	20	-1.0	"
"	6	180	6.5	8	175	6.5	5	0	"	"	27	220	5.9	28	260	7.0	40	1.1	"
"	10	182	6.1	12	188	6.0	6	- .1	"	"	31	270	5.5	32	250	6.4	20	-.9	"
"	14	191	5.5	16	183	5.7	8	-.2	"	"	35	245	6.1	36	270	8.9	25	2.8	"
"	18	204	5.6	20	176	6.9	28	-1.3	"	6	6	213	1.0	8	208	5.9	5	-4.9	♀
"	22	196	6.9	24	187	6.2	9	.7	"	"	16	278	7.0	17	227	4.5	51	2.5	"
"	26	196	6.8	28	230	7.8	34	1.0	"	"	26	251	3.5	27	237	3.1	14	.4	"
"	30	203	6.1	32	177	10.6	26	-4.5	"	"	9	249	5.6	10	242	5.6	7	0	"
"	34	184	6.2	36	188	5.1	4	-1.1	"	"	19	291	5.3	20	254	5.5	37	-.2	"
"	38	175	1.0	40	191	2.5	16	1.5	"	"	29	271	5.8	30	297	7.0	26	1.2	"
"	42	205	2.7	46	177	2.8	28	-.1	"	8	5	221	4.0	6	219	3.2	3	.8	♀
"	48	193	1.2	50	203	1.5	10	.3	"	"	8	284	5.9	9	228	5.1	56	.8	"
"	54	179	2.5	56	195	1.3	16	-1.2	"	"	10	278	4.7	11	277	6.3	1	-1.6	"
5	1	420	6.1	2	440	6.3	20	.2	♂	"	13	267	4.4	14	296	5.4	29	1.0	"
"	5	340	6.1	6	345	6.3	5	.2	"	"	16	278	3.8	18	290	2.0	12	-1.8	"
8	35	272	1.5	37	245	1.5	27	0	♀	9	21	255	4.2	12	260	4.6	5	.4	♂
"	50	285	.5	51	285	1.5	0	1.0	"	10	10	230	5.1	11	206	4.3	24	.8	"
9	3	247	3.5	5	325	2.5	78	-1.0	♂	"	13	194	4.3	14	188	1.7	6	2.6	"

Diff $\bar{W}$ = 21.58 gms. Diff $\bar{M}$ = -.057 of a section
r = +.129 n = 60 p = >.05 Not significant.

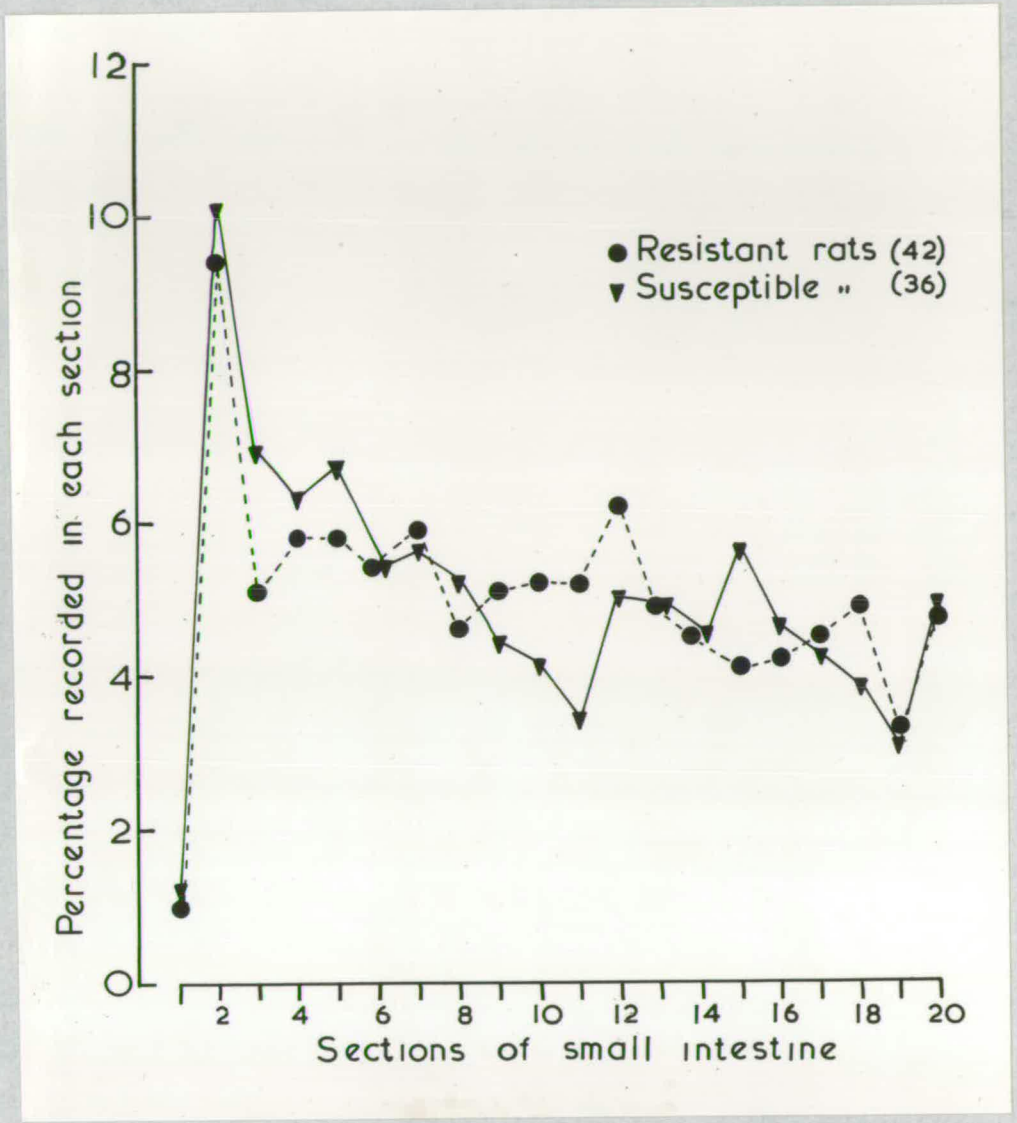
Had there been a significant effect to explain the sex difference observed r = -.250. (See Fisher, 1954, p. 209).

FIGURE 30.



Percentage incidence of Peyer's patches in each section of the small intestine of male and of female rats.

FIGURE 31.



Percentage incidence of Peyer's patches in each section of the small intestine of rats which have had more than one infection (resistant) and of rats which have had only one infection (susceptible).

DISCUSSION.

DISCUSSION

Cultures

It is not difficult to culture Nippostrongylus brasiliensis to the infection stage. Eggs are laid in great profusion and develop to the infective stage in a few days. When larvae are needed for subcutaneous injection it is essential that they be as clean as possible. Severe bacterial contamination can arise from the faeces in which the larvae are cultured. However, any other material is an undesirable contaminent because it can cause an inflammatory reaction at the site of the injection. For this reason charcoal cultures are not good as not only are the faeces and the charcoal very much intermingled making it difficult to separate the larvae clustered on the charcoal from the faeces, but also the charcoal itself is liable to break up on handling resulting in a dust that is laborious to eliminate. Barakat's (1951) technique of culturing on filter papers and utilizing the migration of the larvae allows a very easy separation of larvae from faeces to be made. In this work his technique has been slightly modified to improve the stability of the cultures. Filter paper is apt to bend and sag and should it touch the side of the container the larvae do not stop migrating at the edge but carry on onto the walls of the container. Substituting filter pads, which have greater rigidity, removes this hazard. The support of the filter paper or pad must be such that water can reach the paper without the paper's edges touching the container, in other words it must be permeable to water and it must be flat. Pulp board discs are superior to cotton wool pads in meeting these requirements and they can be set up very rapidly.

Wilson's (1964) method of culturing has the advantage of allowing large quantities of faeces to be cultured without too much labour being spent on setting the system up. However, where only small quantities of faeces are available it is still preferable to use the technique of filter pads supported on pulp board discs.

Transit time.

After the eggs have been laid by the worms the principal source of inaccuracy in egg counts rises from lack of knowledge of the rate of passage from the upper small intestine to the anus. The ideal would be to sample the eggs directly, through an intestinal fistula, but this cannot be done without seriously affecting the host in a way that would probably alter the course of infection. In the absence of knowledge of how long a particular piece of faeces has taken to traverse from the small intestine to the exterior and of how much it has been mixed with faeces that had passed the adult worms before or after, an approximation of the transit time must be made.

When a batch of eggs has been laid in the upper small intestine the eggs will become gradually separated in their passage to the anus. A few will be passed sooner than the majority and a few will be passed later. Most will be passed at the same time and it is this time, the mode, which can be taken as that at which the faeces contain the greatest proportion of the original batch of eggs and smaller proportions of batches laid before or after.

The feeding of marked foodstuffs measures the transit time from mouth to anus, which must by definition be longer than that from small intestine to anus. Thus estimates of the total transit time

provide outer limits for the time from the small intestine to the anus. Probably at least one hour can be allowed for the time spent in the stomach. In the two estimations done in this work the mode time was 10 hours and 14 hours; the median time was 12 hours and 15 hours and the mean time was 13 hours and 17 hours. Thus for practical purposes the transit time can be taken as being about twelve hours between the site of egg laying and passing through the anus.

Intestinal division

In this work the small intestine was divided by referring to the two ends while the organ was under precise standard conditions. This method revealed that the worms parasitized the organ in a consistent manner. Although it is not known what are the factors which determine where a worm attaches itself, it can be assumed that the micro-anatomy and the biochemistry of the region are important. Thus it can be concluded that this method of 'mapping' the small intestine distinguishes between functional zones in the organ.

The evidence collected made it possible to relate some other anatomical features to the arbitrary division of the small intestine. The bile duct was always found in the latter part of Section 1. The short mesentery, which marks the end of the duodenum and the beginning of the mesenteric part of the small intestine is an imprecise structure it being difficult to judge exactly where it occurs, was found to be in the region of the middle of Section 2. Thus Sections 1 and 2 can be regarded as being duodenal sections and Sections 3 to 20 as belonging to the Jejunum-Ileum. The division between Jejunum and Ileum is arbitrary (Sisson, 1917), and could be taken as being at the junction

of Sections 11 and 12.

Whether the development of the micro-anatomy of the intestinal lining is clearly tied to the gross anatomical changes in the embryo is not known. As Meckel's diverticulum was found only once this work can provide no evidence on the point. It is quite conceivable that a vestigal structure like this might vary in its position along the small intestine without the functional zones showing any corresponding variations.

Very much evidence was gathered on the distribution of Peyer's patches in the rat. These are large aggregations of lymphoid tissue and as this tissue has been implicated in the processes of immunity it is possible that there might be a correlation between the positions of Peyer's patches and the reaction of the rats against the worms. However, no relation could be found between the positions or numbers of Peyer's patches and the history of worm infection. Peyer's patches appear to be distributed in a definite pattern, but this only becomes apparent when the results obtained from many rats are grouped together.

The blood supply of the small intestine, while of a constant general pattern, is so variable in detail that it has proved useless as a guide to the regions of the organ. Certainly there are no grounds at all for assuming any relation between the details of the blood supply and the presence of worms. Similarly, the positions of the mesenteric lymph nodes are even more remote from the site of attachment by the worms and it would have been pointless to have looked for any relationship between the two.

The behaviour of the small intestine under different treatments was

revealed by using artificial markers. This showed that, provided the organ has not been irreversibly damaged, the standard treatment in the 'Haruspicator' of 5 grams tension in 1/500,000 Adrenaline saline at 37°C is an extremely satisfactory method for dividing the organ. Probably the most important feature is the bath itself which allows the intestine to find its own position without any drag against a supporting surface. The temperature and the saline serve to maintain the isolated intestine in as constant a physiological state as possible. The adrenaline inhibits any muscular activity. The second most important feature of the Haruspicator method is the application of a small but standard tension. Tensions of the order of five to ten grams are extremely difficult to judge. The small intestine is apt to tear under tensions of over twenty grams, so it is very important to know when the tension has exceeded the maximum intended. The collapsible phosphor-bronze spring developed in this work is ideal for the purpose.

Intestinal nematodes are very exact in their requirements. N. brasiliensis can only parasitize Sections 3 to 10 in any great numbers. Tetley (1937), showed that in the sheep intestine the different species of worms were found at different levels of the organ. He divided the organ into multiples of feet from the stomach, and he presented his results in terms of absolute distance from the pyloric sphincter. As the small intestines he examined were, by inference, of greatly different lengths, c. 65 ft. in one case, 78 ft. in another, possibly 39 ft. in several others, it is difficult to determine the relative positions of the worms precisely. Nevertheless, his results can be summarized

as follows:

Strongyloides and Trichostrongylus were in general found nearest to the pyloric sphincter, then Nematodirus fillicollis followed by Nematodirus spathiger, behind which comes Cooperia curticei. Bunostomum trigonocephalum was found all along the small intestine and Capillaria longipes was found very close to the ileo-caecal valve. In most cases Tetley found compact distributions but in some instances he found the worms of a species very much more spread out. It is not known what are the factors which determine the site of each species but it is clear that within the small intestine these factors cannot be uniformly distributed. The most obvious ones are histological differences and it is in this field that this work must develop. Equally important but more difficult to examine are biochemical differences. The technique of dividing the intestine using the haruspicator should allow for much greater detail to be described than before. It is a pity Tetley did not express his results as proportional lengths of the organ. It is not possible to do this from the results he published because there is no certainty of how far the ileo-caecal valve was from the pyloric sphincter in the several sheep he used. Had he done so his results would have been very much more valuable, as there is an obvious differential distribution between the species of worms and possibly one between different periods of the same infection.

Egg production.

A considerable number of biological variables have had their effect on the concentration of the eggs in the faeces by the time the pellets pass through the anus. It is not practicable to determine most of these

variables. Even when the total number of eggs being passed is determined, thereby eliminating some of the variables, the variations in transit time make inferences drawn about the worm population from the egg output of any value only when expressed in most general terms. Thus a population can be said to have started laying eggs, reached its peak of egg production, and to have stopped laying eggs on particular days. It can be inferred to consist of few worms or many worms and its general pattern can be compared with that of another population.

Africa (1931) and Graham (1932) found that worms were more fecund in rats under two months of age than in older ones. Spindler (1933) showed that worm populations produced more eggs and over a longer period in Vitamin A deficient rats than in normal rats. Schwartz, Alicata and Lucker (1931) showed that fewer eggs were produced in secondary infections than in primary ones. Sarles and Taliaferro (1936) produced data which indicates that less eggs are produced per worm in secondary infections. Chandler (1936) showed that the decrease in egg production in ageing primary infections was in part due to lowered fecundity of the female worms, as when these worms were transferred to the small intestine of a fresh host their fecundity increased.

The results of Experiment 4 show that in adult rats the fecundity of the worms rises sharply and then falls almost equally sharply. The peak level of egg production being almost 7700 eggs per day per female worm.

In secondary infections the egg output was reduced to almost zero and consequently the production per worm was near to zero. There was evidence that the degree of suppression was a function of the size of

of both the primary and the secondary infections for in Experiment 8, no eggs were seen after the secondary dose had been given in the group which had had 2500 infective larvae as the primary dose, though eggs were seen in the groups which had had primary doses of 100 or 500 larvae. Similarly, those groups that were challenged with 100 or 500 larvae did not develop egg counts whilst those challenged with 2500 and 12500 did show egg counts (except in the group with 2500 larvae as the primary dose). However, serial daily killings were not made on rats with secondary infections and it is not possible to draw very precise conclusions on the egg laying activity of the female worms. The evidence does suggest, however, that the process of elimination of the worms from the small intestine may not simply be an extension of the process which inhibits egg laying.

Type of distribution within the small intestine.

During the course of a primary infection this work has shown that the shape of the distribution changes can be classified into four different phases. Phase 1, lasting from Days 1-6 is the one that is probably least complicated by the influence of the hosts' reactions and by damage to the worms. In Phase 2 the worms are more spread out and by Phase 3 the pattern is very much changed. Phase 1 was taken as the pattern most likely to approximate to a simple mathematical distribution. However, it was not possible to fit the data to a Normal, Binomial or Poisson distribution. The distribution is skew and had too many worms about the mode to make a satisfactory fit. Having failed to show a simple form of distribution the point was not pursued.

Tetley (1937) recognized two types of distribution: those in which

the

the worms were distributed Normally and those in which they were not Normal. He judged normality by eye. What he saw can best be described as compact distributions in which many worms were concentrated in a relatively small area of the intestinal wall and dispersed distributions in which the worms were spread over a relatively large part of the intestinal wall.

This work has shown that in N. brasiliensis infections the dispersed distribution is a sequel to the initial compact distribution and that it is associated with the development of resistance by the host. It has also shown that the worms found more posteriorly are not passively on the way out but that some at least are attaching to the small intestine.

The evidence presented here provides support for Tetley's suggestion that the larvae determine the site of attachment, though the mechanism by which this happens is still unknown. The changes in the distribution will be discussed in more detail below.

In a Normal, Binomial or Poisson distribution the most convenient parameters to work with are the mean and the variance. In a skew distribution of the type encountered in this work the mean is not a particularly helpful parameter as it does not state anything specific. However, the mode does state the position where most worms were present. The variance gives some estimate of the amount of scatter but as this is uneven on either side of the mode it is not a particularly useful statistic. In order to get an estimate of scatter and at the same time eliminate the effect of differences in the total size of the population the population in each section has been expressed as a percentage of the whole population and the range has been taken to include those

sections which contained more than 5% of the worms seen in the rats. This has been done in preparing Figures 9 and 16, etc.

Primary Infections

Figure 9 shows that the distribution of worms in a primary infection changes as the infection ages, and that these changes occur at approximately 6, 12 and 18 days after infection. In the phase leading up to Day 6 the worms are concentrated around the mode section and are not present in Section 1. During the next phase the worms are in much the same position except that they have now invaded Section 1 and are appearing in greater numbers in the more posterior sections. In the third phase the worms are spread all along the small intestine and in the fourth phase there are a few worms left at the anterior part of the intestine. A final phase can be assumed in which no worms at all survive. Although Experiment 5 did not extend over so long a period as Experiment 4 the evidence obtained from it showed that at dose rates of 1000-6250 larvae there appeared to be no marked differences in the pattern of the distribution.

The following alternatives could explain the loss of adults from Sections 3 to 10 between Days 12 and 18.

1. The intestinal environment remains constant and the worms become incapable through old age.
2. The intestinal environment changes and the worms can no longer parasitize because of humoral and cellular attack resulting from these changes.
3. The intestinal environment changes and the worms can no longer parasitize because the intestine no longer contains suitable sites of attachment.

Alternative 1. is untenable because Porter (1935), Taliaferro and Sarles (1939, 1942) and Symons and Fairbairn (1963) have shown there to be gross changes in the intestinal wall during an infection. Also, Africa (1931) has excluded old age of the worms from being the operative factor by showing the length of time worms survive depends in part on the size of the initial infestation.

Alternative 2. has strong evidence to support it in that the rate of egg production is reduced indicating interference with the worm, and also that Sarles (1938) found precipitates in and around the intestinal stages of the worm, which may have interfered to an extent with the normal functioning of the worm. Sarles and Taliaferro (1938) found that immune serum passively transferred five days after the start of a primary infection resulted in a fall in the number of worms ~~removed from~~ in the small intestine.

Alternative 3, which involves changes in the intestine which remove suitable sites for the attachment of worms, however, cannot be easily dismissed. These changes could be initiated either directly as the result of damage caused by the worms or indirectly through an immune response. The evidence that changes of this sort take place is that Symons and Fairbairn (1963) have shown that the affected part of the small intestine undergoes great changes in which the villi fuse with each other and so remove the inter-villiary spaces. Taliaferro and Sarles (1942) had referred to the eroding of villi. Symons and Fairbairn, however, regard the lesion as an infilling of the inter-villiary spaces. Porter (1935) had already shown that the worms anchored by pushing their heads deeply between the villi. It is quite reasonable to assume that a

worm with its head deeply between villi is in a better position to withstand the effect of the net downward flow of intestinal contents than one which is attached to an exposed part of the mucosa. Further evidence in support of there being changes in the nature of sites of attachment comes from the events in Section 1 as reported in this paper.

The situation in Section 1 is interesting in that the worms are able to survive in this section for longer than in Sections 5 and 6, although at the start of the infection worms were apparently unable to settle in this section. It is possible that worms under seven days of age are incapable of attaching in this section and that after seven days they acquire the ability to do so.

The evidence from Experiment 7 indicates that worms are capable, in some circumstances, of attaching in Section 1 before seven days. This finding makes it probable that it is not changes in the worms but changes in the intestinal wall which determine whether or not Section 1 can be parasitized. At present there is no evidence as to what these changes might be. To find out more on this point an experiment is being performed in which the histology of the small intestine is being studied in relation to the age of infection. This experiment does not form part of this thesis.

Section 2 has shown itself to be intermediate, in the manner the worms are distributed, between the pattern seen in Section 1 and that seen in the more posterior sections. This may be because, as is obvious, the section is situated immediately between these other regions and that a gradual transition takes place within it, or it may be because the anterior part of the section is in the anatomical duodenum whilst the posterior part is

in the anatomical jejunum. Until it is understood why Section 1 behaves differently from, say, Section 5 there seems to be no point in examining the manner in which Section 2 is transitional between these two regions. However, although Section 2 is transitional in the sense that some worms are found in it before the seventh days and after the eighteenth day, it must not be taken that after the eighteenth day it plays a subsidiary part to Section 1. In fact, more often than not, more worms were found in Section 2 than in Section 1 after the eighteenth day. It is the clear cut pattern observed in Section 1 that makes it so interesting.

Sections 11 to 20 are never very attractive to worms, most of those seen being on the way out from Sections 3 to 10. The average time taken for worms to pass through this region was fifteen hours. The data from Experiment 4 can be further broken down and the average time taken to pass each section estimated. Thus while the worms took an average of 1.8 hours to pass through each of sections 11 to 17, they took only 0.8 hours to pass through each of the last three sections.

The distribution in secondary infections has not been easy to study on account of the very low numbers of worms recovered. If it was certain that no survivors of the original infection were present then the low numbers of survivors from the secondary infection would be of no particular disadvantage. However, the number of first infection survivors is comparatively large and there is always the doubt of which infective dose gave rise to the worms seen. However, in Experiment 7 one rat reacted to such an extent that there can be no doubt that the majority of worms seen even in Section 1 were derived from the second infective dose. The

pattern observed in secondary infections was not similar to that seen in primary infections after eighteen days. It was similar to that seen in primary infections between Days 6 and 18. There was evidence that the centre of population was nearer the pyloric sphincter than in similarly aged primary infections, but the centre was not in Sections 1 and 2. The secondary infections reached their peak very much sooner than primary infections in spite of the worms being delayed in their passage through the lungs and the worms disappeared very much more quickly than in a primary infection. The fact that the worms of a secondary infection are found in a pattern associated with the break-up of a primary infection and not with the later phase of a few persisting survivors must be due in part to there being a large proportion of worms in the actual process of being eliminated. In a primary infection it has already been shown that only a few worms from Sections 3-10 are able to reach Sections 1 and 2 though presumably many are potentially capable of survival in these two sections, for they are able to resume egg laying on transfer to fresh rats and they are able to delay their passage down the posterior half of the small intestine. Thus in a secondary infection only a few worms are presented with the opportunity of attaching in Sections 1 and 2. However, a surprisingly small number take advantage of this. The reason is unclear. It may be that the worms are already too éffété from their passage through the tissues and lungs to be able to attach in Sections 1 and 2 or it may be that under the stimulus of the secondary infection further changes take place in these sections which make them unsuitable for the survival of worms.

Size of Infective dose.

The size of the dose of infective larvae does not have a very marked effect on the distribution of worms in the range of 1000 to 6250 larvae. This work did not provide sufficient evidence to show if the same is true for doses that are smaller than 1000 larvae. Evidence for an effect due to the size of the dose would be primarily an increase in the standard deviation if the mode-position became saturated with worms. For in this event the number of worms settling in the more distant parts would increase relative to the number settling at the mode position. This did not happen. The standard deviations in all three groups were very similar in Experiment 5. There was a slight difference in the mean position of the worms in the groups dosed at different levels. (The mean position is a useful statistic in that it can be estimated in terms of fractions of a sectional length while the mode can only be measured in terms of intervals of whole numbers of sections). However, these changes were small, of the order of 0.5 of a section, compared to the variation in the position of the mean that occurred between rats receiving the same treatment.

In Experiment 7 1123 worms were recovered from one section of the small intestine. It seems that there are more sites available in the small intestine of a susceptible rat than the animal's 'system' can support, and that saturation of a site is not likely to occur in a living animal. Obviously there is a change in the suitability of the sites once worms have been present. The question is raised as to why, if the mode site can support over eleven hundred worms, is there not a more compact distribution in rats harbouring a total number of worms that is

smaller than this figure. Presumably a worm is influenced by the presence of another, or alternatively, at the start of an infection, the presence of worms elicits changes which make more sites available.

The rate of passage of the larvae from the skin to the intestine was found to be dose dependent. A greater proportion of the larvae from the larger doses had reached the small intestine by the third and sixth days than from the smaller doses. This is quite possibly because in the larger doses a particular larva has more chance of following down the pathway broken through the tissues by a preceding larva than in the smaller doses. Between Day 6 and Day 9 there was a significant fall in the number of worms recovered from the small intestine of the group dosed with 6250 larvae in Experiment 5, although in the lower dosed groups there was no significant change in the numbers. This must be because in the 6250 dosed group some mechanism had begun to remove the worms earlier than in the other groups. While there can be no doubt from the results of many workers and of the later experiments in this work that acquired immunity plays a very important part in the resistance of the rats, it is hard to see how giving a dose of 6250 larvae as opposed to 2500 larvae can advance the onset of an acquired immune response from about Day 12 (vide Experiment 4) to Day 8 or earlier. The mechanism must be closely dependent on the size of the dose. It may involve the sensitization of the host along the afferent immunogenic pathways, or it may be independent of those pathways and be simply a reaction to trauma which changes the suitability of the small intestine to the worms. Because no change in the spread of the worms was detected it can be taken that these reactions involve the humoral

transmission of active substances which cause the intestine to react over a relatively wide area. If no such agents were involved it would be expected that the worms would cause most damage in the mode section and that this section would become unsuitable ahead of the others. It is probably true that the worms do cause most direct damage to the mode section but that humoral agents resulting from this cause the sections of intestine on either side of the mode section to become unfavourable for worms.

To find out more about the reactions which eliminate the worm it is necessary to examine the histological changes in the small intestine in relation to the movements of the populations that have been observed in this work.

Sex of worms.

Male and female Nippostrongylus brasiliensis worms do not have the same distribution, either in time or along the intestine. It was found that in a primary infection the percentage of males amongst the adult worms increased from less than fifty to almost one hundred between Day 2 and Day 30. During a primary infection the proportion of males amongst the adults tends to decrease with distance from the pyloric sphincter, though from Sections 11 to 20 consistent results on this point were not obtained. However, between Sections 1 and 10 this decline in the proportion of males is true. Moreover the decline gets more marked as the infection ages. The proportion of males in Section 1 increases more markedly with the time after infection than does the proportion in, for example, Section 6.

It cannot be argued that the persistence of male worms in Sections 1 and 2 in the period after Day 18 is simply because these are the only sites available for worms and that they are never suitable for female worms, because male and female worms are found in almost equal numbers in these sections around Day 9. It must be concluded that the changes that take place in these sections from Day 12 onwards are sufficient to cause the removal of the female worms but that, unlike elsewhere in the small intestine, they do not reach the threshhold necessary to dislodge the male worms. Indeed in primary infections the females are more easily removed than the males along the whole of the anterior half of the small intestine.

The sex incidence in secondary infections does not reflect that seen at any stage of primary infections. In the first few days of secondary infections up to 75% of the adults recovered may be females. This preponderance of females was found even in the first three sections where in Experiment 8 female worms made up 72% of the adults as compared to 50% in similarly aged primary infections.

Site of attachment by larvae

The position in which the early fourth stage larvae were found completely refute Chandler's contention (1935) that the larvae settle in a more posterior position to that taken up by the adults. If Chandler was referring to the very special case of the movement of some of the adults to the duodenum at the end of infection he had no right to make his statement without strongly qualifying it. It is odd that he did not publish the results of his examination of the worm distribution in more detail. This work has shown unequivocally that the larval

Nippostrongylus brasiliensis worms settle immediately after arrival in the small intestine from the lungs in those sections which are later found to be most heavily parasitized by adult worms.

Secondary infections.

The results obtained from secondary infections show that there are changes in the number of worms recovered, the timing of the intestinal phase, the distribution within the intestine and the sex ratio of the worms.

Experiment 6 showed that the peak of the intestinal phase was passed by the ninth day after infection, and that the number of worms recovered was very much lower than in a primary infection of similar age. It showed that the worms were distributed in a similar way to the breakdown phase of a primary infection (i.e. Days 13-18), but that the sex ratio of the worms was not the same was in a primary infection either during the breakdown phase or when the worms were of a similar age. About 70% of the adults in a 15 day old primary infection would be males, the proportion during the first nine days would be 45%. During the first nine days of the secondary infections the males made up less than 30% of the adults. In Experiment 7, in which the immunizing dose had been very large, most rats were harbouring very few worms when examined six days after the secondary dose had been given. Four rats had large populations, however, and in these the distributions were displaced in a similar way to that seen in Experiment 6. The male worms made up 26% of the total adults found.

As most worms had already disappeared by nine days after a secondary infection, those that were left were those that were able

to establish themselves more securely. This presumption ignores that in resistant rats larvae take longer to pass through the skin and lungs and that even after Day 9 some of these stragglers will be passing through the intestine. By and large it is a workable hypothesis. In Experiment 8 rats were killed on the eleventh day so that the vast mass of worms which fail to establish would have already passed and would not mask the finding of the sites where the worms were more firmly established. In this experiment both the primary and secondary doses varied, but no effect was noticed between primary doses of 100 and 2500 larvae; and the number of worms recovered from most of the secondary infections was similar to that expected from the original primary infection. Males made up 21% of the adult worms found in rats with secondary infections. In those rats which had larger populations than usual the number of worms in the most anterior sections was the same as in primary infections, the extra worms being in more posterior sections and in one case the majority of the worms were in the posterior half of the small intestine. In Sections 1, 2 and 3, 28% of the adults were male in the secondary infections whilst 50% were male with similarly aged primary control infections.

The finding of similar sized populations of worms in Sections 1, 2 and 3 in old primary and in secondary infections suggests that other factors than the number of worms presenting themselves are involved in determining the number which parasitize this region. There can be no doubt that worms of the secondary infection try to parasitize these sections. The changes in the ratio of males to

females show that at least some females derived from the secondary infection move into these sections and at the same time some males from the primary infection move out. However, against the hypothesis that there are only a limited number of sites in the anterior sections it must be remembered that before Day 12 in primary infections and occasionally in secondary infections (e.g. Rat 3, Experiment 7) very many more worms can be found in these sections.

The evidence does not allow a satisfactory hypothesis to be formulated to explain the increasing predominance of males in ageing primary infections whilst in secondary infections, even when as long-standing as eleven days, males are very much in the minority.

Transferred stages

In Experiments 9 and 10 young stages of worms which had undergone the early part of their parasitic life in donor rats were transferred to recipient rats so that the phase of migration through the tissues and lungs could be avoided. Thus in all the rats the immunological phenomena will have resulted from the passage of materials from and to the lumen of the digestive tract.

The results showed that there was no distinct advantage to be gained by using either late lung stages or early intestinal stages of the worm. The number of worms arising from these stages which were recovered at post mortem of the recipients was disappointingly small. The first finding of interest was that in a primary infection with transferred stages the worms were found very much further from the pyloric end of the small intestine than in a normal primary infection. In a normal primary infection worms are beginning to be found in the usual

sites in 24 hours. The different position of the transferred stages could be because the passage of the larvae through the skin and lungs can result in a change in the usual sites to make them receptive, even in less than 24 hours after dosing or it could be because the worms that are transferred are different from normal worms. The latter alternative, implying some damage to the worms during the transfer technique, is the more likely.

In spite of there being evidence that the worms have been affected during the transfer the results also indicate that a primary infection by transferred stages alone is capable of conferring some immunity on the recipient rat, as is shown when they are challenged with normal larvae. The evidence also shows that the intestinal phase is susceptible to the acquired resistance of the recipient and that it is not necessary for the worms to have been through the skin and lungs and subject to the host's tissue reactions in these organs for them to be rejected from the intestine.

Sex of host

Hunter and Leigh (1961a) state that they observed a sex difference in the L.D. 50 (Dose which kills 50% of rats at risk) which was independent of weight. Unfortunately these authors have not published any detailed data on the weight-dose-death incidence in the two sexes of rats. They state that the L.D. 50 for male rats is 12,300 and for female rats 9,700 infective larvae. In the description of their experimental procedure they give the range of weights of the male rats to be 230-370 grams and of female rats to be 170-300 grams. If the lethal dose is divided by the centre point of these ranges, which is the only thing that can be done with the data given, the L.D. 50 can be expressed as a number of

infective larvae per gram of live weight. The figures so obtained are: L.D. 50 (male rats) 41.0 worms/gram; L.D. 50 (female rats) 41.3 worms/gram. There can hardly be any justification for claiming a sex difference on this basis.

In Experiments 4 and 5 significant differences between the numbers of worms recovered from the two sexes of host were not evident. The evidence from the two experiments is that the mean positions were 0.7 and 1.0 of a section length further from the pyloric sphincter in female rats than in male rats. The mode positions were correspondingly different. Haley (1958b) notes that there is no difference in the number of worms harboured by the two sexes of rats at 1-2 months of age. There does not appear to be any further evidence available on the effect of the sex of the host in rats. However, several papers have been published on the effects of the sex of abnormal hosts: mouse, hamster and guinea pig. Neafie and Haley (1962) detected a difference in the number of worms recovered from the two sexes of mice after puberty, the females harbouring just over half the average number found in the male mice. Haley (1954, 1957, 1958a, 1958b, 1961b) has studied the effect of sex in hamsters on the number of worms harboured. His results can be summarized as saying that male hamsters harbour 25 times the number of worms harboured by female hamsters. Parker (1961) broke down the resistance of guinea-pigs to this worm by giving cortisone. He found that male guinea pigs harboured more worms than female guinea-pigs.

It would be interesting to know if the biochemistry of the small intestine of the two sexes also shows a similar difference. If this was to be true it might be possible to study the factors determining

the site of a worm's attachment more fully and it is to be hoped that the technique of Haruspication will allow a more detailed and localized study of the biology of the small intestine by disciplines other than helminthology.

Peyer's Patches

Figures 30 and 31 show that the distribution of Peyer's patches in the small intestine of rats follows a pattern which is similar in both sexes and does not change with differences in the host's experience of worms. The number recorded in each rat was 18.7 $\pm$ 3.1. The most frequent site of a patch was close to the short mesentery, that is near the duodenal-jejunal boundary. The number occurring in each section fell slowly from this point to the ileocaecal valve. There is a suggestion that the number increased slightly around Section 12.

The presence of Peyer's patches did not have an effect on the positions occupied by the worms, and the differences in worm distribution in the two sexes of host were not parallelled by differences in the distribution of Peyer's patches.

SUMMARY OF FINDINGS IN THIS THESIS.

SUMMARY OF FINDINGS IN THIS THESIS.

The reaction of the small intestine of the laboratory rat to its nematode parasite Nippostrongylus brasiliensis was studied to find out if this organ behaved as a single unit, reflecting a generalized immune state in the rat, or whether it showed localized changes. The actual study was concentrated on the patterns of distribution of the worms along the length of the small intestine in rats which had had various experimental treatments with the parasite.

The findings of preliminary experiments designed to establish suitable techniques for this study showed that:

i. saturated aqueous solution of sodium chloride is a suitable flotation medium for counting the eggs of this parasite.

ii. twelve hours is a reasonable approximation of the transit time of digesta from the small intestine to the anus.

iii. the small intestine can be divided into proportional parts in a consistent manner if it is placed in saline at 37°C containing 1/500,000 Adrenaline and if a tension of 5 grams is applied. A routine was developed in which the small intestine was divided into twenty parts of equal length. These were designated in consecutive order from the pyloric sphincter to the ileo-caecal valve as Sections 1 to 20.

The principal findings obtained from experiments in which rats had had primary infections were that:

i. eggs were not laid until the fifth day after infection, and that by the ninth day each female worm was laying on average 670 eggs per day. After the ninth day the rate of egg laying declined until

it had reached zero by the fifteenth day.

ii. larvae were found in the small intestine after twenty-four hours. Very few larvae arrived in the intestine after five days. During the first five days larvae were found to be distributed mostly between Sections 4 and 10 (Mode 4.5 [Section 5]; Mean 6.1; Standard Deviation 2.4).

iii. the numbers of worms in each section of the small intestine before the 12th day after infection could not be fitted to Normal, Binomial or Poisson distributions. The distribution between sections showed skewness and positive kurtosis.

iv. the mode position, arrived at on the assumption that all the worms in a section were at the mid point, was considered to be the statistic with most meaning.

v. the worm distribution was found to change as the infection got older and could be conveniently divided into phases of six days each.

Phase 1 (1-6 days); the worms were compactly distributed around Section 6 with no worms in Section 1 and few more posterior than Section 10.

Phase 2 (7-12 days); the worms were still in a compact distribution around Section 6 but had begun to spread out, both towards the anterior and posterior ends of the small intestine.

Phase 3 (13-18 days); the centre of population had moved nearer to the pyloric sphincter, but a large proportion of worms were found in the posterior half of the small intestine.

Phase 4 (19 days onwards); the few survivors are concentrated in Sections 1 and 2.

vi. Section 1 had no worms and Section 2 few worms during Phase 1. During Phase 2 these sections had their peak infection, but in subsequent phases the worm numbers did not dwindle to zero, as happened in the other sections.

vii. the two sexes of worms were not distributed in the same way. Throughout infection the proportion of males to females fell as the distance from the pyloric sphincter increased. However, as the infection grew older there was a relative increase in males all along the small intestine; this increase was more marked in the most anterior sections than in the others.

viii. between the ranges of 1000 and 6250 larvae there was no evidence that the size of the infective dose had any effect on the distribution of the worms, both the mode position and the scatter being almost the same in the three groups. However, proportionately more of the larger dose had reached the small intestine by 6 days than of the other doses.

The experiments on rats which had had two infections showed that:

i. the intestinal stages passed through the process of attachment and rejection more quickly than in primary infections. The number of worms being recovered at 6 days was greatly in excess of those recovered at 9 days.

ii. the distribution of worms in secondary infections did not pass through similar phases to those seen in primary infections. During the period when most worms were recovered they were distributed in a manner most similar to Phase 3 of a primary infection.

iii. relatively more female worms were recovered from rats with secondary infections (up to 80%). This applied to Sections 1 and 2

as well as to the more posterior sections.

iv. after high challenge doses populations of established worms were found at six days, but by 11 days few worms remained. Low primary and secondary doses resulted in some worms remaining at 11 days.

v. Sections 1 and 2 were parasitised at all stages of secondary infections though the number of worms in these sections seldom rose above 30. However, in secondary infections a high proportion of the worms were females, unlike in the later stages of primary infections when male worms were predominant.

vi. in general, secondary infections produced few eggs. However, at six days eggs were produced in rats which had had large secondary infections compared to the primary infection.

Experiments in which worms that had just reached the intestinal stage were transferred to recipient rats as primary and/or secondary infections showed that:

i. transferred late lung or early intestinal stages were equally suitable, though only about 7% of the worms became established.

ii. primary infections of transferred stages were found in a more posterior position in the small intestine than normal primary infections of the same age (Mode Section 10 and 7).

iii. primary infections of transferred stages can result in the rat becoming resistant to secondary infections.

iv. resistant rats are able to reject secondary infections of transferred stages.

The experiment designed to show if rats could be induced to become tolerant of N. brasiliensis did not provide conclusive results, probably

because the size of challenge dose was too small.

An apparent difference in the distribution of worms in the two sexes of host was seen. The population centre in female rats being about one section more posterior than in males. By comparing the position of the centres of population with the weights of pairs of rats of the same sex which had had identical treatments it was found that the differences in weights of the two sexes were not responsible for this phenomenon. It is presumed that there is a true sex difference in the way a host is parasitized. No difference was found in the total numbers of worms recovered from each sex.

The distribution of Peyer's patches was examined in a large number of rats. No sex differences were found, and there was no evidence that Peyer's patches directly influenced the pattern of worm distribution.

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