LABELLED METABOLITES IN THE STUDY OF IMMUNE EXPULSION OF *Nippostrongylus brasiliensis*

A Thesis

Submitted for

The Degree of Master of Science

in

The Faculty of Science

of

The University of Glasgow

by

John MacPhee Maclean, M.I.Biol.

Department of Veterinary Physiology

University of Glasgow.

July, 1977.

ACKNOWLEDGEMENTS

I wish to express my grateful appreciation for the assistance received in the course of this study.

I am particularly indebted to Professor W. Mulligan, under whose supervision the work was carried out, for the advice and encouragement so freely given.

I also thank Mr. R.W. Henney for his collaboration in the experiments described in Chapter I.

Finally I express my thanks to Mr. A. Finnie and his staff for the reproduction of the graphical illustrations contained in the thesis and to Mrs. D.J. McGruer for preparation of the text.

CONTENTS

SUMMARY

One of the features of Nippostrongylus brasiliensis infections in the rat is the sudden expulsion of the worm population commencing at about day 11 of infection, the rats subsequently being highly resistant to further infection.

It has been known for some time that the parasites depend for their nutrition on the uptake of metabolites from the tissue fluids of the host. It was thought that measurements of the uptake of metabolites by the adult N. brasiliensis from the host's tissue fluids might provide an index of immunological damage sustained by the parasites.

A method using metabolites trace labelled with radio-isotopes was employed. The substances used were ^{32}P - sodium phosphate, ^{75}Se- selenomethionine and ^{14}C- glucose. The labelled materials were injected intravenously into the host and the levels of radioactivity in the worms at necropsy, five hours later, were ascertained. Such measurements do not of course represent "net" uptakes of labelled material but allow changes in the rate of transfer of labelled metabolites between the adult N. brasiliensis and the tissue fluids of the host to be measured. The relationship between these changes (or metabolite uptake), other changes known to occur in the parasites prior to their expulsion and the timing of the immune expulsion was examined.

In the initial experiment described in Chapter I, it was observed that the rate of uptake of ^{32}P from the host, following injection with ^{32}P labelled phosphate, appeared to be related to the age of the infection, i.e. there was a greater uptake of

^{32}P in a day 6 compared to a day 9 infection. Following this interesting observation the uptake of ^{32}P by the parasites was measured between day 6 and 13 of infection. After day 8 there was a rapid decline in the uptake of ^{32}P by the worms, the significant point being that the decline in worm metabolic activity as measured in this way preceded worm expulsion by several days.

It is known that worms transferred surgically to "clean" hosts on or before day 9 of infection are able to re-establish and continue normal reproductive activity but parasites transplanted after this time are less able to establish and resume normal functions. In order to investigate whether or not "transplanted" worms were able to recover their original capacity to take up metabolites a series of measurements were made in worms which had been surgically transferred to "clean" hosts between days 6 and 13 of infection. The results of this experiment indicated that worms transplanted after day 9 of infection were progressively less able to recover their ability to take up the labelled metabolite, presumably as a result of irreversible immune damage sustained in the first host.

It is well established that if certain cortisone derivatives are administered to N. brasiliensis infected rats worm expulsion is prevented. When a series of measurements of uptake of ^{32}P by parasites from host rats which had been injected with cortisone were made it was obvious that the ability of the worms to take up labelled metabolites was unimpaired, i.e. the reduced uptake

of labelled metabolites observed in N. brasiliensis as an infection progresses appears to be related to the immune response of the host which also causes the expulsion of the parasites.

In case the results obtained up to this point were peculiar to phosphate metabolism only, a series of measurements were carried out with a labelled amino acid (^{75}Se- selenomethionine). The pattern of uptake of ^{75}Se by the worms bore a remarkable similarity to that previously observed when ^{32}P-sodium phosphate was used as the test metabolite. It seemed likely that the impairment in the uptake by the worms of both phosphate and amino acid, as an infection progressed, was related to developing immunity in the host.

Chapter II described improvements in the procedures used for measuring the uptake of radioisotopically labelled metabolites by N. brasiliensis and the tests carried out to confirm the validity of the techniques.

Using these improved methods, the uptake of metabolites by N. brasiliensis was measured in two experiments described in Chapter III. Where a mixture of ^{32}P-sodium phosphate and ^{75}Se-selenomethionine was injected into the host it was shown that the timing and magnitude of the depression of each metabolite was remarkably similar as immunity developed in a normal infection. The uptake of ^{14}C-glucose was similarly measured and the pattern of ^{14}C uptake observed corresponded closely to that seen when the uptakes of ^{32}P or ^{75}Se was measured. The results of these experiments confirmed that there was a dramatic fall in worm

metabolic activity after day 8 of infection. Since the substances used in these studies, inorganic phosphate, an amino acid and glucose, are involved in different aspects of metabolism and the uptake of each is similarly affected it seems probable that the parasites suffer some general damage which renders them unable to take up metabolites from the host rather than that there is interference with specific metabolic processes. The results give no indication as to how this damage occurs. It could be the result of antibody damage to the worms which affects their general physiology or of a displacement of the worms by the immune reaction of the host to a less suitable intestinal environment, where the diffusion path for metabolites could be somewhat lengthened.

The results of the experiments described in Chapter III appeared to confirm the view that as an infection of N. brasiliensis progresses the increasing immune pressure exerted by the host is responsible for the reduction in the uptake of labelled metabolites. In order to confirm that this is so and that senescence in the parasites is not the cause of the reduced metabolic activity, a series of experiments were carried out (Chapter IV) where the uptakes of labelled metabolites by N. brasiliensis were measured

(a) in second infections where worm expulsion occurs at a much earlier time of infection, and

(b) where the onset of "self-cure" was delayed by a concurrent infection of Trypanosoma brucei and also where the host rats were irradiated prior to infecting with N. brasiliensis in order to delay the worm expulsion.

The same relationship was observed in each case between the onset of worm expulsion and the depression in uptake of labelled metabolites by the parasites.

A study of the results of the experiments described in the study led to the conclusion that measurements of the uptake of labelled metabolites by the worms carried out as described does provide an index of worm damage and that this phenomenon is one of several integrated changes occurring in worm function and structure caused by developing host immunity.

GENERAL INTRODUCTION

The life cycle of the nematode Nippostrongylus brasiliensis was first described by Yokagawa[1]. The parasite occurs naturally in the intestine of the rat and the mouse, the natural hosts being Rattus norvegicus, Rattus rattus and Mus muscularis[2] and the infections can easily be established in experimental rats and mice.

A feature of Nippostrongylus brasiliensis infections in the rat is the sudden expulsion of the worm population starting at about day 11 of a primary infection. This phenomenon has come to be known as the "self-cure" reaction and may be described as the elimination of a burden of parasites by the immune response of the host. The term "self-cure" was first used by Stoll[3] to describe sudden dramatic falls in faecal egg counts in lambs infected with Haemonchus contortus and he assumed this to be due to the expulsion of the worm burden. In Nippostrongylus brasiliensis infections the "self-cure" reaction was first described by Africa[4] and Taliaferro and Sarles[5] demonstrated that the loss of parasite burden was not the result of ageing of the worms but of an immune reaction in the host.

Details of the parasitic and the preparasitic stages of N. brasiliensis and a review of the relevant literature was given by Haley[6]. The eggs are passed in the faeces and hatch out in about 24 hours. By day four after hatching two larval moults have taken place to give third stage (infective) larvae. The infective larvae penetrate the skin of the host (experimental

infections are conveniently produced by subcutaneous injection) and after spending several hours in the hypodermis the larvae migrate, probably via the blood and lymph, to the lungs (12 - 15 hours post infection). There is still speculation about the exact route of migration to the lungs (see review[7]).

The larvae grow in the lungs (18 - 32 hours), then moult to give fourth stage larvae and sex differentiation takes place (32 - 46 hours). The fourth stage larvae migrate up the bronchi and the trachea then via the oesophagus and the stomach to the small intestine (50 - 60 hours) where rapid growth takes place. The final moult occurs at about 100 hours after infection and the adult nematode lives in the anterior portion of the small intestine. Eggs appear at day six of the infection and egg production rises rapidly to reach a plateau, usually from day eight to ten, after which a rapid fall sets in to give very low counts by day 13 and zero by day 20. In parallel with this the worm population falls dramatically after day 12 or 13. This is the classic "self-cure" pattern. The timing of the changes observed in the "self-cure" reaction can vary by one or two days depending on the sex or strain of the host rats.

The immune expulsion is a phenomenon which occurs in many host/parasite systems but it is seen at its most dramatic in rats infected with Nippostrongylus brasiliensis. In many ways this is a good system for laboratory study. The experimental techniques are relatively simple and the prepatent period is short, thus minimising the problems involved in producing adequate supplies of animals and infective materials. Rats infected with N. brasiliensis

have been used as a model in many studies on the mechanisms of immunity to gastrointestinal helminths. A good deal of information has been gleaned on the kinetics of expulsion, related changes in the small intestine of the host and on alterations to worms living in immune rats, but the exact mechanism of the immune expulsion of the worms has not yet been completely elucidated (see reviews [7 - 13]).

Investigations into the immune response have led to much speculation as to the possible factors leading to the expulsion of the worms. The role of humoral antibody in the sequence of events has been investigated. Passive immunity was demonstrated by the transfer of immune serum followed by larval challenge [14, 15] and it was further shown that where adult parasites were transferred to rats which had been passively immunised with hyperimmune serum the expulsion was accelerated [16, 17].

However the latter work showed that relatively large quantities of hyperimmune serum are required to produce evidence of immunity and the efficacy of different batches of immune and hyperimmune serum proved to be very variable. It has also been shown that hyperimmune serum has little or no effect on adult worms <u>in vitro</u>. This accumulated evidence tends to suggest that the presence of circulating antibody alone is insufficient to cause worm expulsion and that some other factor or combination of factors is required to produce the immune expulsion.

In speculating as to how the circulating antibodies might come into close contact with the worms in sufficient quantity to cause immunological damage it is interesting to look at some of

the investigations into the feeding activities of the worms. The report by Taliaferro and Sarles[18] that the gut of the adult worms contained red blood cells led to the assumption that the parasites feed on host blood and tissue. However Rogers[19], and Davenport[20] showed that the characteristic red colour of the adult worms was due to the presence of endogenous haemoglobin which was quite distinctive from that of the host and it was demonstrated[16, 21] that the worms did not feed by sucking blood from the host nor did their feeding activities cause any significant loss of blood across the damaged host intestine. Rogers and Lazarus [22], suggested that the worms feed on host tissue. There is still some debate about the feeding activities of the worms but it now seems probable that the sole source of nutrient is the digestion and absorption via the gut of damaged host gut cell contents and debris (see review[7]). If this is so, it appears that the normal feeding activities of the worms do not provide the substantial transport for humoral antibody.

In a primary infection of N. brasiliensis the sudden termination of the worm population [4, 16] gave rise to the theory that the worm expulsion might involve an anaphylactic reaction in the gut. However when an anaphylactic reaction was produced in the gut of infected sensitised rats using ovalbumin as antigen, the resulting shock alone did not expel the worms [23, 24], although it was shown by Barth and colleagues [23] that if immune serum was administered before the ovalbumin shock the expulsion of the worms occurred though the immune serum alone had had little effect.

These findings supported the hypothesis that the expulsion of the worms was the result of a two stage process [16, 25]. This hypothesis suggested that the first stage, a local anaphylactic reaction, caused an increased capillary permeability which then allowed significant amounts of anti-worm antibody to leak into the gut and exert a direct action on the parasites. Maclean [26] showed that in a primary infection of N. brasiliensis the macromolecular leak into the intestine was relatively massive and reached a peak between days 10 and 12 of the infection, i.e. corresponding in time to the worm expulsion. No suggestion was made about the mechanism of the second stage, i.e. the direct action of antibody on the parasites leading to expulsion.

Probably a disproportionate amount of work has been carried out in studying the changes occurring in the host at the expense of the study of functional changes in the parasite. As yet, the mode of action of humoral and cellular elements on the parasite has not been fully elucidated. Following investigations into structural and other changes in the worms during the course of an infection the hypothesis as presented above has lost some credence. Investigations into changes taking place in the parasite [27, 28] showed that structural changes had taken place in the worms by about day 10 of infection, i.e. before the macromolecular leakwas maximal. The suggestion was made that the immunological damage might precede the macromolecular leak (see review [7]). The actual termination of the infection may involve the expulsion of worms which have been irreversibly damaged by immunological action.

That there are two steps in the termination of a worm population is generally agreed. The presence of IgE antibodies

in immune serum was demonstrated by Ogilvie [29], but it was subsequently shown [30] that IgE alone will not expel worms from neonates or lactating rats and Jones and her co-workers [31] found that rats could be passively protected by antiserum free of detectable IgE. It thus appeared that the action of antibodies alone was not sufficient for worm expulsion although it was later shown that in some circumstances the action of antibodies can cause a gradual loss of worms in neonates or in heavily infected lactating rats (see review [13]). Edwards and co-workers [32] suggested that the worms are attacked by circulating antibodies which produce both the structural changes already referred to and changes in acetylcholinesterase. The suggestion has been made that acetylcholinesterase may have a "biological holdfast" action which allows the worms to maintain their position in the villi of the intestinal mucosa and that anti-acetylcholinesterase antibodies act to interfere with the "holdfast" action forcing the worms towards the lumen of the gut where the environment is less favourable (see review [13]). However this action, if it occurs, is still not sufficient to cause worm expulsion and a further step is necessary for expulsion. The theory was put forward by Jones and Ogilvie [33] that amine release from mast cells acted on the damaged worms to cause expulsion.

Recent evidence has been produced which points to the sequence involved in worm expulsion being that the worms are first damaged but not expelled by circulating antibodies and that the action of cells must follow in order to produce expulsion,

the cells responsible being lymphocytes (see review [13]). The mode of action of the lymphocytes remains a matter for speculation but it was thought that the lymphocytes might exert their action on the worms by collaborating with cells derived from bone marrow. It has been shown [34] that antibody damaged worms can be expelled from the intestines of irradiated rats without the presence of marrow cells, i.e. the lymphocytes alone caused the expulsion, but it is still not known how these cells trigger off the expulsion.

Although the published literature contains a great deal of information on changes in the small intestine of the host associated with the "self-cure" reaction and on changes in worm infectivity, reproduction and morphology resulting from the host's immunity little attempt has been made to investigate functional changes in the worm which might precede or coincide with worm expulsion.

The experimental work in this thesis represents an attempt to study changes in worm physiology during the development of immunity in the host, the index of function chosen being the uptake of labelled metabolites from the tissue fluid of the host.

Nippostrongylus brasiliensis depends for its nutrition on the uptake of metabolites from the host's tissue fluids [22]. Chandler [35] advanced the theory that the nutrition of the parasite was adversely affected by the immune response of the host, possibly due to the action of anti-enzymes developed by the host inhibiting enzymes which enable the nematode to digest and assimilate host tissue. Thorsen [36] produced results which support this theory.

Although the above theories have been expounded, no experimental evidence showing alterations in parasite nutrition during the course of an infection has been produced. It appeared that this was an aspect of the immune response in N. brasiliensis infections which could usefully be investigated and which might give further evidence as to how the immune response of the host caused disfunction in the parasite. One approach to the problem was to look for a method which would allow the uptake of metabolites by the parasite, from the host, to be measured.

The development and use of such a method is described in Chapter I, where the uptake of the radioactively labelled substances ^{32}P sodium phosphate and ^{75}Se l-selenomethionine by the parasites is measured and in Chapter II procedures initially used are further developed and improved.

In Chapters III and IV experiments are described where, using these improved techniques, the uptake of labelled metabolites by the parasite from the host is measured in a number of different circumstances with a view to establishing that there is a relationship between depression of metabolic activity of the worm, as measured in this way, and immune expulsion.

It should perhaps be pointed out that the uptake of labelled substances by the parasite in these experiments can be regarded as only a very crude index of nutritional/metabolic activity. The labelled metabolite is introduced into the host's tissue fluids and the rate of its appearance in the parasite will depend on the ease of equilibrium of that particular substance between

host and parasite tissue fluids. In many cases this will depend on "active transport" systems of the parasite and hence reflect its general metabolic activity. The measurements described in this thesis cannot be equated with net transfer of substances between host and parasite.

GENERAL MATERIALS AND METHODS

A. EXPERIMENTAL ANIMALS

Helminth-free Hooded Lister rats (Animal Suppliers, London, Ltd.) weighing approximately 120 - 150 gm were used in all of the experiments.

The rats were housed in polypropylene cages with wire mesh floors suspended above sawdust trays.

B. PARASITOLOGICAL TECHNIQUES

(i) Culture of Infective Larvae

The culture of *N. brasiliensis* larvae was as described by Jennings and colleagues [37].

Faeces were collected from rats with a patent infection, usually over the period day 7 - 9 of an infection. The faecal pellets were made into a paste with a little warm water and a portion was spread on a circle of filter paper (Whatman No. 1) 7 cm in diameter, the faecal smear being about 3 cm in diameter, care being taken to leave the periphery of the paper clear. The paper was then dipped in water and placed on a foam rubber pad, which had previously been soaked in water, in a plastic petri dish. After replacing the lids the dishes were stored in a humid incubator at 27°C.

After 5 days the larvae can be seen collecting in a fringe round the periphery of the filter paper. The larvae were always harvested between 5 and 10 days after setting up the culture and were used to infect rats on the same day. Harvesting of the larvae was accomplished by flooding the petri

dish with water at 35°C, thus allowing the larvae to swim off into the water. The warm water containing the larvae was collected and filtered under suction in a large Buchner funnel using Green's No. 904 filter paper, 18.5 cm in diameter. This paper was then inverted and placed on an Endecott sieve (mesh 400) in a Baermann apparatus filled with water at 35°C. The larvae swam down the sieve while the faecal debris was retained. The larvae collected at the bottom of the funnel and were run off into a suitable container.

Counts were made by a dilution technique. A 1.0 ml sample was diluted to 100 ml with water and 0.1 ml samples (usually about 10) of this suspension, which had been well mixed were placed on a slide and the larvae counted using a binocular microscope.

(ii) Larval Count

The larval suspension was diluted to 100 or 200 ml, depending on the concentration, with water at 37°C. The dilute suspension was thoroughly mixed and a further 1.0 ml aliquot of this was diluted to 100 ml with water at 37°C. 0.1 ml volumes of the final suspension (usually about 10) were counted under a dissecting microscope, care always being taken to ensure that the larvae were thoroughly mixed before removing the aliquots to be counted. The number of larvae in the original suspension was then calculated and the volume of that suspension adjusted so that 1.0 ml contained the required dose for infecting purposes.

(iii) Infection of Rats with Larvae

The rats, while lightly anaesthetised, were injected in the groin using a 1.0 ml syringe. The level of infection in most of

the experiments was approximately 5,000 larvae per rat. The error involved in the counting procedure is about 10%.

(iv) Maintenance of Parasite

The parasite used in the experiments was maintained in the department of Veterinary Physiology by repeated subcutaneous inoculation of N. brasiliensis larvae in female Hooded Lister rats.

(v) Faecal Egg Counts

A 1.0 gm random sample of faeces was gathered from a 24 hour collection of faeces. This sample was homogenised in 10.0 ml of water, then passed through a 50 mesh sieve. After centrifuging at 1,500 r.p.m. the residue was resuspended in 15 ml of saturated NaCl solution. Both chambers of a McMaster slide were filled with the suspension and the eggs counted. Each chamber has a volume of 0.15 ml. The mean number for the chamber X 100 gives the number of eggs per gramme of faeces.

(vi) Recovery of Worms

All rats were killed by Trilene (I.C.I. Ltd., England) anaesthesia followed by a blow to the head. The skin and abdominal wall were slit along the mid-ventral line and the entire small intestine removed. The first two-thirds was slit longitudinally with blunt scissors, cut into 4 inch lengths and placed in a muslin bag suspended in a 250 ml beaker filled with saline at 37°C. This was then placed in a water bath at 37°C for 1 hour, by which time most of the worms had swum out of the bag and collected at the bottom of the beaker.

(vii) Counting of Worms

Worms collected from a rat as described above were transferred to a Petri dish and counted under a dissecting microscope.

(viii) Collection and Preparation of Worms for Radioactivity Assay

Worms were recovered from the rats essentially as described above, except that the muslin bags containing the worms were incubated in water at 37°C for 30 minutes. (This change was to prevent loss of isotope from the labelled worms during the collecting process.) After 30 minutes most of the water was decanted and the worms were transferred via a wide bore Pasteur pipette to a 250 ml beaker containing ice cold saline. The worms were washed three times in ice cold saline which killed the worms, thus stopping further metabolism, and allowed the remaining unbound radioactivity to be completely removed before the worms were collected on Whatman No. 50 filter paper in a micro Buchner Funnel. The sample thus surface dried was immediately transferred to a glass coverslip* which had previously been degreased and weighed and the weight of the worm sample was ascertained by weight difference. The sample was allowed to dry overnight under cover to exclude dust and finally transferred to a counting vial and stored at -4°C prior to preparation for radioactivity assay.

---

* In the initial experiments (Chapter I) the worms were transferred to a weighed counting vial and the weight ascertained. The worms were then stored at -4°C prior to radioactivity assay.

C. RADIO-ISOTOPIC TECHNIQUES

(i) Radio-Isotopes

Isotopes used were obtained from the Radiochemical Centre, Ltd., Amersham, England, the ^{32}P labelled sodium phosphate in sterile isotonic solution, the ^{75}Se l-selenomethionine in sterile aqueous solution and the uniformly labelled d-[U - ^{14}C] glucose as a freeze dried solid.

The isotopes were diluted prior to the start of an experiment to the required volume and batches stored at -4°C for daily use.

(ii) Preparation of ^{32}P-Sodium Phosphate for Injection

The ^{32}P labelled sodium phosphate was diluted with isotonic saline to give a concentration of 20 µCi ^{32}P per 1.0 ml.

(iii) Preparation of ^{75}Se l-selenomethionine for Injection

The ^{75}Se l-selenomethionine was diluted and made isotonic to give a concentration of 7 µCi ^{75}Se per 1.0 ml.

(iv) Preparation of ^{14}C Glucose for Injection

The ^{14}C glucose was diluted with isotonic saline to give a concentration of 10 µCi per 1.0 ml.

(v) Injection of Labelled Material

The rat tails were immersed in warm water to dilate the veins. The radio-isotope was injected into a tail vein using a 25G x 5/8 needle, the animals receiving 1.0 ml per 150gm body weight.

(vi) Determination of ^{32}P in Worm Samples

The dried worm samples were transferred from the counting vial to 30 ml micro-kjeldahl digestion flasks. Two ml of conc. H_2SO_4, 100.0 mg catalyst (selenium catalyst tablets, BDH Chemicals Ltd., England) and 2 glass balls were added and the

flasks boiled on a micro-digestion stand till the resulting solution was clear. The flasks were allowed to cool and 1.0 ml of distilled water was added. The solutions were then carefully poured into counting vials and the flasks were washed three times into the vials with 1.0 ml distilled water. The samples were then assayed for radioactivity by the Cerenkov method[38] in a liquid scintillation counter.

(vii) Determination of ^{75}Se in Worm Samples

The counting vials containing the dried weighed worms were assayed for radioactivity in a well type scintillation counter.

(viii) Determination of ^{14}C in Worm Samples

Two ml of tissue solubiliser (Soluene 350, Packard Instruments Ltd.) was added to each weighed sample of worms in the counting vials and incubated in a water bath at $60^{o}C$ for 2 - 4 hours or until solubilisation was complete. Ten ml of scintillator (NE 233, Nuclear Enterprises Ltd.) was added to each vial and the samples were assayed in a liquid scintillation counter.

D. CALCULATION OF RESULTS

(i) Corrections to Observed Count Rates

The natural "Background" radioactivity was measured and subtracted from all observed count rates.

(ii) Quench Corrections

Quench correction factors were applied where necessary. Quenching occurs when ^{32}P is counted by the Cerenkov method[38] and in the counting of ^{14}C in a liquid scintillation counter. In

both cases the Internal Standardisation method was employed [39]. This involves adding a quantity of isotope, which has a count rate approximately ten times that of the average sample count already determined, to each sample (internal standards) and to a number of unquenched solutions containing no isotope (unquenched standards). The correction factors are calculated by relating the count rates of the internal standards to the mean count rate of the unquenched standards.

(iii) Worm Radioactivity

Worm radioactivity was expressed as radioactivity counts per minute per mg "fresh worms".

(iv) Statistical Methods

Deviations from the means are expressed as the Standard Deviation (S.D.) of the means.

Students 't' test was used in the analysis of results and differences considered significant where $p < 0.05$.

CHAPTER I

MEASUREMENT OF UPTAKE OF LABELLED METABOLITES FROM THE HOST BY ADULT *Nippostrongylus brasiliensis*

INTRODUCTION

Substances trace labelled with radioactive isotopes have been successfully used to study a wide range of biological activities, including changes taking place in metabolic processes in disease states in man and animals. It seems reasonable to assume that suitable isotopically labelled substances are available which can be used as markers to monitor accurately any metabolic changes occurring in worms during the course of an infection. Essentially such a method will involve administering a substance to the host and measuring the uptake of this substance by the parasite. Any material used for this purpose must satisfy several criteria. It must not have any adverse effect on the host or the parasite and ideally the substance should be, or behave as closely as possible to, one which is implicated in the metabolic process under study. It must also be possible to measure accurately the amount of radioactive label found in the worms at the end of the period of study.

Rogers and Lazarus [22], demonstrated that N. brasiliensis worms depend for their nutrition on the uptake of metabolites from the host's tissue fluids rather than from digesta in the intestine and that if ^{32}P labelled inorganic phosphate was administered intramuscularly to the rats the radioactive label could be detected in worms recovered from the intestine. These

These workers described a method for measuring the quantity of ^{32}P in worms recovered from the intestines of rats 5 hours after oral administration of ^{32}P sodium phosphate. The method involved assaying the radioactivity in very thin layers of worm tissue which had to be of thickness less than 5 mg/sq cm counting area in order to avoid self-absorption of the radioactivity by the sample. The radioactivity was measured by Geiger tube. In this method the wet weight of worms had been found to be approximately 40 mg/1000 worms and the weight of worms assayed was calculated from the numbers used. This method was obviously time-consuming and liable to a relatively high experimental error, but nevertheless it did provide the basis for a more sophisticated approach to the problem of measuring metabolite uptake in N. brasiliensis worms.

Because of the widespread involvement of phosphorus in nematode metabolic activity [40], it appeared that it would be useful to follow the uptake of ^{32}P by the parasites following the intravenous injection of labelled inorganic phosphate into the host. As mentioned in the general introduction some caution should be exercised in interpreting the results of experiments where the uptake of a radioactive label is measured. While the worms are in contact with the labelled metabolite, a two way movement of the label takes place between the parasite and the host's tissue fluid and therefore the measured "uptake" will not represent the "net" transfer of metabolite from the host to the parasite. Nevertheless, any alteration in uptake of the label should indicate a change in the metabolic activity of the worms

and/or a change in the ease of transfer of the substance concerned between the host and the parasite.

An experiment was designed to see whether any changes took place in the uptake of ^{32}P sodium phosphate by N. brasiliensis worms from the host's tissue fluids during the development of immunity. This was accomplished by injecting intravenously into the host, ^{32}P labelled sodium phosphate and measuring the radioactivity of the worms recovered at a known time after the injection of the labelled phosphate. It appeared that the most efficient way to deliver the labelled phosphate to the intestinal tissues of the host was by intravenous injection. The rapid transport provided by this method allowed the assumption to be made that the metabolite was available to the worms from the time of injection, an important consideration when results from a number of rats have to be compared.

In the initial experiment the relationship between ^{32}P uptake by the parasite from the host's tissue fluids and the time interval after injection was investigated in order to establish a standardised procedure for subsequent experiments. The method was then used to measure the uptake of ^{32}P by adult N. brasiliensis on successive days of a primary infection.

UPTAKE FROM THE HOST'S TISSUE FLUIDS OF ^{32}P BY ADULT *N. brasiliensis*

A. TIME/UPTAKE STUDY

This preliminary experiment was carried out firstly to establish whether or not the method was a practical one, and then to ascertain the relationship between the uptake of the radioactive label and the time interval between injection and autopsy.

MATERIALS AND METHODS

Experimental Animals

Sixteen rats which had each been infected with 4,000 *N. brasiliensis* larvae six days previously and 16 which had been given the same number of larvae from the same culture nine days before were used.

Radio-isotope Injection

An isotonic solution of ^{32}P sodium phosphate was prepared so that it contained 20 μCi ^{32}P per ml. The rat was injected via a tail vein with the required volume of this solution as previously described.

Recovery of Worms and Radioactivity Assay

Groups of four rats were killed in series from 1½ hours to 6 hours after injection. In this experiment the worms from each group which had been washed three times in ice-cold saline

were bulked and transferred to a M6 Geiger-Muller tube* for radioactivity measurement. The worms were suspended in a dilute solution of glycerol to prevent them from sinking to the bottom of the tube* during the assay process. The number of worms in each sample was subsequently determined and the results were expressed as radioactivity per worm.

RESULTS AND DISCUSSION

The results are shown in Fig. 1. The most interesting feature is that the worms in the day 6 infection show a better uptake of isotope than those in the day 9 infection. Four and a half hours after the injection of the labelled material the parasites in the latter group of rats showed an uptake of ^{32}P which was only 37% of that in the day 6 infection. Not only does it appear that the procedures adopted provide the basis of a method for measuring the uptake of metabolite by N. brasiliensis worms per se, but that for some reason the day 9 worms consistently show a lower uptake of label. This suggests that the uptake of ^{32}P by adult N. brasiliensis is not uniform throughout the period of an infection and a study of the uptake by adult N. brasiliensis of ^{32}P during the course of an infection might establish the relationship between worm metabolic activity and age of infection. This study is described in the following experiment.

---

* This technique can be used to assay the level of β radiation in a liquid sample. In experiments described later it was replaced by the Cerenkov method.

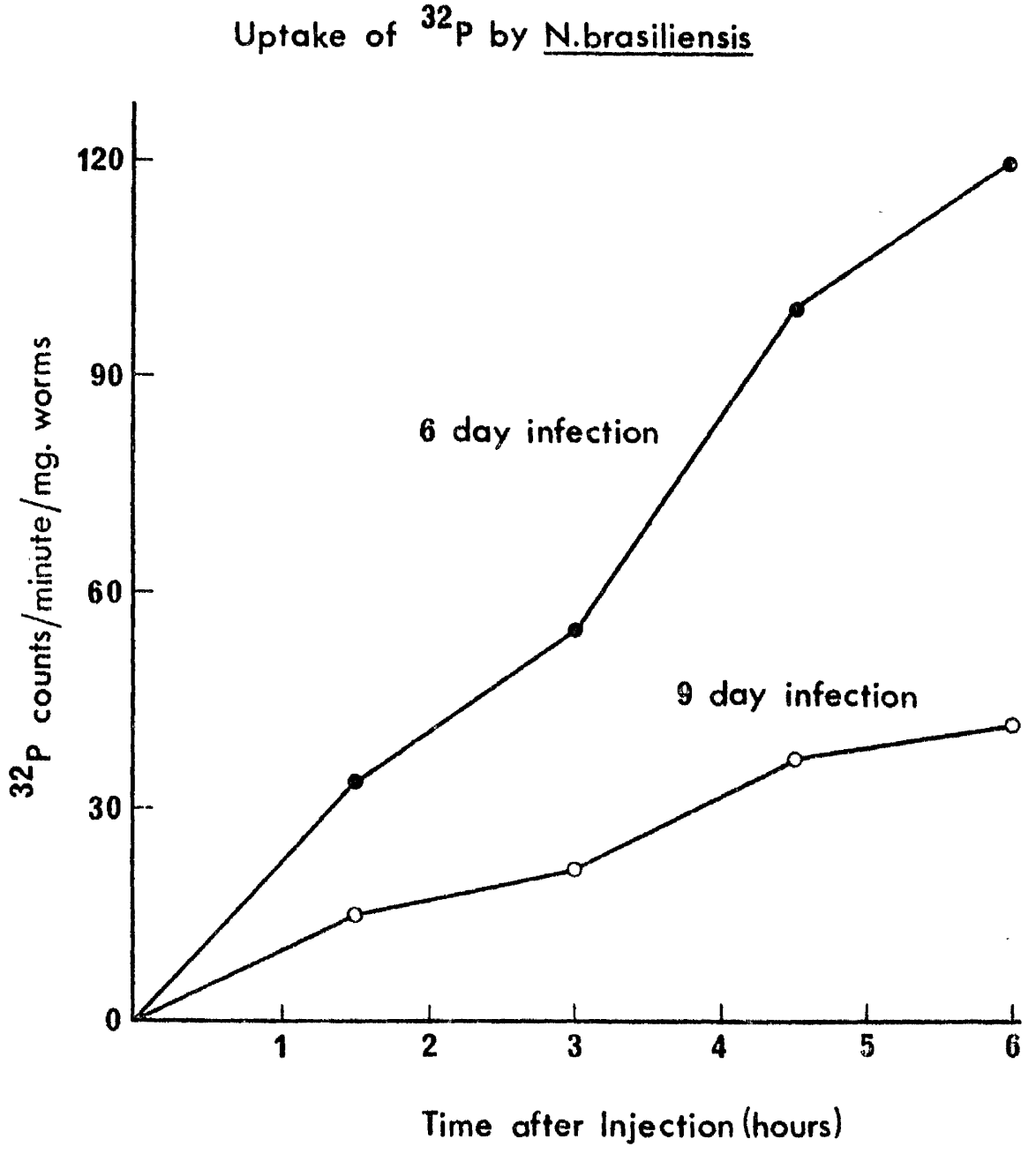

Fig. 1. Uptake of ^{32}P by Adult N. brasiliiensis : A Time Uptake Study

The relationship between uptake of ^{32}P and time after injection of isotope was more or less linear in both groups and for practical reasons it was considered that a five hour interval between injection of the isotope and recovery of the worms would be suitable for all subsequent experiments.

B. UPTAKE OF ^{32}P AT DIFFERENT STAGES OF INFECTION

Employing the techniques used in the previous experiment, the uptake by N. brasiliensis worms of ^{32}P sodium phosphate was measured between days 6 and 13 of a primary infection. The uptake of isotope and the number of worms in each sample were plotted against the day of infection, which allowed the relationship between ^{32}P uptake and worm expulsion to be examined.

MATERIALS AND METHODS

Experimental Animals

Thirty rats which had each been infected with 4,000 larvae were used.

Injection of Radio-isotope

From day 6 till day 13 of the infection (except day 11) groups of four rats were injected with ^{32}P sodium phosphate as in the previous experiment.

Recovery of Worms and Radioactivity Assay

On completion of the ice-cold saline washes the samples were bulked and assayed in a Geiger-Muller tube. The number of

worms in each bulked sample was ascertained and the radioactivity expressed as counts per worm.

RESULTS AND DISCUSSION

The results are shown in Fig. 2. The most striking feature of this experiment is the rapid decline in ^{32}P uptake from day 7 onwards, the day 9 and 10 values being 40% and 10% respectively of the day 7 value. The results are in line with those observed in the previous experiment, where the day 9 value was 37% of that observed on day 6. This is particularly interesting in view of the fact that expulsion of the worms does not begin till day 11 - 12 of an infection and could be regarded as an indication of immunological damage occurring several days before the onset of expulsion.

It seemed important to determine whether the reduced ^{32}P uptake by the parasites from day 7 onwards in the present experiment represents an irreversible change in worm metabolism, a temporary suppression of metabolic activity or simply a reduction in the flux of phosphate from the host's tissue to the worm's. To further investigate the situation, a series of experiments was carried out in which adult worms were transferred from their original host from day 6 onwards ("transplanted worms") and their ^{32}P phosphate uptake measured and compared to that of parasites remaining in the original host ("normal worms"). These are described below.

The procedure used up to the present for assaying the radioactivity of the worms was extremely time consuming and

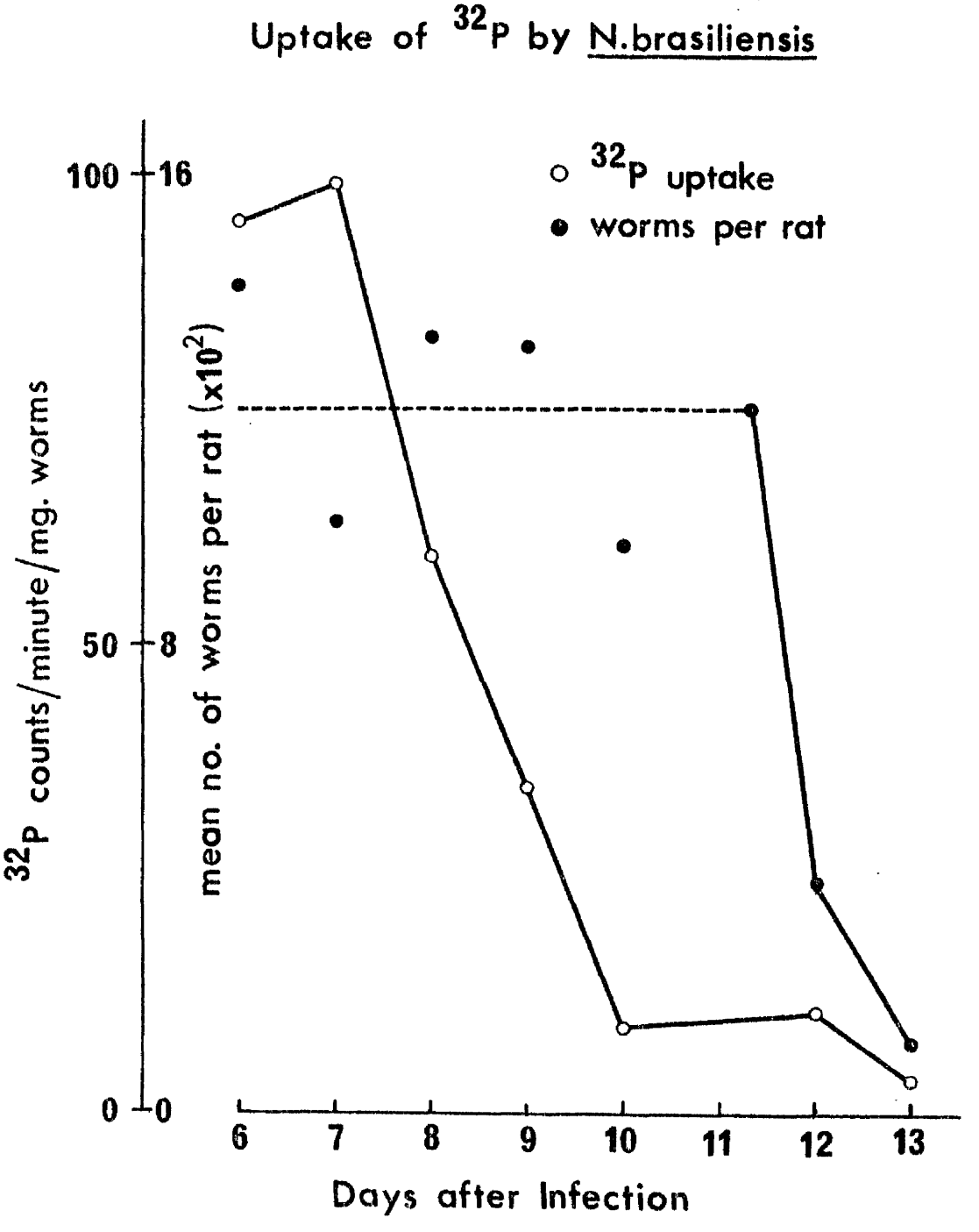

Fig. 2. Uptake of ^{32}P by N. brasiliensis and Mean Worm Burdens per Rat at Different Stages of Infection.

therefore was unsuitable for using with a large number of samples. Counting the number of worms in the samples of recovered worms was also a time consuming process and one which is liable to a relatively high experimental error. The method initially used for assaying the worms for radioactivity and quantifying the worms was therefore abandoned and replaced for all subsequent experiments by the procedures described in the following experiment.

C. UPTAKE OF ^{32}P BY "NORMAL" AND "TRANSPLANTED" WORMS AT DIFFERENT STAGES OF INFECTION

Adult N. brasiliensis were transferred from the host animal at daily intervals between days 6 and 13 of an infection into "clean" rats where the worms would not be under any immunological pressure. In order to allow the transplanted worms to become established in their new environment uptake measurements were delayed until two days after transfer.

MATERIALS AND METHODS

Experimental Animals

One hundred and twenty rats which had each been infected with 4,000 N. brasiliensis larvae were used.

Transfer of Worms

Adult worms were transferred to the small intestine of recipient rats essentially as described by Barth and co-workers [23]. Worms which had been removed from donor rats were examined for

motility before being transferred. The recipient rats were anaesthetised with "Trilene" and the worms were directly injected into the surgically exposed duodenum. The laparatomy incision was closed in two layers with nylon sutures. Worms were transferred into a group of six recipient rats on each day of the experiment.

Injection of Radio-isotope

A group of infected rats was injected from day 6 till 13 of the infection with a solution of ^{32}P sodium phosphate which had been prepared and administered as previously described. Recipient rats were also injected in a similar manner two days after having received the worms.

Collection of Worms

Five hours after injection of the labelled phosphate, the rats were autopsied and the worms were recovered as previously described. On completion of the cold saline washes the worms were transferred by Pasteur pipette to a Whatman No. 50 filter paper in a micro Buchner Funnel. When surface dry, the sample was transferred to a counting vial which had previously been weighed and the sample weight ascertained by weight difference.

Radioactivity Assay

The samples were placed in a liquid scintillation counter and the count rate was determined by the Cerenkov method[38].

RESULTS AND DISCUSSION

The results are shown in Table 1 and Fig. 3. No allowance is made for the fact that the transplanted worms were two days older when the uptake measurements were made. Whether or not this time correction is made it is quite clear that worms transplanted to a normal rat after day 7 show a recovery in ^{32}P uptake. Those in the primary host show the same pattern of decline as in the previous experiment. Worms transplanted on day 8 and 9 show an elevated metabolite uptake but after this there is a rapid fall in the uptake of labelled phosphate. This could be an indication that these parasites are unable to recover their original metabolic activity. This suggests that the worms transplanted after day 9 have suffered some irreversible damage which must be the result of their sojourn in the original host since they were not under any immunological "pressure" after transplanting to the "clean" hosts.

These results provide yet further support for the theory that the fall in ^{32}P uptake by the parasites is in some way related to immunological damage. It therefore seemed logical to study a situation where immunological damage was prevented by the administration of cortisone to the host animals and to see how this affected the ^{32}P uptake at different stages of the infection. Such an experiment is described later.

The method employed for determining the radioactivity count rates in this experiment was superior to that originally used. The Cerenkov method is simple to use and a large number of samples can be processed quickly.

Table 1

^{32}P Uptake by "Normal" and "Transplanted" Worms at Different Stages of Infection

Group		Mean counts per min per mg worm							
		Day of Infection							
		6	7	8	9	10	11	12	13
Normal		104.8	110.2	35.4	10.6	4.3	8.4	5.8	9.4
Worms	S.D.	18.4 (6)	51.8 (5)	14.9 (6)	(6)	3.1 (6)	2.5 (6)	1.7 (6)	4.5 (9)
Transplanted		98.6	68.6	152.2	146.4	109.0	114.8	92.6	74.0
Worms	S.D.	80.0 (6)	12.6 (4)	47.2 (6)	28.8 (5)	(3)	21.4 (5)	(3)	(5)

The figures in parenthesis indicate the number of rats in each group.
Where no S.D. is given the analysis was carried out on a bulked sample from the group.

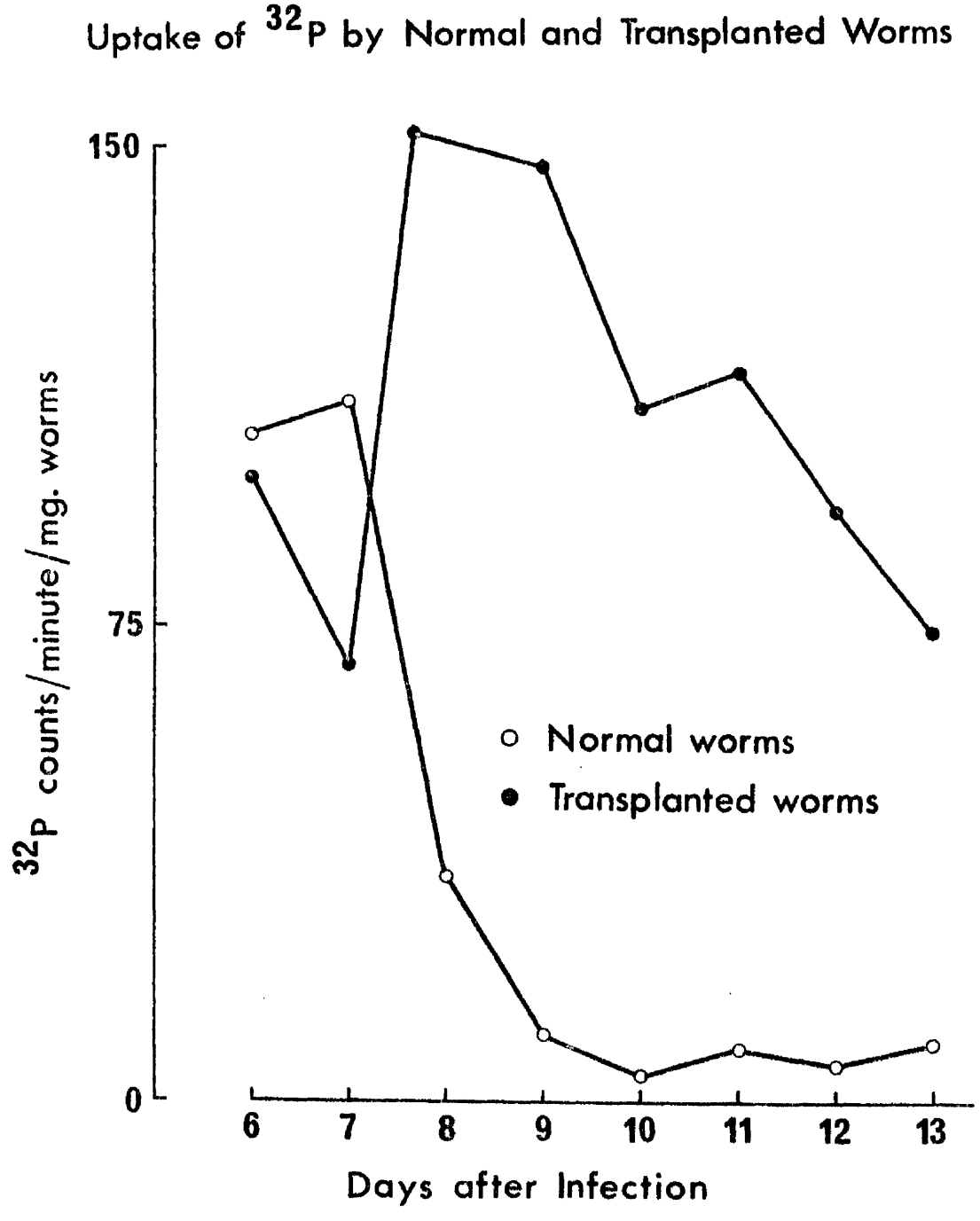
Uptake of ^{32}P by Normal and Transplanted Worms
150
75
0
^{32}P counts/minute/mg. worms
Normal worms
Transplanted worms
6
7
8
9
10
11
12
13
Days after Infection

Fig. 3.

D. UPTAKE OF ^{32}P BY ADULT *N. brasiliensis* IN CORTISONE TREATED AND SHAM-OPERATED RATS

It is well known that if cortisone derivatives are administered to rats infected with *N. brasiliensis*, the immune expulsion of the worms is prevented [41]. It therefore seemed that it would be interesting to study the uptake of ^{32}P labelled phosphate by worms in cortisone treated rats. Post surgical elevation of plasma cortisone has been observed in some species [42] and it is theoretically possible that surgical stress involved in the transfer experiments could, to some extent, modify the response in the new host. To investigate this possibility sham operations were carried out on rats previously infected with *N. brasiliensis* and these were followed by subsequently measuring the uptake of labelled phosphate in the usual way.

MATERIALS AND METHODS

Experimental Animals

Eighty rats which had each been infected with 4,000 *N. brasiliensis* larvae were used. Sham operations were performed on groups of animals on days 7, 8 and 9 and a group were treated with cortisone on days 5, 7 and 9. Metabolite uptake studies were conducted on the "normal" infected group on each day of infection from day 6 to day 13, on the sham operated group on days 9, 10 and 11 and on the cortisone treated group on days 7, 9 and 11.

Injection of Isotope

The isotope was prepared and injected as in the previous experiments.

Recovery of Worms and Radioactivity Assay

The worms were recovered and prepared for radioactivity assay as in the previous experiment. The ^{32}P was measured by the Cerenkov method.

RESULTS AND DISCUSSION

The results are shown in Table 2. In worms from cortisone treated animals the uptake of ^{32}P remained at a high level at day 11 of infection when it had fallen to very low levels in the worms from normal infections. The day 11 uptake in the normal infections was approximately 8.5% of that recorded in the cortisone rats on the same day. The cortisone treatment has clearly prevented the fall in uptake of ^{32}P by the parasites which occurred in the normal infections. Cortisone treatment also prevents the immune expulsion of the worms [41]. This implies that there is a connection between the immune reaction and the depression in uptake of ^{32}P by the parasites as the infection proceeds.

The results of this experiment give very solid evidence for the idea that dramatic fall in ^{32}P uptake by the parasites after day 7 of infection is due to immune pressure on the parasite.

The uptake of ^{32}P by adult N. brasiliensis shows a rapid decline after day 7 of infection, this fall in metabolite uptake

Table 2

^{32}P Uptake by Adult *N. brasiliensis* in "Sham Operated" and Cortisone Treated Rats

Group	Mean counts per min per mg worm Day of Infection							
	6	7	8	9	10	11	12	13
Normal Infection	104.8	110.2	35.4	10.6	4.3	8.4	5.8	9.4
Sham operated				22.0 (4)	17.6 (6)	7.9 (6)		
Cortisone treated		150.8 (4)		154.2 (4)		97.8 (4)		

Figures in parenthesis show the number of rats in each group.
Analyses were carried out on bulked samples from each group.
The mean uptake figures for the normal infection in the previous experiment are included for comparison.

preceding the immune expulsion by several days. Cortisone treatment of the host not only prevents the immune expulsion of the parasites but also enables them to maintain a high level of ^{32}P uptake compared with that recorded in normal infections.

When parasites are transferred to clean hosts prior to day 9 of infection, they appear to be able to recover their ability to take up ^{32}P from the tissue fluids of the new host where they are under no immunological embarrassment. After day 9 of infection the worms are less able to regain their metabolic activity on transfer to a clean host and this is further evidence that the N. brasiliensis are irreversibly damaged by this time in an infection as a result of the host's immune response.

From these experiments the idea emerges that there is a relationship between the ability of the parasites to take up ^{32}P from the host and the onset of host immunity. If this is so it should be possible to demonstrate a similar uptake pattern using another labelled metabolite.

^{32}P labelled sodium phosphate was used as a marker for phosphorus metabolism and it seemed logical to look for a labelled substance which could monitor protein metabolism and thus complement the results obtained using ^{32}P. The gamma emitting analogue ^{75}Se l-selenomethionine has been used as a tracer in the study of protein metabolism in a number of situations, e.g. in rats of different ages [43] and in mice during exposure to cold [44]. It has been shown [45] that following the intravenous injection of ^{75}Se l-selenomethionine the label accumulates rapidly and is incorporated into body proteins, the pancreas, intestine and

liver having the highest uptake of label. Because ^{75}Se is a gamma emitter the radioactivity can be measured easily and accurately. When all factors are considered it appears that ^{75}Se labelled selenomethionine might be an ideal substance for the study of short term variations in the protein metabolism of N. brasiliensis worms during the course of an infection.

In the experiments described below ^{75}Se labelled selenomethionine was used essentially in the same way as the ^{32}P phosphate.

UPTAKE OF ^{75}SE BY ADULT N. brasiliensis

A. TIME/UPTAKE STUDY

This experiment was carried out to investigate the possibility of using ^{75}Se labelled selenomethionine to measure metabolite uptake in N. brasiliensis in rats and also initially to establish the relationship between uptake of ^{75}Se and the time interval between isotope injection and autopsy of rats.

MATERIALS AND METHODS

Experimental Animals

Sixteen rats which had each been infected with 4,000 N. brasiliensis larvae six days previously and 16 which had been given the same number of larvae nine days previously were used.

Radio-isotope Injection

Each rat received by intravenous injection a quantity of ^{75}Se selenomethionine based on the established formula of a 1.0 ml injection per 150 gm body weight where 1.0ml of solution contained 7.0 µCi Se.

Recovery of Worms and Radioactivity Assay

Groups of worms were killed in series from 1½ to 6 hours after injection. On completion of the cold saline washes, the worms from each group were bulked, weighed and transferred to counting vials for radioactivity assay in a scintillation counter.

The results were expressed as radioactivity per minute per mg "fresh weight".

RESULTS AND DISCUSSION

The results are shown in Fig. 4. As in the ^{32}P time/uptake study (see Fig. 1), the worms showed a better uptake on day 6 than on day 9 of infection, the day 9 values being about 30% of the values on day 6. This indicated that again some factor was operating by day 9 of an infection which diminished the ability of the parasites to take up the ^{75}Se labelled amino acid from the host.

It also appeared that the procedures adopted in the previous experiments could be used to give a picture of ^{75}Se uptake by the parasites over the course of infection. Such an experiment is described below.

B. UPTAKE OF ^{75}Se BY ADULT *N. brasiliensis*

This experiment is essentially a repeat of a previous one where ^{32}P sodium phosphate was used, except that the procedures used for quantifying the worms and measuring the radioactivity of the samples are different. The uptake of ^{75}Se was measured daily from day 6 till day 13 of infection.

MATERIALS AND METHODS

Experimental Animals

Thirty rats which had each been infected with 5,000 *N. brasiliensis* larvae were used.

Injection of Radio-isotopes

The rats were injected intravenously with ^{75}Se l-seleno-methionine on a weight basis as described in the previous

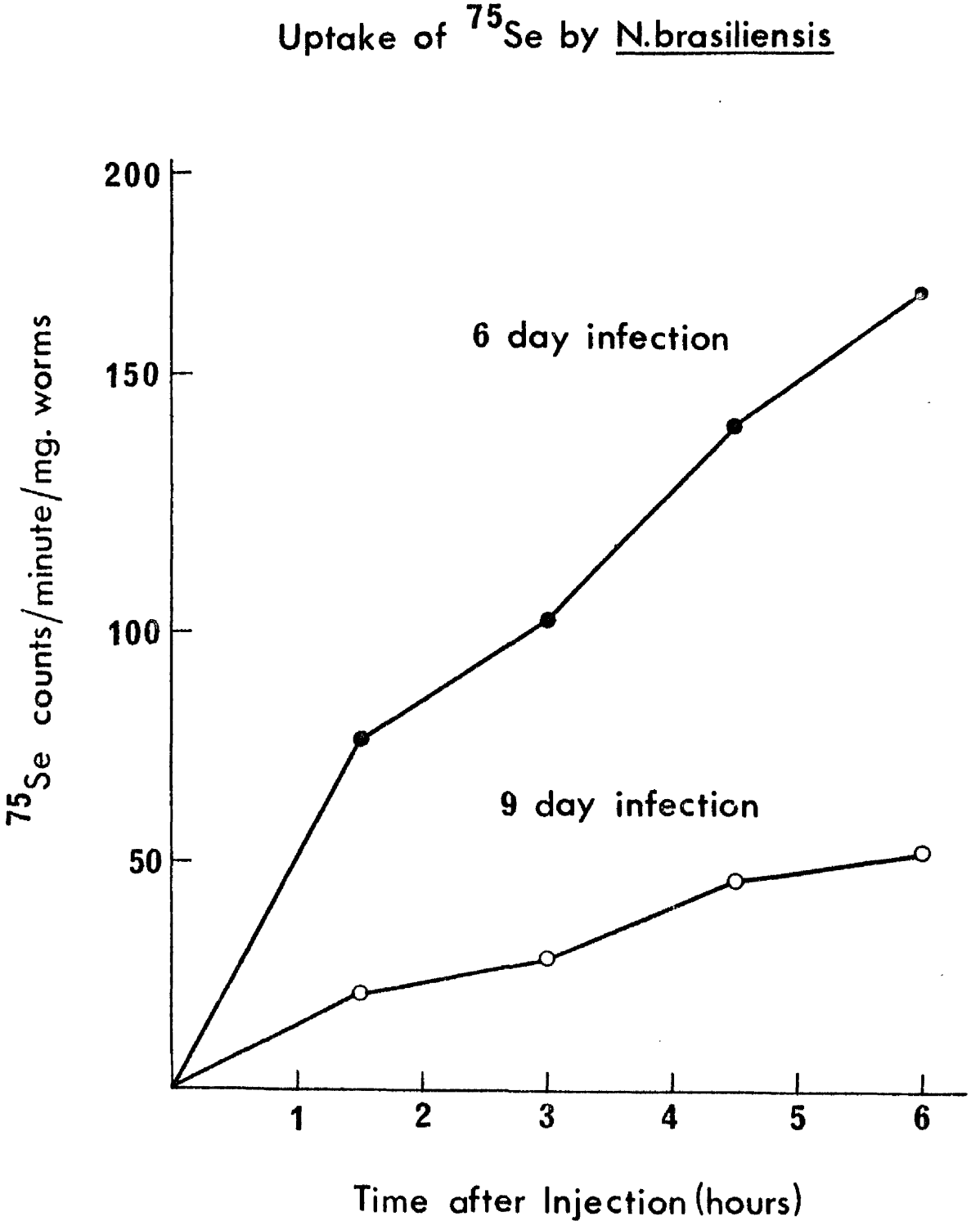

Fig. 4. Uptake of ^{75}Se by Adult N. brasiliensis : A Time Uptake Study

experiment. Four rats were used on each day of the experiment.

Recovery of Worms and Radioactivity Assay

Five hours after injection of the labelled material the worms were recovered and weighed as previously described. The weighed worm samples were transferred to counting vials and the radioactivity assayed in a scintillation counter.

The results were expressed as counts/minute/mg "fresh weight".

RESULTS AND DISCUSSION

The results are shown in Table 3. Fig. 5 shows the comparison between the uptake of ^{75}Se and of ^{32}P, as measured in the previous series of experiments. Except that there is a discrepancy in the day 8 uptake values the general pattern of results is very similar to that observed when ^{32}P was used, i.e. a dramatic fall in the uptake of label between day 7 and 9. Statistically there was less scatter in the ^{75}Se results, the S.D. values being much smaller when related to the mean values in this experiment compared to when ^{32}P labelled phosphate was used.

As pointed out previously, the "uptake" measurements of a labelled metabolite by worms from the host's tissue fluids have to be interpreted with some caution. Although it cannot be assumed that the results represent a measure of "net" transfer of label, one hopes that they reflect "metabolic activity" in some general sense.

Table 3

^{75}Se Uptake by Adult N. brasiliensis at Different Stages of Infection

	Mean Counts* per mg per minute							
	Day of Infection							
	6	7	8	9	10	11	12	13
	82.0	96.0	82.2	24.0	26.8	8.5	7.9	7.8
S.D.	6.5	12.2	23.0	5.0	3.8	1.2	2.3	1.3

* Means were obtained from four rats per group each day of the experiment.

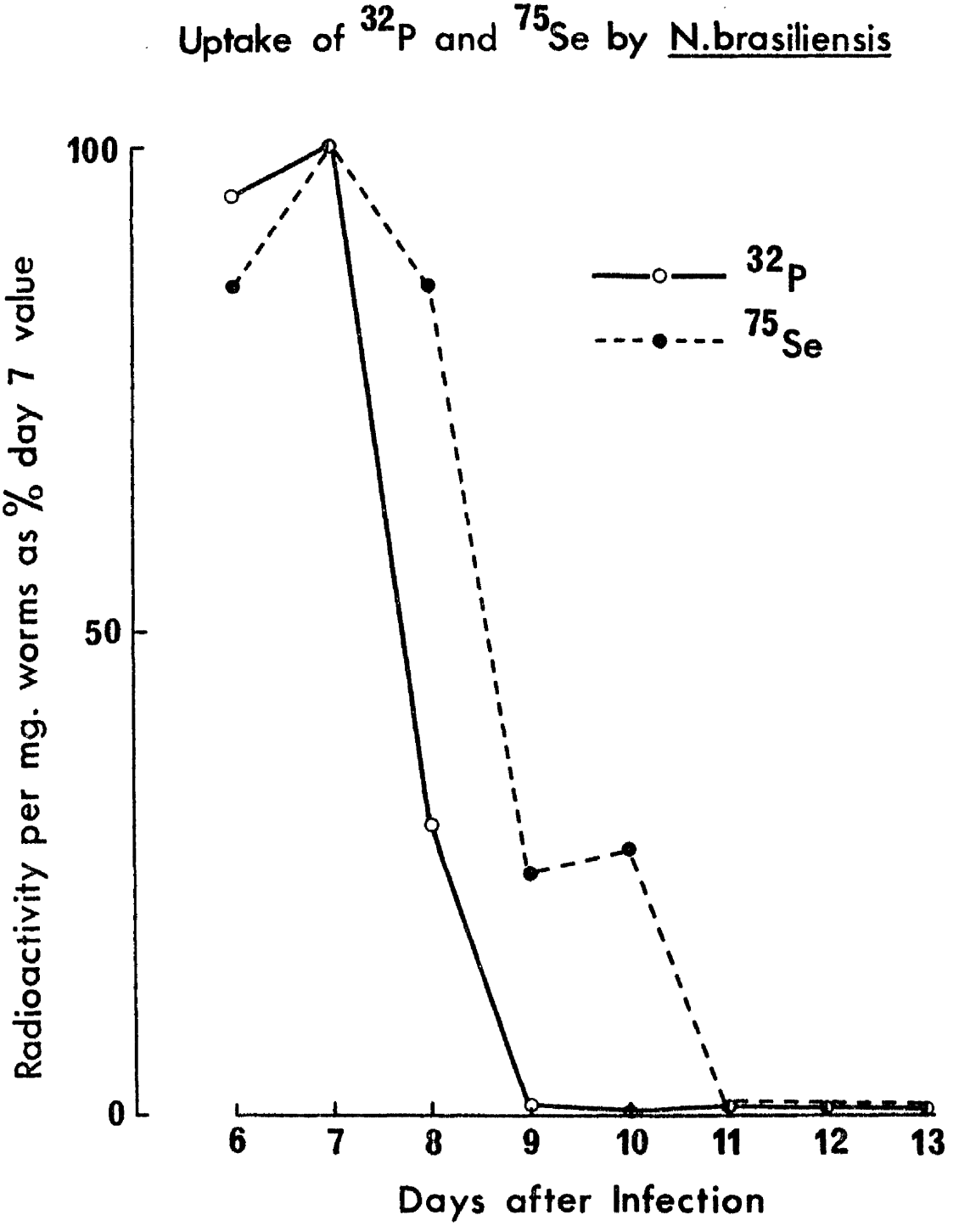

Fig. 5. Comparison of ^{32}P and ^{75}Se Uptakes by N. brasiliensis

It is very gratifying to find that the results with ^{75}Se so closely parallel those obtained with ^{32}P labelled phosphate. It strengthens the idea that the fall in uptake observed from day 7 onwards does reflect some important change in the worm's situation, either an impairment of metabolism or perhaps a physical displacement away from the mucosa which would depress the rate of diffusion of substances from the host's tissue fluids to the parasite.

SUMMARY

The results of the preliminary experiments described in Chapter I indicated that either ^{32}P labelled phosphate or ^{75}Se labelled selenomethionine could be successfully used to measure the uptake of metabolites by N. brasiliensis worms. The techniques employed were relatively simple and by standardising the quantities of isotope injected and the time interval between injection and worm recovery, results obtained from different groups of worms in any experiment can be compared. This allowed a comparison of the uptake of labelled substances by the worms on successive days of an infection to be made.

The results suggest that the parasite is under some immunological pressure at a much earlier stage than had been at one time anticipated and this is in line with the fact that the fall in egg output precedes the fall in worm population by several days [16]. Bearing in mind the time required for eggs to pass from the lumen of the small intestine into the faeces it is

obvious that some functional impairment occurs well before the actual expulsion of the worms.

Such an interpretation of these results is very much in line with the work of Ogilvie and Hockley [27] and Lee [28] who demonstrated that substantial structural changes, which they attributed to immunological damage, were observed by day 10 of infection, i.e. several days before the onset of expulsion.

However, before embarking on further investigations into the suppression of metabolite uptake by N. brasiliensis as an index of immunological damage it seemed that some of the procedures already used required to be critically examined and if possible improved. Such a study is the subject matter of the following chapter.

CHAPTER II

STANDARDISATION OF PROCEDURES USED IN MEASURING LABELLED METABOLITE UPTAKE BY ADULT *Nippostrongylus brasiliensis*

INTRODUCTION

The first objective of this study was to perfect and standardise the method for measuring the uptake by N. brasiliensis worms of labelled metabolites from the host.

Basically the methods described in the previous chapter involve measuring the radioactivity of a known quantity of worms recovered from the host 5 hours after the injection of the labelled metabolite. In the techniques described so far there exist the possibilities of errors in both the measurement of radioactivity and in the precedures used for quantifying the worms. The recorded radioactivity of a sample of worms may be affected by elution of the radioactive label during the process of collecting the worms and also errors in the counting precedure itself.

Initially radioactivity was related to the number of worms in the sample but this was abandoned for the more practical method of weighing the worms. This latter procedure requires the recovered parasites finally to be in the form of a pellet of worms, free from extraneous materials, the weight of which must be measured accurately. In Chapter II these matters are discussed and investigated.

In order to increase the number of worms recovered from each rat, infecting doses of 5,000 N. brasiliensis larvae were used in subsequent experiments and in most of the remaining experiments to be described the number of rats in each experimental

group was increased to at least six. The first change simply produces a greater number of worms per rat which is of some practical importance especially in the latter stages of an infection and the increased number of rats used can be statistically important where the results of uptake studies in worms from different groups of rats have to be compared.

RADIOACTIVITY MEASUREMENTS

Some changes had to be made in the procedures for assaying the worm samples for radioactivity. Selenium-75 emits γ radiation and presents no problems since the radioactivity of the sample can be measured simply by placing the weighed sample of worms in a gamma radiation spectometer (well-type scintillation counter). Phosphorus-32 emits β radiation and this may be detected by using a Geiger-Muller counter. This method was used in the first experiments described in the last chapter but it was abandoned because of practical difficulties in favour of the simpler Cerenkov counting method [38].

The Cerenkov effect arises when β particles travel through a medium with a velocity faster than light and for that medium short durational light pulses (photons) are emitted. The counting method is simple to use and expensive scintillators are not required. The sample is placed in a liquid scintillation counter and its radioactivity determined. With Cerenkov counting the efficiency is not affected by chemical quenching and the background counts are relatively low.

Quenching is a term which describes the reduction in the efficiency of the energy transfer processes between the solution and the photomultiplier tube. Chemical quenching results from the presence of impurities in the scintillator system but quenching can also be caused by the colour of the sample which diminishes the path of the photons and thus reduces the optical efficiency between the sample and the photocell of the photomultiplier tube. The net result of all quenching is that the observed count rate for a quenched sample will be less than that for an unquenched sample containing the same quantity of radioactivity. Quenching occurs when coloured samples are assayed for radioactivity by the Cerenkov method. No account was taken of this phenomenon in the preliminary experiments where worm samples were assayed for ^{32}P. Three methods are commonly used to correct for quenching:

(a) Internal Standardisation Calibration

(b) External Standardisation Calibration

(c) Channels - ratio method.

The internal standardisation method involves counting a series of samples of unknown activity and then recounting after adding a known amount of a non-quenched standard to each sample. A series of unquenched standards are also counted. The quench correction factors may be found by relating the count rates of the internal standards with the unquenched standards.

The external standardisation method utilises an external gamma source which can be moved into a position in the liquid scintillation counter near the sample. A calibration curve

must first be obtained by counting samples of known activity and recounting them after the external source has been positioned near the sample. A curve of counting efficiency versus gross external standard counts is then obtained. Using this curve the efficiency of an unknown sample may be found from the observed count rate of the sample.

The Channels - ratio method involves splitting the counting channel into an upper and a lower part. The effect of quenching is to increase the count rate in the lower channel and to decrease the rate in the upper channel. Since this ratio is independent of activity and depends on the shape of the beta spectrum it is a measure of quenching. A calibration curve has to be obtained, using samples of a known activity but different quench factors, of sample counting efficiency versus channels - ratio. From this curve the efficiency for any sample may be obtained using the channels - ratio value.

Each method has drawbacks and advantages. A calibration curve is not required in the internal standardisation method and the quench correction is obtained by the simple procedure of adding a standard amount of isotope to each sample. However if a large number of samples have to be assayed regularly this method could be very time-consuming and a sample cannot be recounted or used for any other purpose once the internal standard has been added. The three methods referred to have been evaluated [39] and it has been found that careful addition of known amounts of internal standard gave the highest accuracy. It is recommended that an

amount of internal standard to give ten times the average count obtained from the samples being assayed should be added. The automatic external standardisation was shown to be less accurate than the internal standardisation method. The channels - ratio method gave results comparable with internal standardisation but the method was less accurate when dealing with highly quenched samples with a low count rate.

In order to test the accuracy of the internal standardisation method an experiment was designed to simulate the situation obtaining when sulphuric acid digests from a range of quantities of worms containing ^{32}P are corrected for quenching. This experiment is described below.

QUENCH CORRECTIONS IN ^{32}P SOLUTIONS USING THE INTERNAL STANDARDISATION METHOD

A number of coloured (quenched) solutions were prepared so that the range of colours (and quenching) covered those obtained where quantities of worm tissue from 0 - 100 mg were digested in concentrated sulphuric acid. To each solution a quantity of ^{32}P was added, calculated to produce count rates approximating to those obtained from worms recovered from rats five hours after injection with 20 μCi ^{32}P.

These solutions were assayed for ^{32}P. After adding to each solution an amount of ^{32}P which would give approximately ten times the original counts, the solutions were recounted together

with a number of unquenched solutions containing the same amount of isotope (i.e. internal standards).

The quench correction factors were calculated and the original unquenched count rates were corrected. This experiment allowed the accuracy of the internal standardisation method to be evaluated before being applied in subsequent experiments where ^{32}P uptake by N. brasiliensis worms is measured.

MATERIALS AND METHODS

Quenched Solutions

A solution containing 200 µl of blood in 20 ml of water was serially diluted to give twenty solutions containing from 200 µl to 0.5 µl of blood per 20 ml. A quantity of ^{32}P was added to 5.0 ml aliquots of each quenched solution so that count rates of approximately 3,500 counts per minute were obtained in the least quenched sample and the same quantity of ^{32}P was added to six 5.0 ml volumes of distilled water (unquenched solutions).

Radioactivity Assay

The count rates of all samples were ascertained for ten minutes. Using a 10 µl automatic pipette a quantity of ^{32}P (Internal Standard) calculated to give approximately ten times the mean count rate of the quenched samples, was added to each of the quenched samples. The same quantity of ^{32}P was added to five 5.0 ml volumes of distilled water (unquenched standards). The quenched solutions were recounted together with the unquenched standards.

Calculation

The background radioactivity was subtracted from the unquenched samples and the count rates noted for comparison at a later stage.

The background radioactivity was subtracted from the gross counts (A) of the quenched samples to give net counts (B).

From the second counts (C), gross counts (A) were subtracted to give the internal standard counts (D) for each sample. The internal standard counts for each sample were compared with the mean net counts (E) for the unquenched standards, to give the quench correction factors for each sample.

e.g. A - background = B counts/minute (net sample count)

C - A = D counts/minute (Internal Standard counts)

Quench Factor = $\frac{E}{D}$

therefore corrected counts = $B \times \frac{E}{D}$ counts/minute.

RESULTS AND DISCUSSION

The following mean net count rates were obtained:

unquenched samples 3,483 counts/minute

unquenched internal standards (E) 14,525 counts/minute

Table 4 shows the net count rates (B) obtained for each of the quenched samples and the corresponding net count rates (D) for the added internal standards.

The quench correction factors were calculated and applied as shown above. The mean quench corrected count rate (3,539 counts/min) corresponds closely to the mean count rate for the unquenched

Table 4

The Use of Internal Standards in Quench Correction

Quenched Sample	Quenched net/counts/min (B)	Internal Standard net/counts/min (D)	Quench Correction Factor (E/D)	Corrected counts/min
1	3,749	14,389	1.00	3,749
2	3,422	13,748	1.06	3,617
3	3,209	13,107	1.11	3,555
4	3,094	12,740	1.14	3,527
5	3,371	13,538	1.07	3,617
6	3,112	13,235	1.10	3,425
7	3,129	12,892	1.13	3,536
8	3,119	13,326	1.09	3,400
9	2,932	11,595	1.25	3,665
10	2,743	11,425	1.27	3,486
11	2,347	9,787	1.48	3,474
12	2,550	9,845	1.48	3,319
13	2,014	8,380	1.73	3,490
14	1,821	7,349	1.98	3,598
15	1,799	7,338	1.98	3,560
16	1,770	6,974	2.08	3,678
17	1,266	4,991	2.91	3.684
18	1,149	4,843	3.00	3,446
19	727	2,873	5.06	3,675
20	632	2,551	5.69	3,599
			Mean	3,539
			S.D.	100

samples (3,483 counts/min). Thus the effect of quenching has been satisfactorily overcome. It is obvious from the results of this experiment that the internal standardisation method of calculating quench corrections may be used with confidence in subsequent experiments.

Following this investigation the next most critical part of the method appeared to be the procedure used in quantifying the worm sample prior to radioactivity assay and this is the subject of the following experiment.

METHOD OF QUANTIFYING WORMS

In experiments on metabolite uptake radioactivity has finally to be expressed as activity per unit quantity of worms, so the method chosen for measuring the worm sample is critical and must give an accurate measure of the amount of worm material present.

The method ultimately chosen in the experiments described in Chapter I involved collecting the recovered worms in a small Buchner funnel and transferring the surface dried samples to weighed counting vials and reweighing the bottles. These results were expressed as counts per unit weight (mg) of "fresh worms". It was often difficult to place the worm sample on to the bottom of the counting vial. With gamma emitters, e.g. ^{75}Selenium, the geometry of the sample being examined directly affects the magnitude of the count rate obtained in a well type scintillation counter, e.g. if two samples of identical weight and radioactivity are

examined, one placed at the bottom of a counting vial and the other dispersed over the inner wall, the latter will produce a lower count rate. By transferring the worms directly from the Buchner funnel on to a previously weighed degreased cover slip the quantity of worms present can be ascertained by weight difference. After drying overnight, the small hard mass of worms can easily be transferred to a counting vial, thus avoiding any problem of sample geometry.

The coverslip method was also used to advantage in experiments to be described where worms are digested in acid before being transferred, in solution, to counting vials for ^{32}P determination and where worms containing ^{14}Carbon must be solubilised in a counting vial (Chapter III). In the latter case it is essential that the worms are at the bottom of the vial.

The method described above for weighing worms is used in all of the experiments to be described later in this study. However the procedure described was open to some criticism. Relatively small quantities ($<$ 50 mg) were weighed and any weighing error combined with possible differences in moisture content of the samples from time to time, could lead to an unacceptable variation in the amount of worm tissue present per unit weight of sample. Results of tests designed to prove the validity of the method are described below.

These experiments involve selecting another parameter, nitrogen content of the weighed worm samples, which can be ascertained with a high degree of accuracy and correlating these values with the recorded sample weights.

CORRELATION OF NITROGEN CONTENT AND WEIGHT OF *N. brasiliensis* WORM SAMPLES

Minute quantities of nitrogen in tissue can be accurately measured by the Micro-Kjeldahl method. In this process organic nitrogen is converted to ammonium sulphate by digestion with concentrated sulphuric acid, catalyst being added to raise the boiling point of the acid. The ammonium sulphate solution is subsequently made alkaline and steam distilled in a closed system, to produce ammonia which is collected in a boric acid indicator mixture as ammonium borate and this is titrated directly with hydrochloric acid to form ammonium chloride, thus allowing the amount of nitrogen originally present in the sample to be calculated.

Using this method the amount of nitrogen present in a worm sample may be calculated without reference to the weight of worms assayed. If the nitrogen contents of a number of worm samples are measured and show a good correlation with the respective "fresh weights" of worms determined as already described, then the assumption can be made that the recorded sample weights do give an accurate measure of the worm tissue present.

The experiment to be described was planned so that the worm samples used were weighed on five successive days to cover the possibility that variations could occur in moisture content of the samples from day to day which would then affect the proportion of worm tissue in any given sample.

MATERIALS AND METHODS

Adult N. brasiliensis

On five successive days, groups of five rats were given 5,000 N. brasiliensis larvae. Seven days after infection each group was killed and the worms were recovered as previously described. The worms were washed three times in ice-cold saline and then transferred via a wide bore pasteur pipette on to a glass cover-slip which had previously been thoroughly degreased and weighed. The weight of the worms was immediately ascertained by weight difference and the sample was then allowed to stand overnight, under cover to exlude dust, till the worm sample had dried into a small hard pellet which was easily completely transferred to a micro-Kjeldahl digestion flask, which was stored at -4°C to await digestion.

Digestion of Worms

Two ml nitrogen free concentrated sulphuric acid and 100 mg selenium catalyst were added to the worms in the flasks and the samples were digested till the resulting solutions were almost colourless.

Indicator Mixture

This was prepared by adding 4.0 ml of 0.1% bromocresol green in alcohol to one litre of 2% aqueous boric acid.

Distillation and Titration

Each digest was added to a Hoskins distillation apparatus [46], made alkaline with 3.0 ml 40% NaOH solution and the ammonia steam distilled into 10.0 ml of the indicator mixture. The indicator

changed from green to blue. The solutions were then titrated with 0.02N HCl till the blue colour reappeared. "Blank" distillations and titrations were also carried out from time to time, i.e. an alkaline steam distillation containing no sample.

Calculation

From each titration figure the value of the "blank" is deducted. From the chemical equation $HCl + NH_3 \rightarrow NH_4Cl$, it can be deduced that 1.0 ml 0.02N. HCL $\equiv$ 14 x 0.02 mg nitrogen. It therefore follows that the volume (V) of 0.02 N HCl used in the titration

$\equiv$ V x 14 x 20 ug nitrogen

$\equiv$ V x 280 µg nitrogen

Standards

As a check on the accuracy of the method and the purity of the reagents used, ten 5.0 ml volumes of an ammonium salt each containing 500 µg of nitrogen were digested and steam distilled as above and the nitrogen content of each sample calculated.

RESULTS AND DISCUSSION

Table 5 shows the correlation of nitrogen content against the wet weight or "fresh weight" in mg of each worm sample analysed. Statistically the correlations are good, and the evidence indicates that the method chosen for weighing the worms gives an accurate measure of the worm tissue present in a sample.

Table 5

Correlation of Nitrogen Contents and "Fresh Weights" in N. brasiliensis Worms

Fresh Wt. mg	Nitrogen µg		% Nitrogen in Sample
26.9	675.2		2.51
39.5	999.4		2.53
14.6	351.8		2.41
34.5	859.1		2.49
6.3	145.6		2.31
24.5	646.8		2.64
40.6	994.0		2.58
16.9	405.6		2.40
31.3	735.5		2.35
18.6	483.6		2.60
19.3	436.8		2.26
12.3	291.2		2.37
22.2	539.5		2.43
10.1	238.0		2.36
12.2	294.0		2.41
11.4	294.0		2.58
11.4	271.6		2.38
13.8	294.0		2.13
29.8	635.6		2.13
19.2	431.2		2.25
19.6	464.8		2.37
30.4	677.6		2.23
30.0	753.2		2.44
38.8	942.8		2.43
24.5	605.2		2.47
		Mean	2.40
		S.D.	0.1325
		S.E.	± 0.0265

The results of this experiment remove any doubts which may have been harboured regarding the accuracy of the method adopted for quantifying the recovered radioactive worms. The method used for weighing the worms is described in the General Materials and Methods and is used in the remaining experiments to be described in this study.

The accuracy of the micro-Kjeldahl method for estimating nitrogen was tested by preparing ten samples of ammonium salt solution, each containing 500 µg nitrogen and assaying these solutions. The results of this experiment are shown in Table 6. It can be seen that mean value recorded for the nitrogen content of these samples, 490.16 ± 5.14 µg, is very close to the theoretical value and the margin of error is very small. These results indicate that accurate and repeatable results can be obtained from the micro-Kjeldahl method.

Table 6

Analysis of Ammonium Sulphate Standards

Titration net (ml HCl)		Nitrogen µg
1.78		498.0
1.74		487.2
1.78		498.0
1.73		484.4
1.70		476.0
1.71		478.8
1.78		498.0
1.67		467.6
1.88		526.4
1.74		487.2
	Mean	490.16
	S.D.	16.28
	S.E. ±	5.14

ELUTION OF RADIOACTIVE LABEL FROM WORMS DURING RECOVERY FROM THE GUT

The procedures described in Chapter I for measuring metabolite uptake using iosotpic markers involved injecting intravenously into the host rat an amount of isotope related to the body weight of the rat, killing the rat after 5 hours and recovering the worms by incubating cut sections of gut in muslin for 30minutes at 37°C. The time of incubation was reduced rather arbitrarily from the normal 1 hour in the procedure described by Jennings and co-workers [37] in order to limit any elution of isotope from the worms. The worms must be recovered from the intestine of the host and washed free from unbound activity which is present in the intestine of the host. Since metabolic activity is ultimately expressed in terms of worm radioactivity at autopsy it is important to know whether or not elution of the radioactive label takes place during the period of worm collection. If there is any significant loss of label from the worms during the incubation period, the radioactivity of worms collected from different rats and on different days of an experiment could not be compared unless the collection times were strictly observed, a procedure which is difficult to follow in practice. The possibility that elution of the isotope occurred during the collection period was not investigated in the preliminary experiments. Experiments

designed to detect any elution of radioactive label at this point in the procedure are described below.

The aim of these experiments was to determine whether or not there was any significant loss of radioactive label from the N. brasiliensis worms while they were incubating in water at 37°C during the 30 minute collection process. Because of the possibility that the uptake of metabolite may vary slightly on any day of an infection and to compensate for possible experimental errors, this experiment was designed so that each sample of worms finally assayed for radioactivity contained worms pooled from 30 rats, i.e. the radioactivity of the worms was constant, the only variable being the incubation time.

A. ELUTION OF ^{32}P

MATERIALS AND METHODS

Experimental Animals

Thirty rats which had each been infected 9 days previously with 5,000 N. brasiliensis larvae were used.

Injection of Radioisotope

The rats were divided into six groups (1 - 5, 5 - 10, etc.) and the rats in each group were injected with 1.0 ml (20 μCi) ^{32}P sodium phosphate per 150 g weight at one minute intervals.

Collection of Worms

Five hours after injection each group of rats was killed and cut sections of the intestines of the five rats were pooled and incubated at 37°C for up to 60 minutes.

The worms from each group were treated as follows:

At 10, 20, 30, 40, 50 and 60 minutes after incubation had begun a number of worms were removed. These six samples were washed three times in ice-cold saline and their "fresh weight" ascertained as previously described.

The six weighed samples thus obtained for each incubation period were finally pooled and their weights aggregated. This gave six samples collected from the original 30 rats.

Radioactivity Assay

These samples were digested in 2.0 ml concentrated sulphuric acid and 100 mg selenium catalyst as described in the General Materials and Methods. The resulting clear solutions were transferred to counting vials and the ^{32}P count rates determined by the Cerenkov method. Quench corrections were calculated by the internal standardisation method. The results were expressed as counts/minute/mg "fresh weight" worms. These results were then correlated against incubation times.

RESULTS AND DISCUSSION

It can be seen from Table 7 that there was no significant difference in the radioactivity of the parasites recovered for incubation periods between 10 and 50 minutes. The reduction in the recorded radioactivity after 60 minutes incubation may be the first evidence of elution of label from the worms but since the time used in the experiments is 30 minutes no problems arise. In the following experiment the possibility of elution of ^{75}Se during the collection process is investigated.

Table 7

Elution of ^{32}P from Worms During Collection Process

Incubation Time	Worm Weight mg	Counts/min/mg "Fresh Weight"
10	42.2	36.5
20	58.4	30.7
30	64.0	28.2
40	59.2	24.3
50	60.8	30.6
60	68.1	18.4

B. ELUTION OF ^{75}Se

The procedures followed in this experiment are identical to those described above except that worms are collected, weighed and assayed directly for ^{75}Se in a scintillation counter.

MATERIALS AND METHODS

Experimental Animals

Thirty animals which had been infected 8 days previously with 5,000 *N. brasiliensis* larvae were used.

Injection of Radioisotope

The rats were divided into groups as in the previous experiment and injected with 1.0 ml (7 μCi) ^{75}Se l-selenomethionine per 150 g weight.

Collection of Worms

Six weighed worm samples, corresponding to the incubation times of 10, 20, 30, 40, 50 and 60 minutes, were collected from the thirty rats exactly as in Experiment A.

Radioactivity Assay

The weighed samples were allowed to dry overnight and were transferred to counting vials. These were assayed for ^{75}Se in a scintillation counter. The results were expressed as counts/minute/mg "fresh weight". These results were then correlated against incubation times.

RESULTS AND DISCUSSION

The results (Table 8) were similar to those obtained when

Table 8

Elution of ^{75}Se from Worms During Collection Process

Incubation Time	Worm Weight mg	Counts/min/mg "Fresh Weight"
10	150.1	91.7
20	116.1	85.0
30	168.1	92.5
40	91.1	96.3
50	116.0	96.3
60	205.6	70.6

^{32}P was used. Again there was a drop in the radioactivity of the worms which had been incubating for 60 minutes in saline at 37°C. This is not surprising and suggests that after 50 minutes elution of the label occurs. However since the arbitrary incubation time chosen was 30 minutes, for all practical purposes there is n o problem of elution. From a practical viewpoint it is advantageous to know that small variations in incubation times can be ignored.

The final problem requiring investigation concerns the difficulty in obtaining "clean" worm samples for weighing prior to radioactivity assay. In the preliminary experiments it was often difficult to obtain collections of worms free from grit and other extraneous gut debris. The inclusion of this matter in a sample would introduce a high experimental error and in the experiments described in Chapter I some samples had to be discarded for this reason. An experiment showing how the problem can be minimised is described below.

UPTAKE OF LABELLED METABOLITES BY ADULT *N. brasiliensis* IN STARVED AND *ad. lib.* FED RATS

It is important when measuring uptake of metabolites by *N. brasiliensis* worms using the techniques described in this study to be able to collect samples of worms which are free from grit and other extraneous matter so that the weight recorded for the sample is the true weight of worm tissue present in the sample. It has been observed that when rats are starved for five hours prior to autopsy, the worm sample subsequently collected was more likely to be free from extraneous matter than a sample from an *ad lib* fed rat.

The objective of this experiment was to see whether or not starving the host animals for the five hours between isotope injection and autopsy affected the metabolic activity of the parasites. Both ^{32}P sodium phosphate and ^{75}Se labelled selenomethionine are used as markers in the experiment to be described.

A. UPTAKE OF ^{32}P

MATERIALS AND METHODS

Experimental Animals

Twenty five rats which had been infected with 5,000 *N. brasiliensis* larvae 6 days previously were used.

Injection of Radioisotope

Each rat was injected with 1.0 ml (20 μCi) ^{32}P sodium

phosphate per 150 g weight. The rats were divided into two groups, food being removed from one, the other allowed to feed ad lib.

Collection of Worms

Five hours after injection the rats were killed and the worms recovered and weighed as previously described. The worm samples were transferred, when dry, to micro-Kjeldahl digestion flasks.

Radioactivity Assay of Worms

The weighed worm samples were digested in sulphuric acid as already described and the resulting solutions transferred to counting vials for radioactivity assay by the Cerenkov method. Corrections for quenching were made by the internal standardisation method.

RESULTS

The results are shown in Table 9. There is a remarkable similarity in the mean uptake values recorded for the two groups, 119.72 counts/min/mg and 119.92 counts/min/mg for the worms from the ad lib fed and starved rats respectively. These results indicate that starving the rats has, as might be expected, no effect on the metabolic activity of the parasites.

Table 9

Uptake of ^{32}P by Adult *N. brasiliensis* in *ad lib* Fed and Starved Rats

	Worm Weight mg		Radioactivity counts/min/mg worms
Rats fed *ad lib*	22.4		13.9
	22.7		15.4
	12.4		11.9
	26.8		16.6
	39.4		19.8
	34.5		17.2
	17.0		14.1
	6.7		15.6
		Mean	15.55
		S.D.	2.39
starved rats	9.3		25.3
	7.6		13.6
	26.1		17.8
	34.1		20.0
	18.8		14.1
	21.7		10.8
	24.5		12.6
	20.1		14.5
	16.9		16.7
	31.3		13.6
		Mean	15.90
		S.D.	4.24

B. UPTAKE OF ^{75}Se L-SELENOMETHIONINE

MATERIALS AND METHODS

Experimental Animals

Eighteen rats which had been infected with 5,000 *N. brasiliensis* larvae 9 days previously were used in this experiment.

Injection of Radioisotope

Each rat was injected with 7 µCi (1.0 ml) ^{75}Se l-selenomethionine per 150 gm weight. The rats were divided into two groups, food being removed from one, the other fed *ad lib*.

Collection of Worms

Five hours after injection the rats were killed and the worms were recovered, weighed, dried overnight and transferred to counting vials.

Radioactivity Assay

The counting vials containing the worms were assayed for radioactivity in a scintillation counter.

RESULTS AND DISCUSSION

The results (Table 10) again show that there is no difference in the metabolic activity of the worms recovered from the two groups of rats. The mean uptake values recorded in both groups are almost identical. These results are in line with those obtained using ^{32}P as a marker.

Since worm metabolism in starved rats is unaltered and worm samples virtually free from extraneous matter can be obtained from such rats, in all remaining experiments to be described the rats are starved for the 5 hours between isotope injection and autopsy.

Table 10

Uptake of ^{75}Se by Adult *N.brasiliensis* in *ad lib* Fed and Starved Rats

	Worm Weight mg		Radioactivity counts/min/mg worms
Rats fed *ad lib*	11.4		141
	13.8		118
	10.1		108
	18.0		98
	29.8		137
	14.7		128
	13.7		125
	8.1		118
	5.9		126
	19.6		132
	5.8		86
		Mean	119.72
		S.D.	16.70
Starved rats	19.2		108
	13.9		92
	19.6		135
	30.4		134
	30.9		130
	23.5		122
	9.8		92
	5.6		89
	20.1		155
	14.4		110
	12.9		107
	18.7		65
		Mean	119.92
		S.D.	24.70

CHAPTER III

METABOLITE UPTAKE MEASUREMENTS USING THE IMPROVED TECHNIQUES DESCRIBED IN CHAPTER II

(A) Simultaneous Uptake of ^{32}P and ^{75}Se

(B) Uptake of ^{14}C-Glucose

INTRODUCTION

The results of the preliminary experiments where the uptake of ^{32}P inorganic phosphate and ^{75}Se labelled seleno-methionine was measured in separate experiments indicated that there is a good correlation between the uptake of both metabolites. It is reasonable to assume that this reflects the true situation, i.e. the pattern of uptake of both substances is adversely affected by a common factor. However the situation can best be confirmed by carrying out the uptake measurements of both radioactive labels simultaneously, in the same group of rats, using the improved techniques described in Chapter II.

An experiment is described below where the uptake by adult N. brasiliensis of both ^{32}P and ^{75}Se from the host's tissue fluids is simultaneously measured to see if the results of this experiment confirm the findings of the individual experiments reported in Chapter I. In addition these results are further compared with those of a study using ^{14}C labelled glucose.

UPTAKE OF RADIOACTIVELY LABELLED METABOLITES BY ADULT *N. brasiliensis* FROM THE HOST'S TISSUE FLUIDS

A. SIMULTANEOUS UPTAKE OF ^{32}P AND ^{75}Se

The objective of this experiment was to measure simultaneously the uptake of ^{32}P sodium phosphate and ^{75}Se l-selenomethionine by *N. brasiliensis* worms between days 6 and 13 of a primary infection and to correlate the results with the known pattern of egg production, worm expulsion and other changes known to occur with the onset of the immune expulsion of the worms.

In this experiment the ^{32}P worm radioactivity was related to both the "fresh weight" of worms in a sample and to the nitrogen content of the sample, thus allowing a straight comparison of the two methods for quantifying the worms to be made.

MATERIALS AND METHODS

Experimental Animals

Seventy five rats which had each been infected with 5,000 *N. brasiliensis* larvae were used.

Radioisotope Injection

A solution was prepared which contained 20 µCi ^{32}P and 7 µCi ^{75}Se per ml. Sterile precautions were observed and the solution was divided into 10 ml volumes and stored at $-4^{o}C$ till required. The isotope mixture was injected intravenously on the basis of 1.0 ml per 150 gm weight. On days 6 - 8 of the infection eight rats were used and on days 9 - 13 ten rats were used.

Worm Collection

Five hours after injection the rats were killed and the recovered worms were weighed, dried overnight and transferred to counting vials which were stored at -4°C till the end of the experiment.

^{75}Se Assay

On completion of the experiment the sample vials containing the weighed worms were placed in a scintillation counter and the ^{75}Se count rate determined. The results were described as counts/minute/mg "fresh weight".

^{32}P Assay

(a) Following the measurement of the ^{75}Se count rates the samples were transferred to micro-Kjeldahl flasks and digested with 2.0 ml concentrated sulphuric acid and 100 mg selenium catalyst till the solutions were almost colourless. The digests were then carefully transferred to counting vials and the flasks washed into the vials with 2 x 1.0 ml distilled water. The samples were then assayed for ^{32}P in a liquid scintillation counter by the Cerenkov method. Quench corrections were made using internal standards.

(b) Following the determination of the ^{32}P count rates above, each sample was transferred to a steam distillation apparatus, made alkaline with 3.0 ml 40% NaOH and the ammonia produced steam distilled into 10.0 ml of bromocresol green indicator mixture. The indicator changed from green to blue and this

solution was titrated with 0.02 N HCl till the blue colour reappeared. "Blank" distillations and titrations were also carried out from time to time. The nitrogen content of each sample was calculated as described in Chapter II.

The results were expressed:

(a) as counts/minute/mg "fresh weight" and,

(b) as counts/minute/µg worm nitrogen.

RESULTS

The results of the measurement of ^{75}Se uptake are shown in Table 11. The overall picture is very similar to that seen when ^{75}Se uptake was measured in the preliminary experiment reported in Chapter I. The day 10 value shows a drop to approximately 30% of the uptake at day 6 of infection.

The results of the ^{32}P uptake measurements are shown in Tables 12 and 13, expressed in terms of worm weight and worm nitrogen respectively. Except for a divergence on day 8, there is a remarkable similarity in the uptake of both labelled metabolites (see Fig. 6). Statistically the results are good. The numbers of rats in each group are high and the S.D. values are relatively low till about day 10 of infection, when in both the ^{32}P and ^{75}Se uptake measurements a greater scatter is observed. This may be due to the fact that slight variations probably occur in the timing of the immune response in individual animals and if the assumption is correct that changes in uptake of metabolite is a result, either directly or indirectly, of this host immunity then it is not surprising that there are slight variations in the timing of the consequential changes in metabolite uptake. A

Table 11

Uptake of ^{75}Se by Adult *N. brasiliensis*

Day of Infection	No. of Rats in Group	Mean ^{75}Se counts/min/mg "fresh weight"	S.D.
6	8	14.8	1.5
7	8	14.7	2.6
8	7	11.3	2.3
9	10	9.9	2.2
10	10	4.4	2.1
11	10	2.3	1.2
12	10	0.97	0.58
13	10	0.81	0.37

Table 12

Uptake of ^{32}P by Adult *N. brasiliensis*

Day of Infection	No. of Rats in Group	Mean ^{32}P counts/min/mg "fresh weight"	S.D.
6	8	94.0	9.0
7	8	99.8	23.4
8	6	95.7	13.8
9	10	65.8	14.1
10	10	32.8	18.8
11	10	25.3	16.7
12	9	7.7	4.3
13	10	4.1	1.8

Table 13

Uptake of ^{32}P by Adult N. brasiliensis

Day of Infection	No. of Rats in Group	Mean ^{32}P count/min/mg worm nitrogen	S.D.
6	8	4017	407
7	7	3891	989
8	7	4249	624
9	10	2597	618
10	10	1308	841
11	10	1111	733
12	10	271	157
13	10	176	75

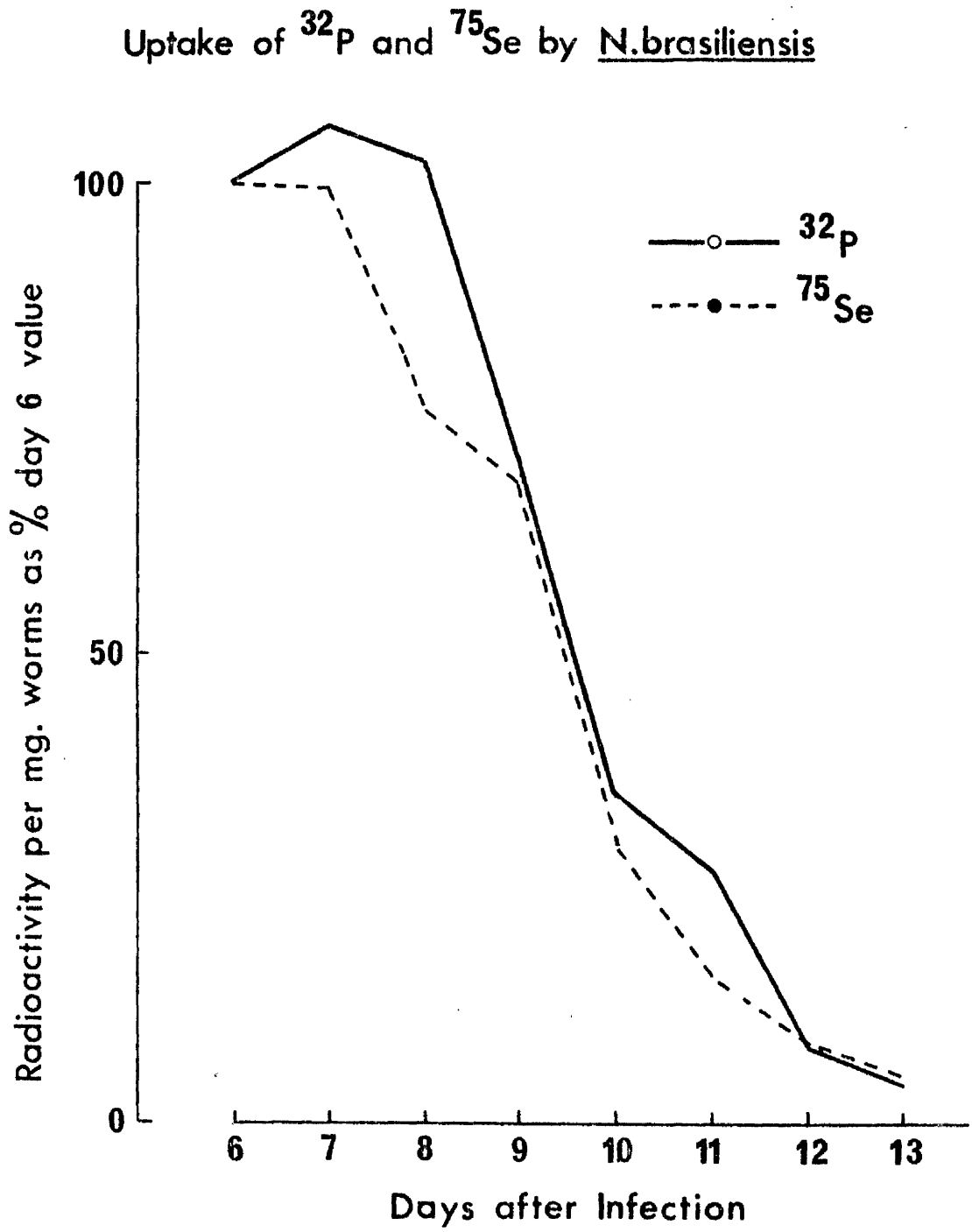

Fig. 6. Simultaneous Uptake of ^{32}P and ^{75}Se by N. brasiliensis

A statistical analysis of the results on days 7, 10 and 13 of the infection is shown in Table 14. It is obvious from these results that there is no significant difference in the uptake of the two labelled metabolites. This confirms the interpretation of the results obtained in the experiments described in Chapter I when the same measurements were made in separate experiments.

A direct comparison of the two methods employed for quantifying the amount of worm tissue in a sample can be seen in Fig. 7. Again there is practically no difference in each set of results. This experiment shows conclusively that the method adopted for quantifying the radioactive worms, the "fresh weight" method, is satisfactory and there is no advantage to be gained from using the more complicated and time-consuming nitrogen content method.

B. UPTAKE OF ^{14}C LABELLED GLUCOSE

Experiments have been described where the uptake of ^{32}P and ^{75}Se by adult N. brasiliensis from the host's tissue fluids has been measured. There is a remarkable similarity in the way in which the uptake of these metabolites altersduring an infection. In this experiment the uptake of a further labelled metabolite, ^{14}C d-glucose was measured and the results correlated with those obtained in the studies of metabolite uptake already carried out using ^{32}P sodium phosphate and ^{75}Se l-selenomethionine in similar infections.

Table 14

Mean ^{32}P and ^{75}Se Uptakes Expressed as % Day 6 Values

Group		Day of Infection		
		7	10	13
^{32}P	No. of Measurements	8	10	8
	Mean	93.8	30.9	3.9
	S.D.	21.9	17.7	1.7
	S.E.	± 7.7	5.6	0.6
^{75}Se	No. of Measurements	8	10	8
	Mean	99.5	29.2	5.5
	S.D.	17.2	14.7	2.4
	S.E.	6.1	4.6	0.9
	"t" test	N.S.	N.S.	N.S.

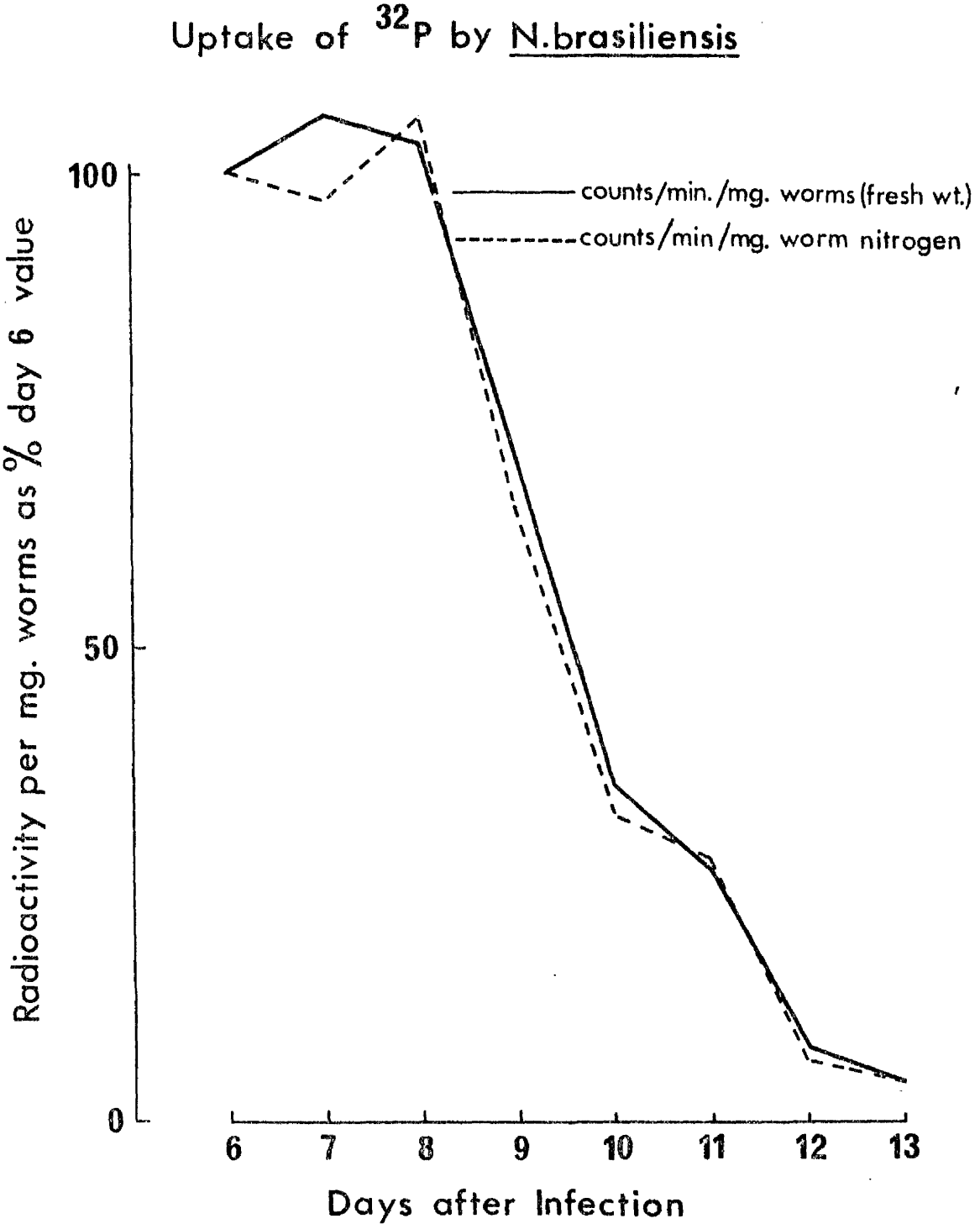

Fig. 7. Comparison of ^{32}P Uptakes Expressed in terms of Worm Weight and Worm Nitrogen

MATERIALS AND METHODS

Experimental Animals

Fifty rats which had each been infected with 5,000 larvae were used.

Radioisotope Injection

An ampoule of freeze-dried d-(u-^{14}C) glucose was diluted to 100 ml with sterile isotonic saline and stored at -4^{o}C in 10 ml volumes till required. Each day of the infection from day 6 till day 13, a group of rats were injected intravenously with the prepared radioactive glucose solution on the basis of 1.0 ml (10 µCi ^{14}C) per 150 g weight.

Worm Collection

Five hours after injection the rats were killed and the recovered worms were weighed, dried overnight and transferred to counting vials which were stored at -4^{o}C till the end of the experiment.

Worm Digestion

Two ml of Soluene 350 tissue solubiliser was added to the samples in the counting vials and these were placed in a water bath at 60^{o}C for 2 - 4 hours or until the worms had dissolved completely.

^{14}C Determination

Ten ml liquid scintillator was added to each vial and the ^{14}C count rates were determined in a liquid scintillation counter. Quench corrections were made by the internal standardisation method and the results were expressed as counts/minute/mg "fresh weight".

RESULTS

The results are shown in Table 15. A rapid fall in the uptake of ^{14}C between day 8 and 12 of the infection was observed. These results show an uptake pattern which is almost identical to that seen when ^{32}P labelled phosphate or ^{75}Se selenomethionine is used to monitor worm metabolism. A comparison of the uptake of the three metabolites used in these experiments is shown in Fig. 8.

DISCUSSION

The results confirm the findings of the experiments described in Chapter I. The timing and magnitude of the fall in uptake by the worms of each of the labelled metabolites is remarkably similar. There is no evidence of any significant difference in the uptake of any of the substances used. When account is taken of the different involvement in metabolism of the three substances tested, inorganic phosphate, an amino acid and glucose, it seems reasonable to assume that the metabolism or situation of the parasite is affected in some general way by the immune pressure exerted by the host and that any other labelled metabolite tested will show the same changes in uptake by the parasites.

The fall in uptake of metabolite by the adult N. brasiliensis precedes the start of worm expulsion by several days and the timing of the depression in metabolic activity coincides with

Table 15

Uptake of ^{14}C-Glucose by Adult N. brasiliensis

Day of Infection	No. of Rats in Group	Mean ^{14}C Count/ min/mg "fresh weight"	S.D.
6	5	540	49
7	6	506	47
8	6	495	152
9	5	284	116
10	6	204	89
11	6	94	58
12	6	61	17
13	7	52	10

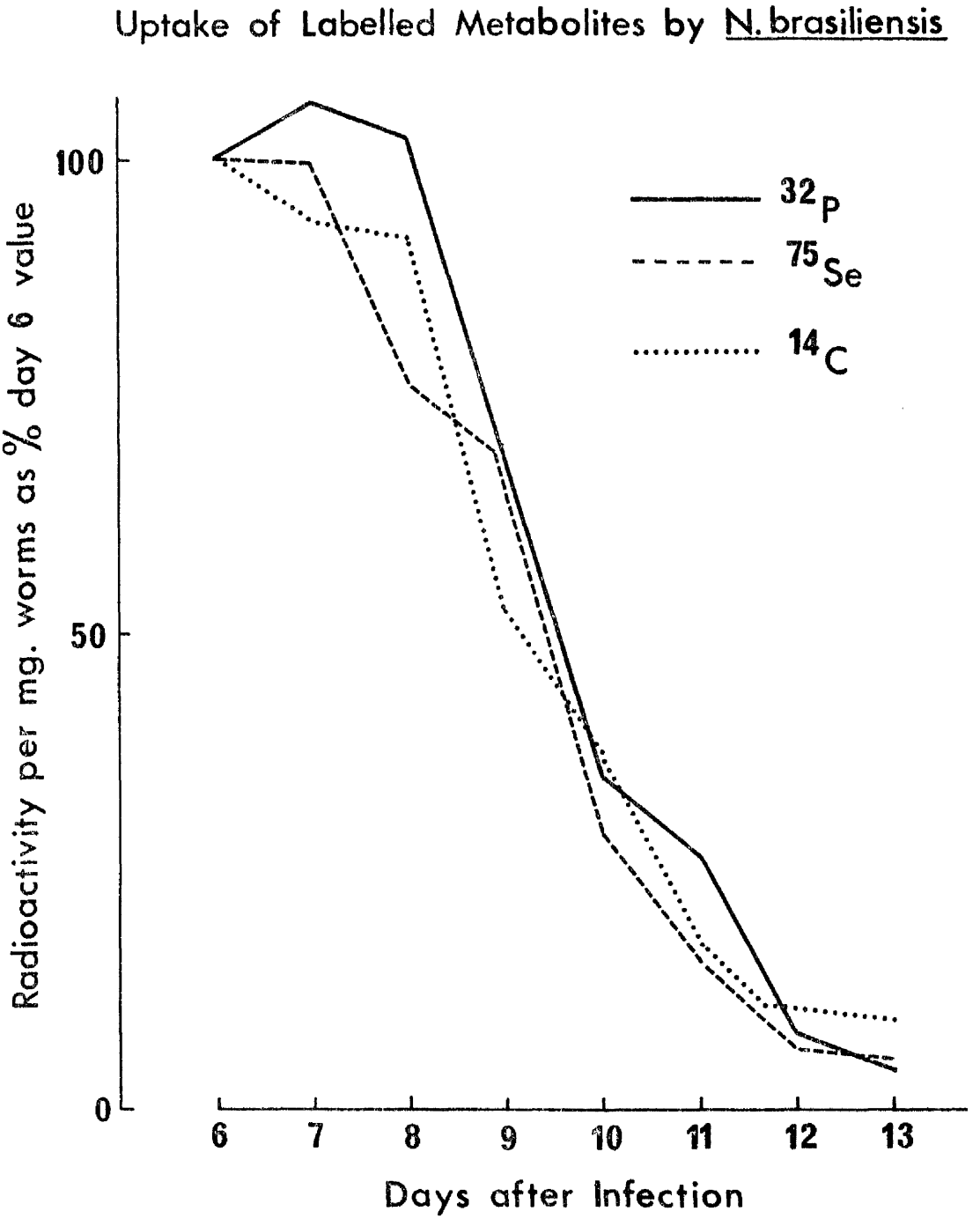

Fig. 8. Comparison of Uptakes of Labelled Metabolites

the structural changes observed in the worms by about day 10 of infection [27, 28]. It was also shown [27] that worms transferred to a fresh host after day 9 of a primary infection are less able to re-establish and recover egg production than those transplanted prior to day 9. The results of an experiment described earlier where the uptake of ^{32}P was measured in adult N. brasiliensis which had been transferred to fresh hosts on successive days of an infection show that worms transferred after day 9 are less able to recover their capacity to take up the ^{32}P than worms transplanted prior to day 9. These findings show that there is a close parallel between the effect of immunity on the capacity of the worms to take up metabolite from the host and on their ability to re-establish themselves and resume normal egg production. After day 9 of infection adult N. brasiliensis appear to have undergone irreversible damage due to the immune response of the host and this damage manifests itself in several ways, e.g. changes in morphology and egg production [27, 28] and in a reduced ability of the adult worms to take up metabolites from the host's tissue fluids as the infection progresses.

The changes referred to above may be secondary changes brought about by the immune pressure of the host on the parasite. It is possible that the immune response of the host may cause a displacement of the parasites from the intestinal villi into the lumen of the gut where the environment is less suitable for normal metabolism. Brambell [47] showed that such a movement of the parasites did occur. It was shown by Roberts and Fairbairn [48]

that N. brasiliensis has an aerobic metabolism. The more anaerobic conditions in the lumen of the gut possibly causes structural changes to occur in the worms due to the inability of the parasites to metabolise lipid which leads to a build up of lipid droplets in the worms [28]. A general impairment in worm function would follow leading to a drop in egg production and a reduced ability to take up metabolites from the host. It may simply be that displacement of the parasites away from the gut wall lengthens the diffusion path for metabolites passing from the host's tissue fluids and the parasite with a corresponding depression in uptake as measured over a standard time.

CHAPTER IV

THE RELATIONSHIP BETWEEN DEPRESSION IN METABOLITE UPTAKE AND WORM EXPULSION

INTRODUCTION

The changes observed in the uptake of labelled metabolites by the parasites in a primary infection appear to be an integral part of the changes in worm physiology associated with the development of host immunity but the possibility exists that they are merely coincidental in time with the onset of the immune response. The results of experiments to be described where the uptake of ^{32}P sodium phosphate and ^{75}Se Selenomethionine by adult N. brasiliensis is measured, where the onset of expulsion is brought forward and where it is delayed should give evidence to support or refute this view.

When rats are given a second infection of N. brasiliensis larvae, the numbers of larvae reaching maturity are reduced and the worm expulsion occurs earlier in the infection. It has been shown [49] that in second infections the phase of worm expulsion or "Loss Phase 2" occurs between days 5 and 10 of infection. In the experiment described below the uptake of ^{32}P and ^{75}Se by worms in a second infection was studied and the relationship between metabolic activity and worm expulsion was examined.

UPTAKE OF ^{32}P SODIUM PHOSPHATE AND ^{75}Se L-SELENOMETHIONINE BY *Nippostrongylus brasiliensis* WORMS IN A SECOND INFECTION

In the previous experiment it was shown that the uptake of metabolites by *N. brasiliensis* worms during a primary infection became depressed at about day 8 of the infection, i.e. 4 days prior to the onset of the immune expulsion of the worms. This experiment was designed to examine the relationship between metabolite uptake and worm expulsion where the expulsion process occurs several days earlier as in the case of second infections. In order to ensure that any deviation in metabolite uptake observed in worms from a second infection, compared with a primary infection, was not due to experimental error, a number of uptake measurements were carried out on worms from a primary infection using the same materials.

MATERIALS AND METHODS

Experimental Animals

Thirty rats which had been given an initial infection of 5,000 *N. brasiliensis* larvae 28 days previously were each infected with a second dose of 10,000 larvae per rat. At the same time a group of 20 rats were each given an initial dose of 5,000 *N. brasiliensis* larvae. This second group acted as "controls" for the experiment.

Radio-isotope Injection

A solution containing 20 µci ^{32}P sodium phosphate and 7 µci ^{75}Se l-selenomethionine per ml was prepared and stored at -4°C till

required. Six rats harbouring second infections were used on days 5, 6 and 7 of the infection and six of the "control" rats were used on days 6, 8, 10 and 13. The injections were given on the basis of 1.0 ml per 150 g body weight as in earlier experiments.

Worm Collection

The rats were killed five hours after injection of the isotope and the worms were recovered, weighed, dried and stored at -4°C till the end of the experiment.

Radioactivity Assay

The samples were assayed for ^{75}Se and ^{32}P as previously described, ^{32}P quench corrections being made by the internal standardisation method.

RESULTS AND DISCUSSION

The results of the ^{32}P and ^{75}Se uptakes are shown in Tables 16 and 17 respectively. It can be seen that the level of metabolite uptake in the second infection worms at day 5 is approximately 50 % of the levels found at day 6 or 8 in worms from a first infection. By day 7 in the second infection, worm expulsion was well under way and was complete by day 8. In both groups the metabolic activity fell to the same low level at the time of worm expulsion, day 7 in the second infection and day 13 in the primary infection. A comparison of the results for the uptake of both labelled metabolites in the first and second infections is shown in Fig. 9.

N. brasiliensis in a second infection is under immune pressure throughout the larval and adult stages. The comparatively

Table 16

Uptake of ^{32}P by N. brasiliensis in First and Second Infections: Group Means

	Day of Infection	No. of Rats in Group	Mean ^{32}P Count per min per mg "fresh wt"	S.D.
First Infection	6	6	753	119
	8	6	735	45
	10	4	278	17
	13	3	57	15
Second Infection	5	5	353	23
	6	6	84	27
	7	6	60	*

* Two samples of worms, each collected from 3 rats were counted.

Table 17

Uptake of ^{75}Se by *N. brasiliensis* in First and Second Infections: Group Means

	Day of Infection	No. of Rats in group	Mean ^{75}Se Counts per min per mg "fresh wt"	S.D.
First Infection	6	6	170	26
	8	6	148	18
	10	4	58	7
	13	3	10	4
Second Infection	5	5	90.0	11.7
	6	6	12.4	5.3
	7	6	10.0	*

* Two samples of worms, each collected from 3 rats were counted.

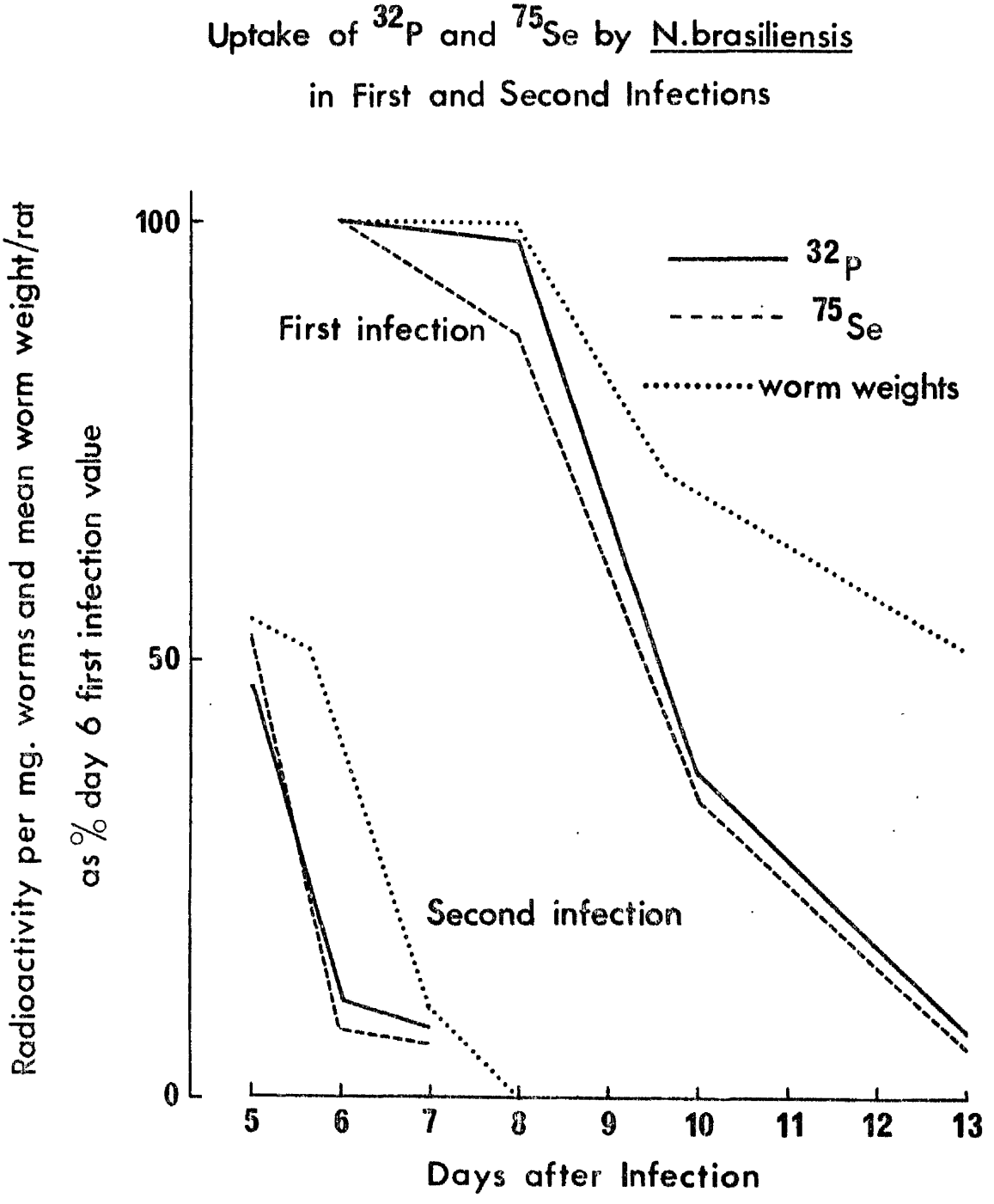
Uptake of ^{32}P and ^{75}Se by N.brasiliensis
in First and Second Infections
Radioactivity per mg. worms and mean worm weight/rat
as % day 6 first infection value
100
50
0
First infection
Second infection
^{32}P
^{75}Se
worm weights
5
6
7
8
9
10
11
12
13
Days after Infection

Fig. 9.

low level of metabolite uptake on day 5 in a second infection is clearly the result of the action of the host immunity on the parasites, i.e. the impairment in metabolite uptake at day 5 is not the result of the age of infection but of the immune response of the host.

It has been shown (see review[7]) that worms taken from a second infection are capable of re-establishing in clean rats and resuming normal egg production. These worms are also capable of longer survival in the new host compared with worms transplanted from a normal infection. The evidence suggests that worms from a second infection are less immunogenic than normal worms. It would be interesting to measure the uptake of metabolite in second infection worms after transfer to clean hosts to see if the parasites recovered their normal metabolic activity. This is an experiment which could easily be conducted using the techniques developed in this study.

So far it has been shown in normal N. brasiliensis infections and in second infections, where the worm expulsion occurs earlier, that there is a corresponding time relationship between the depression in the metabolic activity of the N. brasiliensis and the other observed changes in worm physiology [27, 28]. The assumption has been made that all of these changes are the result of immune pressure exerted by the host on the parasite.

If a situation can be engineered where the uptake of labelled metabolites by adult N. brasiliensis is measured in rats where the immune response is delayed, a more complete picture of the relationship between worm metabolism and the host's immune response will be seen.

Following evidence that some protozoal infections appear to exert an immunosuppressive effect on the response of the host to other antigens, a study was made by Urquhart and colleagues [50] of the effect of Trypanosoma infections on the immune response of the host to a N. brasiliensis infection which had been superimposed on rats infected with Trypanosoma brucei. It was shown that the normal immune expulsion of adult N. brasiliensis worms did not occur. The numbers of worms found in the intestines of the T. brucei infected rats at day 18 of the infection had not fallen from the initial plateau level. The production of circulating and reaginic antibodies had been grossly impaired and the population of mast cells in the intestinal villi had not risen as in normal infections.

It appears that a situation can be produced where the immune response of the host to N. brasiliensis infections can be significantly suppressed and it would be interesting to measure the uptake of metabolites by the worms in rats immunosuppressed in this way.

An experiment is described below where parasite metabolism was monitored in normal infections and in rats infected with T. brucei and N. brasiliensis infections (double infected rats). These measurements were correlated with faecal egg counts and worm numbers remaining in the rat during the experiment and the relationship between metabolite uptake and immune expulsion established.

THE EFFECT OF IMMUNOSUPPRESSION ON THE UPTAKE OF ^{32}P SODIUM PHOSPHATE AND ^{75}Se SELENOMETHIONINE BY ADULT *Nippostrongylus brasiliensis*

^{32}P and ^{75}Se Uptakes in Rats Infected with 5,000 *N. brasiliensis* Larvae Three Days after Trypanosome Infection

In this experiment an attempt was made to inhibit the immune response of the host by infecting rats with trypanosomes three days prior to the *N. brasiliensis* larvae being administered. The metabolic activity of the worms harboured by these rats was compared to that of worms in a normal infection, with a view to examining the relationship between any delay in worm expulsion and metabolite uptake. *N. brasiliensis* faecal egg counts, which give a good indication of the effect of immunity on the worms, were made at day 7 of the infection and at intervals from day 11 till the end of the experiment. On day 14 of the *N. brasiliensis* infection the trypanosomes were eliminated from a group of the double infected (immunosuppressed) rats to allow metabolite uptake measurements to be made after the removal of the organisms reponsible for the suppression of the immune response to *N. brasiliensis*.

MATERIALS AND METHODS

Trypanosome Infections

Eighty rats were infected by intraperitoneal injection of a strain of *Trypanosoma brucei* derived from a stabilate of TREU 667. Each rat received 0.8×10^6 trypanosomes. The double infected

rats to be used on each day of the metabolite uptake studies were screened for trypanosomiasis by examining fresh wet blood under a high power objective.

N. brasiliensis Infections

Three days after being infected with trypanosomes each rat was given 5,000 N. brasiliensis larvae. At the same time a group of 20 normal rats were given 5,000 larvae each, the latter group acting as controls for the experiment.

Isotope Injection

An isotope mixture containing 20 µci ^{32}P sodium phosphate and 7 µci ^{75}Se 1-selenomethionine was prepared and stored as previously described and the injections were given on the basis of 1.0 ml per 150 g body weight. On alternate days from day 6 till day 16 of the N. brasiliensis infection six of the double infected rats were used and the control rats were used on days 6, 8, 10 and 12.

Berenil Treatment

In order to eliminate the trypanosome infection in a group of the double infected rats, 24 of these rats were treated intraperitoneally on day 14 of the N. brasiliensis infection with 0.15 ml of a solution containing 22.8 mg Berenil (diminazene aciturate, Hoechst Pharmaceuticals) per 1.0 ml.

N. brasiliensis Faecal Egg Counts

In the double infected rats, counts were made on day 7 of the infection and on alternate days till day 31.

In the control rats egg counts were made on day 7 and on days 11 and 13 of the infection.

Egg counts were made in the treated rats on days 17, 19 and 24.

Worm Counts

On days 24 and 31 of the infection a count of the worms remaining in the rats of the double infected group was made.

Worm Collection and Radioactivity Assay

The procedures previously described were followed and the samples were assayed for ^{32}P and ^{75}Se.

RESULTS AND DISCUSSION

Faecal Egg Counts

On day 7 of the N. brasiliensis infection the egg counts for the control and double infected groups were 233 x 10^3 and 275 x 10^3 eggs per gramme respectively. In the control group the counts on days 11 and 13 were 6.4 x 10^3 and 1.4 x 10^3 respectively, in keeping with the pattern normally found at the time of "self-cure". In the double infected rats the egg counts were 134.7 x 10^3 on day 11, 38 x 10^3 on day 13 and from day 17 till day 24 when the last counts were made the egg counts remained at the very low level of approximately 0.2 x 10^3 eggs/g. faeces (see Fig. 10). The fact that N. brasiliensis eggs are produced at the rate of 200 eggs/g faeces between days 17 and 24 of infection in the double infected rats indicates that a greater number of parasites survived the immune expulsion than is the case in a normal infection.

Worm Counts

After day 16 of the N. brasiliensis infection the numbers of worms remaining in the double infected and treated groups were insufficient to give samples of the minimum weight necessary

for proceeding with metabolite uptake studies even if the daily collections of worms from each group were pooled and consequently such measurements were discontinued. There was a wide variation in the numbers of worms recovered after day 16 and it was obvious that by this time the "self-cure" reaction was well established. The numbers of worms recovered from the double infected and treated rats on days 24 and 31 of the infection are shown in Table 18. These numbers are significantly higher than those found after "self-cure" in a "normal" primary infection. The presence of worms in these numbers at day 31 indicates that the immune response of the rats has been somewhat attenuated by the trypanosome infection.

Trypanosome Counts

All double infected rats used in this experiment were found to be parasitaemic with respect to trypanosomes. Two days after Berenil treatment the treated rats were found to be free of trypanosomes.

Metabolite Uptake

^{32}P Uptake

The results are shown in Table 19. There is a significant difference on days 10 and 12 of infection between the uptake of ^{32}P by the parasites in both groups of rats, i.e. 64 and 46 counts/min/mg in the normal infection and 307 and 362 counts/min/mg in the immunosuppressed rats on days 10 and 12 respectively.

^{75}Se Uptake

The results are shown in Table 20. Slightly larger differences are shown in the uptake of ^{75}Se between the two

Table 18

N. brasiliensis Recovered From Double Infected and Treated Rats

	Day of Infection	
	24	31
	63	101
	218	153
Double Infected	274	378
		124
		106
		432
		60
	37	108
Treated	8	66
	377	24
		53

Table 19

Uptake of ^{32}P by Adult *N. brasiliensis* in Normal and Immunosuppressed Rats
Group Means

Group	Mean ^{32}P Counts/min/mg "fresh weight"					
	Day of Infection					
	6	8	10	12	14	16
Normal Infection	439	400	64	46		
S.D.	140 (4)	83 (4)	16 (4)	4 (4)		
Immuno-suppressed	414	459	307	362	95	52
S.D.	44 (6)	50 (6)	145 (5)	118 (5)	65 (4)	3 (6)
't' test	N.S.	N.S.	$p < 0.05$	$p < 0.01$		

Figures in parenthesis refer to the number of rats in each group.

Table 20

Uptake of ^{75}Se by Adult N. brasiliensis in Normal and Immunosuppresed Rats
Group Means

Group	Mean ^{75}Se Count/min/mg "fresh weight"					
	Day of Infection					
	6	8	10	12	14	16
Normal Infection	122	87	15	6.1		
S.D.	31 (4)	15 (4)	10 (4)	2.2 (4)		
Immuno-suppressed	122	115	87	104	7.2	9.4
S.D.	18 (6)	7 (6)	17 (5)	24 (5)	0.4 (4)	1.7 (6)
't' test	N.S.	$p<0.01$	$p<0.001$	$p<0.001$		

Figures in parenthesis refer to the number of rats in each group.

groups compared with the uptake of ^{32}P above. On days 10 and 12 respectively, the uptake of ^{75}Se by the parasites in the normal infection are 15 and 6.1 counts/min/mg and in the immunosuppressed group, 87 and 104 counts/min/mg.

The pattern of uptake in the control group is similar to that observed in previous experiments, with a rapid fall in metabolic activity between days 8 and 10 of the infection. In the immunosuppressed rats the fall in metabolic activity does not occur till after day 12 when there is a dramatic fall and by day 14 or 16 of the infection the level of metabolite uptake is similar to that in a normal infection at the time of the expulsion of the parasites. A comparison of the uptake of both labelled metabolites and of N. brasiliensis faecal egg counts in the immunosuppressed and control rats is shown in Fig. 10.

It is apparent that the "self-cure" had commenced before the double infected animals were treated to eliminate the trypanosomes and no conclusions can be drawn from this part of the experiment.

The onset of worm expulsion in the double infected rats appears to have been delayed by about four days if either fall in egg production or depression of labelled metabolite uptake is taken as an indication of worm disfunction resulting from the immune response of the host. As in the normal infection the fall in metabolic activity of the worms harboured by the immunosuppressed rats preceded the expulsion of the worms by several days.

It should be noted that although worm expulsion in the immunosuppressed rats had commenced by day 16 of infection there were still a substantial number of worms remaining in the rats

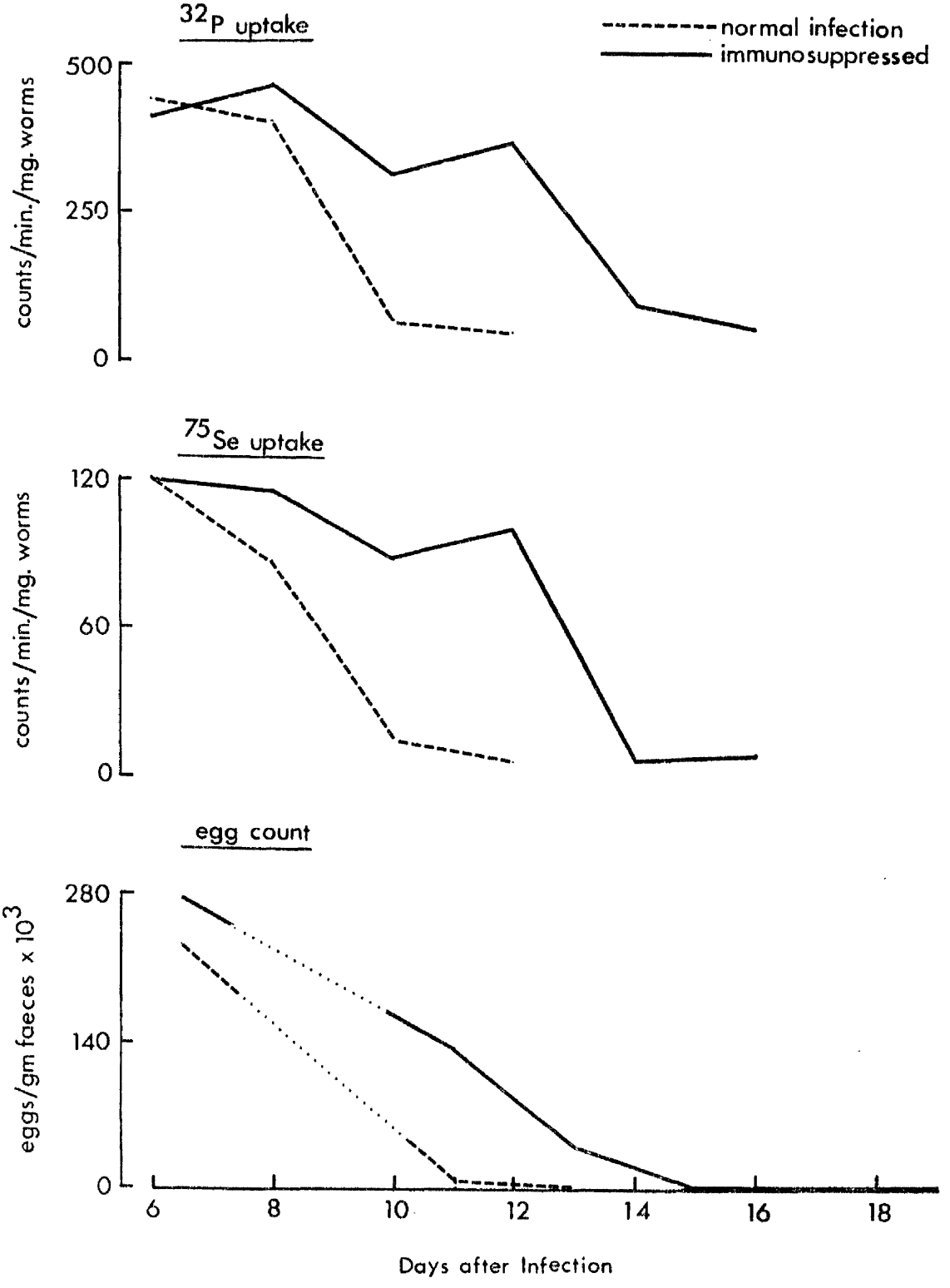

Fig. 10. Uptake of Labelled Metabolites by N. brasiliensis and Faecal Egg Counts in Normal and Immunosuppressed Rats.

at day 31 of infection. It was not possible to continue measuring the uptake of labelled metabolites by the N. brasiliensis in the double infected rats after day 16 of the infection since the numbers of worms remaining in the rats had fallen to levels below which the measurements could be made, unless a very large number of double infected rats had been available from which bulked samples of worms could be gathered and prepared for radioactivity assay. However since the level of uptake of labelled metabolites by these worms had fallen to a very low level at day 16 of infection (see Fig. 10) it is likely that the worms remaining in the immunosuppressed rats after the expulsion of the majority of the worms were, in fact "damaged" worms which would show levels of metabolite uptake similar to those observed at the time of "self-cure" in both groups of rats (Fig. 10).

It is obvious that the degree of immunosuppression produced in this experiment was limited in intensity and duration. However the relationship previously observed in normal and second infections between the timing of the reduced uptake of labelled metabolites by the parasites and their eventual expulsion was again evident in the present experiment where both phenomena were delayed by 3 - 4 days compared with normal infections.

Urquhart and colleagues [50] found that where 1,000 N. brasiliensis larvae were given to rats three weeks after infecting with trypanosomes, worm expulsion had not occurred at day 18 of infection in the double infected animals and the production of circulating antibody (IgG) and reaginic antibody (IgE) was grossly impaired. While there is some speculation as to how a trypanosome

infection might affect the immunity to a superimposed N. brasiliensis infection, these findings suggested that the antibody mediated or first stage of the worm expulsion process was inhibited. The exact mechanism involved is not of prime importance to this study. These experiments [50] did not include investigations into structural changes occurring in the parasites during the period of study or measurement of worm metabolite uptake. These workers [50] also found that cell mediated immunity did occur in the double infected rats, though not to the same extent as in animals not infected with T. brucei. It is not possible to directly compare the results of the experiments described in this study with the work of Urquhart and co-workers [50] since in the latter case the N. brasiliensis was superimposed on T. brucei infections of three weeks duration.

The results obtained from the T. brucei induced immunosuppressed rats indicate, without elucidating the factors operating, that the reduced metabolic activity of the adult N. brasiliensis observed in normal infections, as measured by the uptake of radio-isotopically labelled metabolites, is delayed by several days due to the temporary inhibition of the immune response of the host to the N. brasiliensis and that there is a corresponding delay in the onset of worm expulsion, i.e. the delay in the reduced worm metabolic activity is a direct result of the later immune response of the host. These results give further support for the theory that the changes observed in the uptake of labelled metabolites by the worms as an infectiom progresses is the direct consequence of the immune pressure exerted by the host. Again the disfunction in worm physiology as measured by the uptake of labelled metabolites occurred several days prior to the onset of expulsion of the parasites.

Other methods are available for suppressing or inhibiting the immune response of an animal, e.g. thymectomy, treatment with antilymphocyte serum or the use of immunosuppressive drugs, but the latter substances may be toxic and could affect the parasite as well as the host. Where the primary aim is to establish or enhance a parasitic infection or delay the termination of such an infection, the practice of subjecting the host to ionising radiation has been widely used (see [51, 52, 53]).

An experiment is described below where the rats were irradiated prior to receiving a N. brasiliensis infection, in order to produce an immunosuppressive effect and the uptake of both ^{32}P and ^{75}Se by the adult N. brasiliensis was measured during the infection.

UPTAKE OF ^{32}P SODIUM PHOSPHATE AND ^{75}Se SELENOMETHIONINE BY ADULT N.brasiliensis IN IRRADIATED AND NORMAL RATS

Jones and Ogilvie [33] showed that a radiation dose of 750 rad destroys a factor, not transferred by antiserum, which is essential for worm expulsion. Radiation doses of 300 - 500 rad [51] and 440 - 500 rad [54] have been used to produce immunosuppression in trypanosome infections in rats. The chief limiting factor in the choice of radiation dose is the susceptibility of the gut epithelium to large doses [54]. It has been shown [51] that large doses of radiation tend to cause a breakdown of the intestinal mucosa and since this is the habitat of N. brasiliensis caution was exercised in selecting the radiation dose. A dose, 370 rad,

thought to be sufficient to cause some degree of immunosuppression and yet have the minimum deleterious effect on the parasites was chosen.

The effect of sublethal doses of radiation on antibody forming capacity is transitory (see [52]) and recovery starts after 7 days and can be complete after two months even after relatively large doses. The immunosuppressive effect of radiation is most marked between 24 and 48 hours after irradiation [54]. In order to produce the maximum immunosuppressive effect during the larval stages of the N. brasiliensis infection when the stimulation of host immunity normally begins, the host rats were irradiated immediately prior to receiving the N. brasiliensis infection.

In the experiment described below the uptake of ^{32}P and ^{75}Se by adult N. brasiliensis was measured in irradiated and normal rats between days 6 and 15 of infection.

MATERIALS AND METHODS

Experimental Animals

Sixtysix female hooded Lister rats approximately 150g weight were used.

Irradiation

Fifty rats were each irradiated in a ^{60}Co radiation source for 11 seconds so that each rat received a whole body radiation dose of 370 rad.

N. brasiliensis Infections

The fifty irradiated rats and sixteen normal rats (to act as controls for the experiment) were infected with 5,000 N. brasiliensis larvae each.

Radioisotope Injection

A solution containing 20 µci ^{32}P sodium phosphate and 7 µci ^{75} Se selenomethionine was prepared and stored at -4°C in 10.0 ml aliquots till required. On days 6, 8, 10 13 and 15 of infection six of the irradiated rats were used and on days 6, 10 and 13 four of the normal infected rats were used. Each rat was injected on the basis of 1.0 ml isotope solution per 150g body weight.

Recovery of Worms and Radioactivity Assay

Five hours after injection the worms were recovered, washed, weighed and prepared for radioactivity assay as previously described.

The samples were assayed for ^{75}Se in a scintillation counter.

Following ^{75}Se assay the samples were digested in sulphuric acid as described previously and assayed for ^{32}P in a liquid scintilllation counter by the Cerenkov method, quench corrections being made by the internal standardisation method.

The results were expressed as counts/minute/mg "fresh weight".

RESULTS AND DISCUSSION

In the irradiated rats comparatively large worm burdens were found at day 15 of infection, but by day 17 expulsion of the worms was almost complete, i.e. the "self-cure" in the irradiated rats had been delayed by about four days as a result of the irradiation of the host.

The results of the uptake of ^{32}P and ^{75}Se by the parasite are shown in Tables 21 and 22 respectively and a comparison of these

Table 21

Uptake of ^{32}P by Adult *N. brasiliensis* in Irradiated and Normal Rats

Group	Mean counts/min/mg "fresh weight"				
	Day of Infection				
	6	8	10	13	15
Normal Infection	764		340	60	
S.D.	105 (4)		228 (4)	9 (4)	
Irradiated	448	513	467	133	42
S.D.	93 (6)	80 (5)	171 (6)	71 (5)	10 (6)
't' test p	0.01		N.S.	N.S.	

* The figures in parenthesis refer to the number of rats in each group.

Table 22

Uptake of ^{75}Se by Adult N. brasiliensis in Irradiated and Normal Rats

Group	mean counts/min/mg "fresh weight"				
	Day of Infection				
	6	8	10	13	15
Normal Infection	125		51	*	
S.D.	18 (4)		36 (4)		
Irradiated	84	90	68	16	3.1
S.D.	16 (6)	17 (5)	16 (6)	10 (5)	1.2 (5)
't' test	p 0.01		N.S.		

* Radioactivity statistically not significant. The numbers in parenthesis refer to the number of rats in each group.

results in both groups of rats is shown in Fig. 11. It should be noted that studies of metabolite uptake by the N. brasiliensis in the normal infected group were conducted on days 6, 10 and 13 and changes occurring in the uptake of metabolite between days 6 and 10 and on days 11 and 12 are not recorded. The N. brasiliensis in the normal infected rats act as controls for the experiment.

Two interesting points arise from the results of the metabolite uptake studies.

(a) At day 6 and 8 of infection the level of metabolite uptake in the irradiated group was lower than in the normal infection (see Fig. 11). On day 6 there is a statistically significant difference between the two groups ($p < 0.01$). This initial depression in metabolite uptake may be due to radiation damage to the epithelium of the host's gut and is perhaps an indication that the results of experiments where animals have been subjected to radiation should be interpreted with caution.

(b) Given that there is an initial depression in the uptake of metabolite by the parasites in the irradiated hosts, the fall in uptake normally associated with the onset of the "self-cure" was delayed by 2 - 3 days.

It is obvious that the relatively low level of radiation to which the rats were subjected has produced a transient and low level of immunosuppression but nevertheless the aim of the experiment, to establish that there is a relationship in time between the suppression of the uptake of metabolites by the adult N. brasiliensis and the eventual expulsion of the worms, is satisfied. These results complement those obtained when immunosuppression was

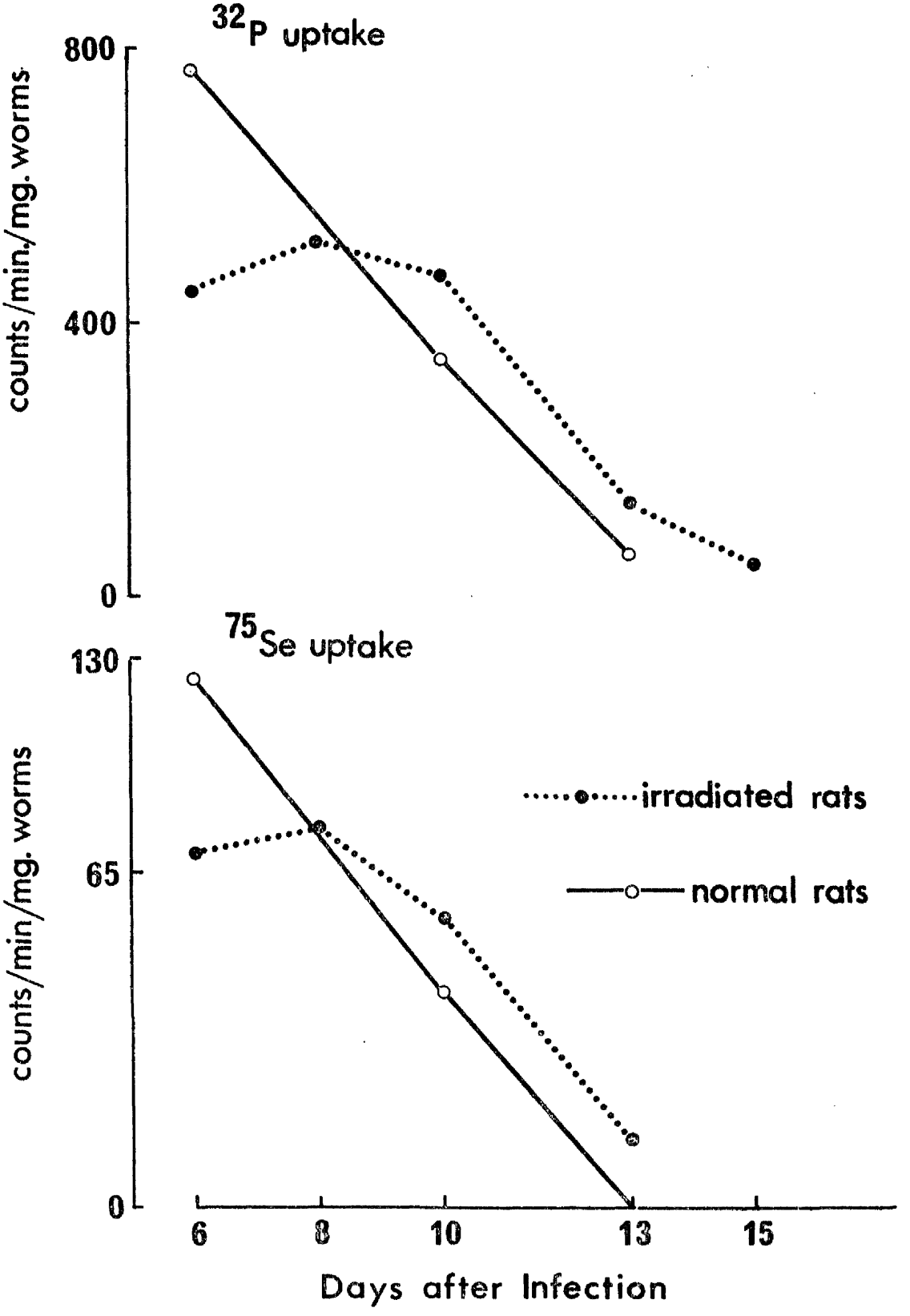

Fig. 11. Uptake of Labelled Metabolites by N. brasiliensis in Normal and Irradiated Rats.

induced by infecting rats with T. brucei prior to receiving the N. brasiliensis infection.

A study of the experiments described in Chapter IV shows that a delay in worm expulsion is reflected in a delay in the depression of worm metabolic activity, and conversely, where the expulsion process is accelerated as in second infections, there is a correspondingly early depression in the uptake of nutrient. All the indications are that the depression of worm metabolic activity, the simultaneous reduction in N. brasiliensis egg output and the structural changes observed in worms by day 10 of infection [27, 28] occur as a result of the immune response of the host to the infections. In normal infections, the changes in the above parameters occur simultaneously and it seems unlikely that there is a specific anti-enzyme antibody as suggested by Chandler [35] operating against the capacity of the parasites to assimilate nutrients.

GENERAL DISCUSSION

Immunity to gastrointestinal parasites, which has been described in many systems, manifests itself in several ways. A proportion of the larvae may be destroyed or inhibited in the larval stages; adults may be destroyed *in situ*, egg laying capacity can be reduced or the parasite may be eliminated by the spontaneous expulsion of the adults by the host. Rats infected with *N. brasiliensis* have been used as a model in many studies on the mechanism of immunity to gastrointestinal helminths. Although all the factors involved and the exact sequence of events leading to the termination of a *N. brasiliensis* infection have still to be elucidated, a great deal of information has been amassed on the circulating, reaginic and cellular antibodies involved and on the kinetics of expulsion (see reviews [7, 8, 9, 10, 11, 12, 13]).

Until comparatively recently most of the studies into immunity to *N. brasiliensis* have concerned changes occurring in the host due to the presence of the worms. To gain a better understanding of the factors leading to worm expulsion it is necessary to study the functional aterations taking place in the parasite as immunity develops. Ultimately the effectiveness of the immune response can best be measured in terms of damage inflicted on the parasite.

In studies on the effect of immunity on the parasites in *N. brasiliensis* infections Ogilvie and Hockley [27] observed that marked

changes occur in the gut cell structure of the adult worms as immunity develops. These correspond to alterations in worm infectivity and reproductive capacity and are visible before the start of worm expulsion. Worms transferred to clean rats after this time are less able to establish and resume normal functions. These changes were shown to be the result of host immunity and not senescence, i.e. the parasites had sufferred irreversible alterations in structure and function prior to the start of "self-cure". The modifications observed in the cells of the N. brasiliensis intestine could have serious consequences for the worms, e.g. a reduction in the absorption of nutrients. Since changes in the structure of the gut cells correspond with modifications in infectivity and reproductive capacity, each presumably the result of the developing host immunity, structural changes in the gut cells give a measure of the damage inflicted on the parasites due to host immunity.

Lee [28], in investigations into changes occurring in adult N. brasiliensis due to the development of immunity found that alterations in nematode cytology occur and that these can be correlated with the onset and development of host immunity. The most striking features noted were evidence by day 12 of infection of reabsorption of spermatozoa by the male digestive tract and the appearance of lipid droplets widely distributed throughout the parasite.

Chandler [35], believing that immunity had an adverse effect on the nutrition of the parasite put forward the theory that anti-enzymes develop which act against the digestive enzymes of the

parasite. Thorsen [36] produced evidence to support this idea but the theory has never been substantiated. It seemed that the relationship between the nutrition of the parasites and the development of host immunity was a subject which could profitably be pursued if a suitable method could be found for making the necessary measurements.

Rogers and Lazarus [22], had shown that ^{32}P labelled inorganic phosphate injected intramuscularly into host rats could be detected in worms recovered from the gut five hours later. This suggested that the parasites depend on the host's tissue fluids for their nutrition. Using this basic idea, a method was developed and used to measure the uptake of metabolites by adult N. brasiliensis from the tissue fluids of the host.

Techniques using substances trace labelled with radioactive isotopes have been extensively used in in vivo studies of metabolic processes in animals and micro-organisms. Isotopic methods allow the measurement of biological processes to be made which would be difficult or impossible by other methods. Tracer techniques have the great advantage that they can be used to conduct in vivo studies and by remvoing body fluids, tissues or micro-organisms from the animal after the administration of the labelled substance and measuring the radioactivity, metabolic changes can be accurately measured as they occur. In choosing a radioactive label for tracer experiments it is important to ensure that the half-life ($t\frac{1}{2}$) of the isotope is sufficiently long so that the radioactivity does not decay away before the end of the experiment, that the label is not toxic and that the cost is not prohibitive since some

labelled substances can be extremely expensive. Because of the relatively high efficiency achieved in determining the radioactivity of samples, very small quantities of labelled material can normally be used and in most cases sample preparation is simple. Disadvantages are few except for the expensive equipment required to measure the radioactivity of samples, but most laboratories using radioactive isotopes have access to such equipment.

Although the method used in the experiments described in Chapter I appeared to serve the purpose well, some of the procedures were open to criticism and it was desirable to make changes to improve the techniques. The time and effort spent in studying and perfecting the materials and methods was rewarding, in that the results of the experiments described in Chapters III and IV could be accepted and interpreted without doubts that observed changes in the uptake of metabolites by the parasites could be distorted by faulty techniques.

In Chapter III, using the methods finally adopted, a study was made of how development of host immunity affects the uptake of metabolites by the adult N. brasiliensis. One of the advantages of radio-isotope techniques is that, provided different types of energy emitters are used, e.g. γ and β, or the energy levels of the isotopes are different, each isotope can be separately assayed. This allows more than one labelled substance to be used simultaneously to measure more than one parameter. Using this technique, infected rats were injected with a mixture of ^{32}P inorganic phosphate and ^{75}Se selenomethionine in several experiments

to study the uptake of ^{32}P and ^{75}Se by adult N. brasiliensis. This double isotope technique not only saves time and effort by producing two sets of results from one experiment but gives results which can be confidently compared since both measurements are made in the same group of worms or rats.

When metabolite uptake by adult N. brasiliensis was measured in a normal infection it was seen that, by day 10 of infection, the uptake of both ^{32}P and ^{75}Se had fallen to approximately 30% of the day 6 value. When the uptake of ^{14}C from injected ^{14}C-glucose was measured the results were remarkably similar to those obtained using ^{32}P phosphate and ^{75}Se selenomethionine, (see Fig. 8). The fall in worm metabolic activity is well established by day 10 of infection and reaches a very low level by day 12 or 13 when rapid expulsion of the worms occurs.

It appears that there is a general impairment of worm metabolism, rather than interference with specific metabolic processes. The timing of the reduction in uptake of metabolites by the parasites corresponds closely to the structural and other changes known to occur in the adult N. brasiliensis by day 10 of infection [27, 28]. These workers showed that worms transferred to clean rats after day 9 of infection were less able to establish and resume reproductive processes than worms transplanted before this time. When uptake of metabolite by transplanted worms was measured, a similar picture emerged, i.e. there was a correlation between the ability of the worms to resume reproductive processes and to regain a normal level of metabolic activity.

The idea put forward by Chanlder [35] that developing host immunity has an adverse effect on the nutrition of the parasite is undoubtedly true but, rather than being the result of specific interference with the digestive processes of the parasite, it seems probable that the cause is a general decrease in worm metabolic activity which in turn is only one of several inter-related changes occurring in worm structure and function. These alterations may only be secondary changes brought about by displacement of the parasites to a less favourable part of the intestine as a result of some process triggered off by the immune response of the host, e.g. local oedema. What clearly emerges is that there is a reduction in the uptake of labelled metabolites by the parasites which is evident from day 8 of infection and this phenomenon is the result of developing host immunity.

The uptake of ^{32}P and ^{75}Se was measured in second infections where the parasites are under immunological pressure throughout the larval and the adult stages. In these second infections the metabolic activity of the worms at day 5 was approximately 50% of that at day 6 in the normal infection measured at the same time, and dropped to a very low level by day 6, two days prior to the expulsion of the worms. These results suggest that at day 5 of a second infection the immune pressure of the host has substantially altered the metabolism of the worms and again the expulsion of the worms was preceded by a severe deterioration in the metabolic status of the parasites. The same relationship is observed in a second infection as in the primary infections between reduced metabolite uptake and worm expulsion, reinforcing the theory

that the reduced worm metabolism is caused by the immune response of the host and not by any other factor, e.g. worm senescence.

Uptake of ^{32}P and ^{75}Se by adult N. brasiliensis was measured where the immune response of the host was suppressed or attenuated by superimposing the N. brasiliensis infection on rats previously infected with T. brucei. However the degree of immunosuppression produced was limited and did not correspond with the results obtained by Urquhart and co-workers [50], who found that N. brasiliensis expulsion had not commenced at day 18 of infection in trypanosome infected rats.

Nevertheless the results of this experiment showed that where the immune response of the host was delayed by immunosuppression the timing of the reduced metabolic activity of the parasites was delayed as well as the expulsion of the worms. Again the timing of the reduced uptake of labelled metabolites by the N. brasiliensis was shown to be related to the timing of the immune response of the host and not to the age of infection.

A final experiment was carried out where the uptake of both ^{32}P and ^{75}Se by adult N. brasiliensis was measured in rats where the expulsion of the worms was delayed by whole body irradiation of the host rats prior to the administration of the larvae. Because large sub-lethal doses of radiation can disrupt the cellular structure of the gut epithelium [51, 54] these rats were subjected to the relatively low radiation dose of 370 rad.

The effect of this comparatively low level of irradiation was to produce a delay of about 4 days in the worm expulsion. For some reason, perhaps associated with irradiation damage to the

intestinal epithelium, the level of metabolite uptake in the irradiated rats at day 6 of infection was about 60% of the corresponding measurements in the normal (control) rats. The depression in metabolite uptake by the worms in the irradiated rats occurred at about two days later than normal but the rate of fall in uptake was similar to that found in the control rats (Fig. 12). Again a direct relationship between depression in worm metabolic activity and expulsion was demonstrated.

An analysis of the results of the experiments conducted into changes in the uptake of metabolites by adult N. brasiliensis as immunity develops, shows beyond reasonable doubt, that one of the consequences of the immune response of the host is a rapid fall in the uptake of metabolites by the parasites from the tissue fluids of the host.

These results, however, do not give any indication as to the exact mechanism operating to cause the uptake of labelled metabolites by N. brasiliensis to fall as an infection progresses. This could arise as a result of antibody damage to the worms which could also be responsible for the structural and functional changes noted by Ogilvie and Hockley [27] and Lee [28]. On the other hand these changes [27, 28] and the apparent impairment of the ability of the worms to take up metabolites could be the result of the immune response of the host causing a displacement of the worms from the walls of the intestinal villi to a less favourable intestinal environment where normal metabolism is more difficult to maintain. It has been shown that such a movement occurs [47]. N. brasiliensis has an aerobic metabolism [48] and the more anaerobic conditions in the lumen of the gut may have an adverse effect on the physiology

of the parasites leading to the changes in worm function already noted. Yet again the reduced uptake of metabolites by the parasites could simply be the result of displacement of the worms from close contact with the tissue fluids of the host further into the lumen of the gut where the diffusion path of metabolites would be lengthened causing a reduced uptake of labelled metabolite in a given time, leading to the assumption that the ability of the worms to take up metabolites had per se been impaired.

The next obvious step is to try and clarify this point. Perhaps the best approach is to study the uptake by the parasites of labelled metabolites introduced directly into the lumen of the gut. As such substances will be in direct contact with the worms any fall off in uptake cannot be attributed simply to lengthening of the diffusion path. Such experiments are now under way.

REFERENCES

1. Yokagawa, S. (1922). Parasitology, 14, 127.
2. Haley, A.J. (1961). J. Parasit., 47, 727.
3. Stoll, N.R. (1929). Am. J. Hyg., 10, 384.
4. Africa, C.M. (1931). J. Parasit., 18, 1.
5. Taliaferro, W.H., Sarles, M.P. (1937). Science, N.Y., 85, 49.
6. Haley, A.J. (1962). J. Parasit., 48, 13.
7. Ogilvie, B.M., Jones, V.E. (1971). Expl. Parasit., 29, 138.
8. Urquhart, G.M., Jarrett, W.F.H., Mulligan, W. (1962). Adv. vet. Sci., 7, 87.
9. Mulligan, W. (1968). "Immunity to Intestinal Helminths: the 'Self-cure' Reaction". In Immunity to Parasites. Sixth Symposium of the British Society for Parasitology. (Ed. by A.E.R. Taylor). Blackwell Scientific Publications, Oxford.
10. Soulsby, E.J.L. (1968). "The Reaction of the Host to Parasitism". (Ed. by E.J.L. Soulsby). Proc. 3rd. Int. Conf. World Assoc. Adv. Vet. Parasitol., 1967.
11. Jarrett, E.E.E., Urquhart, G.M. (1971). Int. Rev. trop. Med., 4, 53.
12. Murray, M. (1972). "Immediate Hypersensitivity Effector Mechanisms. II. In vivo Reactions". In Immunity to Animal Parasites (Ed. by E.J.L. Soulsby). Academic Press, London.
13. Ogilvie, B.M., Love, R.J. (1974). Transplant Rev., 19, 147.
14. Sarles, M.P., Taliaferro, W.H. (1936). J. inf. Dis., 62, 337.
15. Neilson, J.T.M. (1965). Ph.D. Thesis, University of Glasgow.

16. Mulligan, W., Urquhart, G.M., Jennings, F.W., Neilson, J.T.M. (1965). Expl. Parasit., 16, 341.

17. Ogilvie, B.M., Jones, V.E. (1968). Parasitology, 58, 939.

18. Taliaferro, W.H., Sarles, M.P. (1939). J. inf. Dis., 64, 157.

19. Rogers, W.P. (1949). Aus. J. scient. Res. B. 2, 157.

20. Davenport, H.E. (1949). Proc. R. Soc. B. 136, 271.

21. Neilson, J.T.M. (1969). Parasitology, 59, 123.

22. Rogers, W.P., Lazarus, M. (1949). Parasitology, 39, 245.

23. Barth, E.E.E., Jarrett, W.F.H., Urquhart, G.M. (1966). Immunology, 10, 459.

24. Ogilvie, B.M. (1967). Immunology, 12, 113.

25. Urquhart, G.M., Mulligan, W., Eadie, R., Jennings, F.W. (1965). Expl. Parasit., 17, 210.

26. Maclean, J.M. (1974). M.I. Biol. Thesis, University of Glasgow.

27. Ogilvie, B.M., Hockley, D.J. (1968). J. Parasit., 54, 1073.

28. Lee, D.L. (1969). Parasitology, 59, 29.

29. Ogilvie, B.M. (1964). Nature, Lond., 204, 91.

30. Jarrett, E.E.E., Jarrett, W.F.H., Urquhart, G.M. (1966). Nature, Lond., 211, 1310.

31. Jones, V.E., Edwards, A.J., Ogilvie, B.M. (1970). Immunology, 18, 621.

32. Edwards, A.J., Burt, J.S., Ogilvie, B.M. (1971). Parasitology, 62, 339.

33. Jones, V.E., Ogilvie, B.M. (1971). Immunology, 20, 459.

34. Ogilvie, B.M., Love, R.J., Jarra, W., Brown, K.N. (1977). Immunology, 32, 521.

35. Chandler, A.C. (1937). Am. J. Hyg. 26, 309.

36. Thorsen, R.E. (1953). Am. J. Hyg. 58, 1.

37. Jennings, F.W., Mulligan, W., Urquhart, G.M. (1963). Expl. Parasit., 13, 367.

38. Parker, R.P., Elrick, R.H. (1966). Int. J. Appl. Radiat. Isotopes, 17, 361.

39. Rogers, A.W., Moran, J.F. (1966). Anal. Biochem., 16, 206.

40. Rogers, W.P., Lazarus, M. (1949b). Parasitology, 39, 302.

41. Ogilvie, B.M. (1965). Parasitology, 55, 723.

42. Le Quesne, L.P. (1967). "The Response of the Adrenal Cortex to Surgical Stress". In The Human Adrenal Cortex, C.I.B.A. Foundation Study Group, No. 29, Churchill Ltd., London.

43. Mende, T.J., Viamonte, L. (1965). Gerontologia, 11, 213.

44. Yousef, M.K., Luick, J.R. (1969). Can. J. Physiol. Pharmacol., 47, 273.

45. Awwad, H.K., Potchen, E.J., Adelstein, S.J., Dealey, J.B. (1966). Metabolism, 15, 370.

46. Hoskins, J.L. (1944). Analyst 69, 271.

47. Brambell, M.R. (1965). Parasitology, 55, 313.

48. Roberts, L.S., Fairbairn, D. (1965). J. Parasit., 51, 129.

49. Jarrett, E.E.E., Jarrett, W.F.H., Urquhart, G.M. (1968). Parasitology, 58, 625.

50. Urquhart, G.M., Murray, M., Murray, P.K., Jennings, F.W., Bate, E. (1973). Trans. R. Soc. Trop. Med. Hyg., 67, 528.

51. Taliaferro, W.H., Taliaferro, L.G. (1951). J. Immunol., 66, 181.

52. Taliaferro, W.H., Taliaferro, L.G., Jaroslow, B.N. "Radiation and Immune Mechanisms", (1963). Academic Press, New York and London.

53. Thornburn, C.C. (1972). "Isotopes and Radiation in Biology", Butterworth and Co. Ltd., London.

54. Targett, G.A.T. (1973). "Maintenance in Animals, Enhancement of Parasitaemia by Immunosuppression". In "Techniques with Trypanosomes", Churchill Livingstone, Edinburgh and London.

APPENDIX

Table I

Uptake of ^{75}Se (counts/min/mg "fresh weight") by Adult N. brasiliensis

Rat No.	Day of Infection							
	6	7	8	9	10	11	12	13
1	14.4	13.1	12.7	12.9	4.1	1.0†	0.51	0.70
2	15.1	13.9	9.3	11.7	6.5	1.9	0.83†	0.55
3	16.0	18.2	15.7	9.9	7.7	2.9	0.68	0.78
4	16.1	15.4	9.0	11.9	6.0	2.7	0.32†	0.20
5	16.9	17.1	9.7	12.6	5.8	2.8	1.32	1.12
6	13.8	15.4	11.6	8.1	4.3	4.7	2.05	0.78
7	12.4	14.9	11.3	8.4	2.9	1.0	1.08†	1.42†
8	13.7	9.7		8.9	1.3	1.1		0.95†
9				6.5	1.3	2.3		
10				8.2	4.0			
Mean	14.8	14.7	11.3	9.9	4.4	2.3	0.97	0.81
S.D.	1.5	2.6	2.3	2.2	2.1	1.2	0.58	0.37

† Sample collected from two rats.

Table II

Uptake of ^{32}P (counts/min/mg "fresh weight") by Adult *N. brasiliensis*

Rat No.	Day of Infection							
	6	7	8	9	10	11	12	13
1	95.9	99.1	100.3	89.5	30.8	8.1†	4.8	3.0
2	107.7	120.8	94.7	75.5	53.2	37.1	6.7†	3.0
3	92.7	107.9	119.9	63.0	60.0	20.0	7.5	2.7
4	104.3	119.9	80.3	86.0	50.6	26.3	3.8†	2.7
5	95.6	110.0	85.5	71.5	41.1	24.6	12.2	2.9
6	79.8	113.3	93.2	54.4	38.6	48.3	14.8	6.1
7	87.5	67.5		54.7	8.5	48.9	3.8	6.8†
8	88.5	60.1		58.5	9.7	9.0		5.1†
9				48.4	23.0	5.1		
10				56.5	12.9			
Mean	94.0	99.8	95.7	65.8	32.8	25.3	7.7	
S.D.	9.0	23.4	13.8	14.1	18.8	16.7	4.3	

† Sample collected from two rats.

Table III

Uptake of ^{32}P (counts/min/mg worm nitrogen) by Adult N. brasiliensis

Rat No.	Day of Infection							
	6	7	8	9	10	11	12	13
1	4238	2463	4256	3712	1282	371†	154	104
2	4663	3828	4212	2870	2216	2174	220†	158
3	4150	4684	5381	2604	2500	719	265	91
4	4243	4273	3552	3335	2109	1169	145†	156
5	4081	4418	3551	2923	1710	1055	388	110
6	3425	4958	4542	2119	1612	1887	571	311
7	3790	2612	4252	2312	356	1938	155†	262†
8	3545			2103	407	379		189†
9				1684	345	305		
10				2309	540			
Mean	4017	3891	4249	2597	1309	1111	271	176
S.D.	407	989	624	618	841	733	157	75

† Sample collected from two rats

Table IV

Uptake of ^{14}C-Glucose (counts/minute/mg "fresh weight") by Adult *N. brasiliensis*

Rat No.	Day of Infection							
	6	7	8	9	10	11	12	13
1	548	522	608	475	331	57	40	67
2	485	484	531	288	274	213	52	41*
3	564	420	659	193	203	70	50	50
4	605	529	524	187	199	87	90	56
5	496	545	406	275	93	68	71	46
6		533	239		122	71	60	
Mean	540	506	495	284	204	94	61	52
S.D.	49	47	152	116	89	58	17	10

* Sample collected from two rats.

TABLE V

Uptake of ^{32}P by N. brasiliensis in First and Second Infections (counts/min/mg "fresh weight"): Individual Results

	First Infections			
Rat No.	Day of Infection			
	8	10	12	13
1	880	687	255	42
2	591	747	277	58
3	927	754	283	72
4	741	715	296	
5	657	697		
6	807	810		
Mean	753	735	278	57
S.D.	119	45	17	15

	Second Infection		
Rat No.	Day of Infection		
	5	6	7
1	353	46	51+
2	341	61	68+
3	328	109	
4	390	80	
5	355	117	
6		89	
Mean	353	84	60
S.D.	23	27	

+ Sample prepared from three rats.

Table VI

Uptake of ^{75}Se by *N. brasiliensis* in First and Second Infections (counts/min/mg "fresh weight"): Individual Results

First Infection

Rat No.	Day of Infection			
	6	8	10	13
1	192	127	60	5.4
2	136	146	49	9.8
3	193	164	66	14.2
4	169	171	57	
5	140	127		
6	191	152		
Mean	170	148	58	10.0
S.D.	26	18	7	4.0

Second Infection

Rat No.	Day of Infection		
	5	6	7
1	109	7.1	10.0
2	93	11.4	10.0*
3	79	20.7	
4	84	6.5	
5	85	15.0	
6		13.9	
Mean	90.0	12.4	10.0
S.D.	11.7	5.3	

* Sample collected from 3 rats

Table VII

Uptake of ^{32}P by N. brasiliensis in Normal and Immunosuppressed Rats: Individual Results

Group	Rat No.	Uptake - counts/min/mg "fresh weight" Day of Infection 6	8	10	12	14	16
Normal Infection	1	448	493	70	42		
	2	606	296	54	45		
	3	263	428	84	52		
	4	437	381	48	46		
	Mean	439	400	64	46		
	S.D.	140	83	16	4		
Immuno Suppressed	1	365	469	347	341	187	55*
	2	390	437	95	499	39	52
	3	478	461	253	467	60	53
	4	448	378	350	281	95	47
	5	379	530	491	222		
	6	421	480				
	Mean	414	459	307	362	95	52
	S.D.	44	50	145	118	65	3
	't' test	N.S.	N.S.				
				$p < 0.05$	$p < 0.01$		

* Sample collected from 3 rats.

Table VIII

Uptake of ^{75}Se by Adult N. brasiliensis in Normal and Immunosuppressed Rats: Individual Results

Group	Rat No.	Uptake - counts/min/mg "fresh weight" Day of Infection 6	8	10	12	14	16
Normal Infection	1	147	110	9.3	7.3		
	2	134	84	8.2	7.9		
	3	77	72	29.8	2.8		
	4	128	83	12.9	6.5		
	Mean	122	87	15	6.1		
	S.D.	31	16	10	2.2		
Immuno suppressed	1	105	122	102	107	6.9	11.9*
	2	114	117	81	115	7.9	8.0
	3	152	118	62	136	6.9	8.3
	4	135	102	103	90	7.2	9.4
	5	108	115	88	73		
	6	118	116				
	Mean	122	115	87	104	7.2	9.4
	S.D.	18	7	17	24	0.4	1.7
	't' test	N.S.	$p < 0.01$	$p < 0.001$	$p < 0.001$		

* Sample collected from 3 rats

Table IX

Uptake of ^{32}P by Adult N. brasiliensis in Irradiated and Normal Rats

Group	Rat No.	counts/min/mg "fresh weight"				
		Day of Infection				
		6	8	10	13	15
Normal Infection	1	693		621	68	
	2	767		207	68	
	3	682		108	52	
	4	912		422	52	
	Mean	764		340	60	
	S.D.	105		228	9	
Irradiated	1	372	447	406	48	33
	2	406	557	755	182	26
	3	614	481	264	217	52
	4	358	632	389	145	43
	5	464	448	417	75	46
	6	471		571		52
	Mean	448	513	467	133	42
	S.E.	93	80	171	71	10
	't' test	$p < 0.01$		N.S.	N.S.	

Table X

Uptake of ^{75}Se by Adult N. brasiliensis in Irradiated and Normal Rats

Group	Rat No.	counts/min/mg "freshweight"				
		Day of Infection				
		6	8	10	13	15
Normal Infection	1	122		92	*	
	2	148		29	*	
	3	103		13	*	
	4	127		71	*	
	Mean	125		51		
	S.D.	18		36		
Irradiated	1	63	73	54	3	3.0
	2	75	94	94	22	3.0
	3	107	86	61	29	1.1
	4	74	118	58	17	3.7
	5	93	77	73	9	4.5
	6	89		68		*
	Mean	84	90	68	16	3.1
	S.D.	16	17	16	10	1.2
	't' test	$p < 0.01$		N.S.		

* Radioactivity statistically not significant.