OBSERVATIONS ON THE HOST RESPONSE TO SOME HELMINTH INFECTIONS

A Thesis

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SUMMARY

Immunological aspects of the host-parasite relationship were investigated in two systems: Nippostrongylus brasiliensis in the rat and Haemonchus contortus in sheep and are described in Sections I and II of this thesis respectively.

The objective of the work described in Section I was to study the uptake of labelled metabolites by the parasites under a variety of different circumstances to test the validity of such measurements as an index of immunological worm damage. This was largely successful in that in all the circumstances examined, worm expulsion was preceeded by a marked depression in their metabolite uptake. However the converse was not always true, i.e. a depressed metabolite uptake was not necessarily followed by expulsion. For instance in both neonatally infected rats, in rats treated with Depoprovera (a long acting progesterone) and also in female rats in pro-oestrus, worm expulsion was significantly delayed although metabolic damage evidently occurred at the normal time. This finding is important since it implies that immunologically damaged worms are still able to persist in the host and that a further, possibly non-immunological mechanism, is needed to bring about their subsequent physical expulsion.

One of the most interesting features of this section was the observation of significant 'strain' differences in the immune response of the host to N. brasiliensis, i.e. the Aberdeen strain of rat appeared to selfcure earlier than the London strain. An opportunity arose to pursue this line of research in relation

to an economically important helminth disease in East Africa, namely H. contortus in sheep.

The experiments described in Section II of the thesis demonstrate that in Merino sheep in East Africa there are significant strain differences in susceptibility to H. contortus and that these strain differences are linked to haemoglobin type. It was shown that sheep of haemoglobin type A are more resistant than those with haemoglobin B and that AB animals occupy an intermediate position. These differences were demonstrated by parasitological, haematological and pathophysiological measurements. However this strain difference is not absolute and appeared to be dependent on the size of the challenge infection. At certain levels of infection the strain difference in susceptibility tended to disappear. It has been shown that haemoglobin B gene is predominant in Kenya, although the incidence of the A gene tends to increase in the wetter areas. One possible reason for this gradient of haemoglobin genes could be related to the higher incidence of H. contortus in wetter areas which might favour the selection of the A gene which confers more resistance to H. contortus. The practical application of this strain difference in resistance to haemonchosis is discussed.

GENERAL INTRODUCTION

Although the majority of the estimated 500,000 nematode species are non-pathogenic free-living in soil or water, many have become parasitic in plants, arthropods, molluscs and vertebrates and constitute one of the major remaining scourges of mankind. For example, of the total amount of food produced in the world it is evident that a very large proportion is consumed each year by nematodes through the destruction of plant tissues, by causing poor growth and lowering the production in food animals or by direct deprivation within the human body. Indeed, nematodes in domestic animals have caused losses amounting to hundreds of millions of pounds throughout the world by their exploitation of the internal niches offered by the eye, mouth, tongue, alimentary canal, liver, lungs or body cavity.

Important progress has been made however during the last seventy years in the development of a series of chemotherapeutic compounds which were found to be toxic to the adult, and some to the immature nematode. The early remedies such as sodium arsenite, copper and nicotine sulphate, although reasonably effective unfortunately proved very toxic to the host. A later group of chemicals, which included carbon tetrachloride, were generally more effective but the side effects caused by them also discouraged their use. The advent of phenothiazone from the late 1930's marked the first of the broad spectrum anthelmintics (Gordon, 1945). The compounds developed later were even more efficient, less toxic and increasingly effective against the immature parasitic stages and included organophosphorous chemicals such as Neguvon and Loxon (Hebden and Hall, 1965;

Kingsbury and Curr, 1967). Thiabendazole, the first of the benzimidazoles, was the first anthelmintic which enabled a true diagnostic application to detect and measure the economic effects of parasitic diseases caused by gastro-intestinal nematodes in sheep and cattle. Additional benzimidazoles have been introduced, to which thiophenate is the latest addition (Eichler, 1973).

However, the repeated use of the same anthelmintic compound has also led to problems concerned with the development of resistant strains of parasites. Some parasites seem far more prone to develop resistance than are others. Natural selection and the reproduction of resistant individuals evidently can occur relatively quickly and effectively as is examplified by the development of resistance to H. contortus by phenothiazine (Drudge et al., 1964) and O. circumcincta to organophosphorous anthelmintics (Douglas and Baker, 1968). Two types of resistance to chemicals have been described; 'specific resistance' which is characteristically due to a single dominant or recessive gene, and 'polygenic resistance' (Hoskins and Gordon, 1956; Plapp, 1970). The resistance of some strains of H. contortus to thiabendazole appears to be an example of specific resistance whilst the resistance of strains of H. contortus to morantel tartrate may be an example of polygeneic resistance.

Although the use of anthelmintics has led to a considerable reduction in the annual losses from animal production, the repeated use of drugs is an expensive high cash-investment exercise which is not available to many farmers of

the world, since intensive and continual usage and high standards of husbandry and management are prerequisites for success (Lindahl et al., 1971). Accurate forecasting techniques warning of imminent outbreak of diseases such as offered by Allonby (1974) and Thomas and Star (1977) for acute haemonchosis could alleviate the expenditure by the farmer but again may be too sophisticated as yet for many countries.

Animals can be reared and maintained worm-free by keeping them indoors throughout life, e.g. poultry in battery cages or by folding grazing animals on uncontaminated pasture. However, the economics of this latter option are such that this method is unrealistic and unprofitable for most farming systems.

Immunological control by vaccination and the utilisation of the host's capacity to resist parasites has so far been limited to a very few species. Vaccination against babesiosis and anaplasmosis is now a well established procedure in Australia (Callow and Mellors, 1966; Callow, 1971) and in recent years more than a million doses of vaccine have been supplied annually. Vaccines against parasitic bronchitis in cattle (Jarrett et al., 1957) and in sheep (Jovanović et al., 1965) and hookworm in dogs (Miller, 1968) are now commercially available, whilst the development of others such as those against bovine theileriosis, ovine schistosomiasis, avian coccidiosis and avian syngamiasis have given promising recent results. A single course of vaccination using one of a variety of forms of antigens, e.g. irradiated larvae, attenuated adults or an artificial infection terminated by drugs given at a predescribed age, can render the animal resistant. However, although effective, large scale

immunisation programmes are expensive and require a great deal of organisation, with technical and financial backing. This approach although followed in Australia, USA and some parts of Europe must be considered economically unrealistic as yet in most of the developing countries.

With the present rapid increase in the world's human population and an estimated 500 million people suffering at present from malnutrition it is paramount that the production of protein from animal sources should be increased as much as possible. This can be achieved by intensification, by improved management and particularly by improved disease control. At least 7 million square kilometres of grazeable land in Africa are capable of supporting 120 million head of cattle, but remain unproductive chiefly because of trypanosomiasis and East Coast fever.

As well as the need to multiply the numbers of good stock, decisions must be made as to the breed of the animals to be used. The choices open to the government of a developing country at the moment appear to be four fold. The first is the selection within the indigenous tropical breeds; upgrading them is a long and slow process. The second choice is the importation of exotic livestock. This is expensive and can bring rapid results but many of these 'quality producers' are unable to survive the exposure to a plethora of tropical diseases due to an absence of innate immunity, which makes this choice economically less sound, unless the best management procedures can be guaranteed.

Mass importation of tropically adapted livestock from other tropical countries could be a third choice, although many tropical countries are critically short of improved livestock and there is generally little livestock surplus for export. The fourth choice is the cross-breeding of tropical livestock with exotic breeds and the evolution of stabilised or semi-stabilised crossbreeds. This choice is a compromise to improve the production capacity of the animal but to retain some innate immunity to tropical diseases. If there were no immunity to tropical diseases there would be probably no livestock in most of Africa. Therefore studies of the possible use of the host's own resistance may ultimately prove of more practical application in the development of cross-breeding and immunisation programmes designed to reduce parasitic disease.

The presence of genetic and heritable resistance in sheep to trichostrongyle worms has been observed by various authors (Gregory et al., 1940; Emik, 1949; Whitlock and Madsen, 1958; Scrivner, 1964a, b). Many host-parasite systems have been investigated and most breed-differences appear to confer differences in susceptibility, as for example in bovine theileriosis (Radley, 1976) and babesiosis, (Daly and Hall, , 1955; Johnston, 1967), avian coccidiosis (Rosenberg, 1941; Long and Rose, 1965), and ascariasis (Ackert, 1942), murine trypanosomiasis (Jennings and Urquhart, 1977) and trichuriasis (Wakelin, 1975a, b) and rodent malaria (Zuckerman, 1966) and nippostrongylosis (Katiyar and Sen, 1969).

Although a proportion of these breed differences could possibly be attributed to variation in age, sex, acquired resistance and perhaps grazing habits, resistance has been clearly demonstrated to be heritable. Ross et al. (1959) demonstrated heritable resistance to Haemonchus in cattle in Nigeria, and Whitlock (1955b) successfully bred sheep with low susceptibility to Haemonchus. Sheep, chickens, rats and mice have been bred resistant respectively to Ostertagia circumcincta (Scrivner, 1967), Ascaridia lineata (Ackert et al., 1936), Brugia pahangi (Sucharit and MacDonald, 1973) and Trichuris muris (Wakelin, 1975b), and Zuckerman (1963) has been able to develop mice strains with both high and low resistance to Plasmodium berghei.

A better understanding of the immunological aspects of host-parasite interactions is essential not only from the point of view of extending the possibilities of vaccination but also in the implementation of management procedures which take into account the host's own capacity to develop immunity against helminths.

This is essentially the subject of this thesis which consists of two sections. Section I deals with a series of experiments on the mechanism of the immune expulsion of Nippostrongylus brasiliensis from the small intestine of the rat. This system has been used as a 'model' by many workers interested in understanding the nature of the immune response to gastrointestinal parasites. One of themost interesting features of this programme was the observation of significant

'strain' differences in the immune response of the host to this parasite. An opportunity arose to pursue this line of research in relation to an economically important helminth disease in sheep in East Africa.

This is developed in Section II of the thesis in which a series of experiments on the strain differences of Merino sheep in their resistance to Haemonchus contortus infection is described. A detailed introduction is presented at the beginning of each section.

S E C T I O N I

STUDIES ON THE MECHANISM OF IMMUNE EXPULSION OF *Nippostrongylus brasiliensis* FROM THE RAT

INTRODUCTION

Nippostrongylus brasiliensis was first described by Travossos in 1914. It is believed to have originated and to have a cosmopolitan distribution in Brazil. This parasite has subsequently been observed in Australia, China, Panama, U.S.A., Canada, Phillipines and also England (Balfour, 1922; Dudgeon, 1922). It is a nematode endoparasite of Rattus norvegicus, Rattus rattus and Mus musculus (Haley, 1961). N. brasiliensis larvae have been experimentally introduced into a number of laboratory animals. Haley (1961) observed that the worms reached full sexual maturity when the larvae were introduced into the cotton rat, golden hamster, chinchilla, gerbil and rabbit. Adult worms did not develop in the deer mouse, vole or guinea pig (Lindquist, 1949, 1950; Newton et al., 1959; Gharib, 1961). In guinea pigs reared 'worm-free' or cortisone treated then sexual development of the worms occurred normally (Newton et al., 1959; Parker, 1961).

The size and duration of N. brasiliensis infection is influenced by breed, age, sex and diet of the host animal (Haley, 1958). For example, only 4% of infective larvae reached the adult stage in hamsters compared with the rat (Haley, 1958), 14% of the larvae attained maturity in mice (Solomon and Haley, 1966), whereas very few worms developed into adults in the rabbit (Thorson, 1953). The adult worms were stunted and the prepatent period long in the cotton rat and golden hamster (Lindquist, 1950). There was no difference

between male and female mice in resistance to the infection and no change in the sex ratio of the adult worms (Solomon and Haley, 1966). There are, however, similarities between the effect of the innate resistance shown in the hamster and the acquired resistance in the rat since both forms of resistance affect the female worm before the male parasites (Haley, 1958; Africa, 1931).

In the laboratory rat N. brasiliensis completes a life-cycle within three weeks. This provides a means for rapid and productive experimentation and hence N. brasiliensis infections in the rat is a convenient laboratory model in which to study the host-parasite relationship.

The life-cycle of N. brasiliensis consists of two moults in an external non-parasitic phase followed by two moults in a parasitic phase. Haley (1962) describes in detail the worms' development. Rhabditiform larvae hatch from the egg within 24 hours, and moult to the second stage larvae in a further 48 hours. After a second moult the larvae become third stage filiariform infective larvae which penetrate the skin and pass via the blood stream to the heart and lungs (Yokagawa, 1922) or possibly via the lymphatic system. During migration 40 - 60% of the larvae are lost (Jarrett et al., 1968a) and only 55% of the remaining larvae reach maturation (Haley, 1962). Once in the lungs the larvae moult again and sexual differentiation occurs. These fourth stage larvae migrate to the intestine via the bronchi, trachea, oesophagus and stomach. The fourth and final moult occurs 90 - 108 hours post-infection. The adult

worms become sexually mature, copulate, and the female worms produce eggs which are carried externally in the faeces. Each female worm can produce one thousand fertile eggs every twenty four hours (Phillipson, 1969).

The parasites distribute themselves in 'pockets' throughout the jejunum (Brambell, 1965) and apply themselves closely to the mucosa where the oxygen level is high (Rogers, 1949a). The worms move from one position to another to copulate and to find new sites. The N. brasiliensis adult worms begin to feed by pressing their cuticular ridges on to the villi. This results in the damage of the microvilli and epithelial cells of the intestine (Lee, 1969b). The worms do not suck blood nor cause significant blood loss (Mulligan et al., 1965; Neilson, 1969a). Absorption of food via the cuticle is unlikely (Roberts and Fairbairn, 1965) and the worms probably feed solely by absorption through the gut. The digestive enzymes of the parasite consist of aminopeptidase, non specific esterase (Lee, 1969b, 1970) and three isoenzymes of acetylcholinesterazes (Edwards et al., 1971). These enzymes are poured on to the intestinal cells and the pre-digested cell debris is sucked up with the parasites pumping and glandular oesophagus (Jamuar, 1966; Lee, 1969b). As well as their use in digestion the acetylcholinesterazes are believed to act as a 'biochemical holdfast' allowing the worms to maintain their position by affecting nerve transmission to the muscles of the intestinal tract and halting peristalsis (Sanderson and Ogilvie, 1971).

As the host becomes immune the worms alter their position so that they are found mainly anteriorly and to some extent towards the distal ileum (Brambell, 1965). The ribosomes in the gut cells of the worms which are known to be actively involved in absorption and in protein synthesis become replaced by large vacuoles (Lee, 1969a; Jamuar, 1966). Immune precipitates may begin to block the lumen of the gut of the worms (Taliaferro and Sarles, 1939, 1942) although Ogilvie and Hockley (1968) and Lee (1969a) were unable to confirm this. The reproductive tract then ceases to function. At this time egg production by the female worms ceases and spermatozoa are reabsorbed by the male worms (Lee, 1969a).

Worm expulsion then follows but the changes within the worms are not themselves responsible for worm elimination. The female worms are expelled earlier than the male worms (Africa, 1931) possibly since they are more antigenic (Ogilvie, 1965a). At the time of expulsion the number of mast cells and globular leukocytes in the host's intestine increases significantly (Jarrett et al., 1968). An anaphylactic reaction is thought to occur involving mast cells, reagins and allergens, thus facilitating the leakage of protective antibodies into the intestine. A number of worms which are believed to be less antigenic (Ogilvie, 1969) remain in the intestine as a residual population. The worms consist mainly of male parasites and are then concentrated in the region just posterior to the pyloric sphincter (Brambell, 1965).

The age, sex and general condition of the host and previous experience of infection, as well as size

of infection, appears to influence both the timing and rate of expulsion and the size of the residual population. Female rats were able to expel their worm population earlier than male rats (Murray et al.,1971). Worm expulsion was delayed in lactating rats (Connan, 1970) and was either delayed or did not occur in rats treated with cortisone or antilymphocytic serum or in rats thymectomised in neonatal life (Ogilvie, 1965b; Kassai et al., 1968; Jones and Ogilvie, 1971; Ogilvie and Jones, 1967).

Large single infections of N. brasiliensis larvae resulted in a sudden expulsion of adult worms whereas in small infections the worms were expelled more gradually (Haley and Parker, 1961; Jarrett et al., 1968a). If rats were continually exposed to a small number of larvae worm expulsion did not occur as dramatically. The residual population was larger and eggs were continued to be produced (Jenkins and Phillipson, 1972).

Neonatally infected rats did not expel their worm burden as rapidly and the prolonged survival of the adult worms was dose dependant (Jarrett et al., 1966) and the residual population was also larger. The response to second infection was more complete and depended not only on the response of the initial infection but also on both the age of the rat at challenge and the size of the challenge (Jarrett and Urquhart, 1969).

The mechanism of immune expulsion is believed to be a two stage process (Urquhart et al., 1965; Jones and Ogilvie, 1971; Murray et al., 1971; Kelly and Dineen, 1972).

The first stage in the expulsion was regarded as the development of a hypersensitivity in the gut which resulted in a local anaphylactic reaction with increased permeability to plasma macromolecules at the sites occupied by the worms. Worm damage and expulsion was thought to result from the action of anti-worm antibodies on the parasites. More recent results are difficult to reconcile with this simple hypothesis, e.g. significant immunological damage to the parasite appears to occur quite early in the infection (Ogilvie and Hockley, 1968; Lee, 1969), i.e. before the major macromolecular leak occurs. The fact that a depression in egg count always preceeds the actual expulsion also suggests some degree of worm damage prior to the actual elimination. This early immunological damage is very difficult to quantitate. Lee (1969) described the damage in terms of ultrastructural changes observable in the worms. More recently Henney et al., (1971, 1973) have attempted to measure functional changes in the parasite associated with the development of immunity in the host. Their method depends on the measurement of the uptake by the parasites of certain labelled metabolites (^{32}P phosphate and ^{75}Se-methionine) injected into the host. Preliminary studies with this method have indicated a significant fall in the metabolite uptake preceeding the actual worm expulsion.

The object of the experiments described in this section was to conduct further studies on the 'metabolite uptake' method with a view to assessing its validity as an index of impaired metabolic activity of the parasite in the immune host. It seemed worth while to carry out a series of experiments

under circumstances where worm expulsion is altered in time to find out if there is always a consistant relationship between the fall in metabolite uptake and worm expulsion. Thus the following series of experiments was performed (i) in male and female rats (where there is a time difference in the onset of expulsion), (ii) in young rats (where expulsion is delayed), (iii) in female rats at different stages of the oestrus cycle (where endocrinological factors are likely to cause variations in the onset of expulsion) and (iv) in rats treated with drugs likely to interfere with expulsion.

GENERAL MATERIALS AND METHODS I

SECTION I

MATERIALS AND METHODS

INDEX

A. GENERAL

1. Experimental Animals

(a) Experimental Rats

Male and female Hooded Lister rats were supplied by Animal Suppliers, London; and the Rowett Research Institute, Aberdeen. The rats were transported by rail, and were placed in quarantine for ten days prior to experimentation. The rats used in each experiment weighed 150 - 200g body weight unless otherwise stated.

(b) Nippostrongylus brasiliensis

The N. brasiliensis culture was supplied by the Department of Parasitology, the Veterinary School, University of Glasgow. The culture was subsequently maintained at the Department of Veterinary Physiology, University of Glasgow, and passaged through either Albino or Hooded Lister rats.

2. Housing, diet and environmental conditions

A maximum of ten rats was kept in metal wire cages measuring 30 x 60 x 20 cm. A grid floor allowed faeces and urine to pass out of the base of the cage to be collected in a metal tray beneath. When collection of faecal

pellets was required paper towels were placed on the tray, and faeces were removed daily or twice daily for analysis. The urine was absorbed into the paper. The ambient temperature was maintained at 65°C, and a system of 12 hours light and 12 hours darkness was imposed. All rats were fed on 'Diet 41 pellets' (W. Primrose and Son, Glasgow). Food and water were always present in plentiful supply.

3. The culture of N. brasiliensis larvae

Faecal pellets were collected from rats having a patent infection of N. brasiliensis by placing paper towels in the metal trays under the cages on days 7 - 9 post infection. The faecal pellets were separated from the discarded food pellets and shavings, and lightly washed in warm water. The sponges and Petri dishes (Oxoid Ltd.) were autoclaved prior to being used. This procedure was adopted to eliminate the frequent appearance of a fungal growth during culture of the N. brasiliensis larvae which appeared to be consuming the larvae, and hence resulting in low yields. The fungal spores were believed to be possibly contained within the food pellets or in the shavings. The washed pellets were placed into a mortar and mixed with a small volume of warm water. A little of this faecal paste was smeared thinly, using a spatula, in a circular patch on the middle of a 7 cm. diameter Whatmans No. 1

filter paper. Care was taken to keep the peripheral edges of the filter paper clean. The entire filter paper and faecal smear was dipped into warm water (35°C) and then placed on a water-saturated piece of foam material (5 cm x 5 cm) which had been placed in the middle of the base of a disposable Petri dish. The Petri dish lid was replaced and the dishes were placed in an incubator at 27°C. A dish of water was also placed in the incubator to maintain a high humidity. The N. brasiliensis larvae were harvested 8 - 10 days after the faecal material had been spread. After removal of all the Petri dish lids the base of the Petri dishes containing the foam sponge, the filter paper with the faecal smear, and the larvae situated at the edge of the filter paper, were flooded with warm water (35°C). After 10 minutes the sponge and filter paper were squeezed and discarded. The larvae were now in the warm water which also contained loose faecal matter and consequently it was necessary to concentrate and to filter out the larvae. The water was passaged through strong filter paper held in a large Buchner funnel under pressure. The N. brasiliensis larvae collected on the filter paper in large numbers. The filter paper containing the larvae was inverted and placed on top of a fine Endecott sieve with a mesh size of 400. The sieve was then placed at the top of a Baermann funnel which was filled with warm water (35°C). A bright light was directed on

to the sieve from below. The larvae were attracted to the light and collected at the base of the funnel. One hour later the larvae were released into a measuring cylinder. The larvae were allowed to settle and the water was poured off and replaced by physiological saline (0.85% NaCl).

The number of larvae present was estimated by first diluting the larvae with physiological saline to 100 ml. The larvae - saline mixture was well shaken and 0.025 ml was removed using an autozero pipette. The number of larvae contained in this volume was counted at 40 x magnification using a binocular microscope. The number counted was multiplied by 40, thus assessing the number of N. brasiliensis larvae in 1 ml. Five of these readings were made and the mean value was used to calculate the estimated total number of larvae present. The larvae-saline mixture was then diluted or concentrated by the removal or addition of physiological saline until a concentration of 4000 - 4500 per ml was achieved. This concentration was used to infect the rats in the following experiments, unless otherwise stated.

4. The experimental infection of rats with N. brasiliensis

The N. brasiliensis larvae held in physiological saline at the required dilution were placed in a graduated cylinder. The mouth of the cylinder was fitted with a rubber bung through which a needle was placed (Henney, personal communication). The larvae were mixed by

invertion of the cylinder. One ml was withdrawn from the cylinder using a 1 ml syringe inserted into a needle fixed in the rubber bung.

Each rat was placed in a large glass beaker with a lid, and lightly anaesthetised using 'Trilene' (trichloroethylene B.P., I.C.I. Pharmaceuticals Ltd.) soaked into absorbent paper. The rats were thus rendered more manageable. The larvae were injected subcutaneously into the groin of each rat using a 2 cm long, 20 gauge disposable needle which was large enough to allow the larvae to pass through it. Care was taken that the larvae did not leak out from underneath the skin and a finger was placed over the site of entry immediately the needle was withdrawn and held there for about 15 seconds.

5. The recovery of *N. brasiliensis* adult worms from the small intestine of the rat

Rats were placed in a large jar containing 'Trilene' and anaesthetised. They were removed from the jar and their necks were dislocated. The ventral abdomen was incised so as to expose the small intestine and the length of the alimentary canal between the pylorus and the ilio-caecal junction was removed. Using round-tipped scissors, the gut was slit longitudinally along its length then cut transversely into 5 cm pieces. These pieces of gut were placed in a bag made of a double thickness of muslin gauze. The bag containing the gut pieces was

suspended in a 250 ml beaker containing warm physiological saline (37°C), and the beaker and contents were placed in a water bath (37°C).

The N. brasiliensis adult worms were seen to dislodge themselves from the intestine and intestinal contents and pass through the gauze and collect at thebase of the beaker. In the experiments in which radioisotopes were used the intestine was incubated for 30 minutes to reduce the length of time in which isotopic leakage occurred and also to standardise this loss. In all other experiments the intestine was incubated for 60 minutes. The gauze containing the intestine was removed and discarded. The N. brasiliensis worms were allowed to settle, and the saline was gently poured off.

6. Worm burden analysis

Analysis of the worm burden was assessed in one of a number of ways depending upon the subsequent aims of each particular experiment. In some cases only the numbers of N. brasiliensis adult worms present in the small intestine of each rat on a particular day post-infection was required. The N. brasiliensis adult worms were placed in a measuring cylinder in physiological saline. A series of aliquots of N. brasiliensis-in-saline were removed and placed on a Petri dish which had been marked out into squares to facilitate counting. The number of worms in each aliquot and by summation in

the whole sample, was counted and recorded. In the radioisotope experiments, the N. brasiliensis adult worms were washed three times in cold saline to kill the worms and thereby halt ^{32}P metabolism, and to remove any debris. The N. brasiliensis worms were drawn into a 10 ml disposable syringe and passed out slowly on to Whatman No. 50 filter paper which had been previously soaked in physiological saline and then placed in a micro-Buchner funnel. The N. brasiliensis worms were filtered and surface dried under pressure using a water pump. The small pile of worms which collected on the filter paper was picked up using a small spatula, and placed at the bottom of a labelled pre-weighed counting bottle. The bottle and worms were reweighed and the fresh wet weight of the N. brasiliensis worms was calculated.

7. Faecal Egg Counts

Faecal nematode egg counts were performed by a modified McMaster technique (Gordon and Whitlock, 1939; Whitlock, 1948). A number of faecal pellets were selected at random from the tray of faeces removed from under the cages of experimentally infected rats. Three groups of these faecal pellets were placed in a 100 ml beaker and 42 ml of water was added. After the faecal pellets had softened in the water the entire contents were poured into an overhead macerator and the faecal pellets broken up finely. The liquid faeces mixture was poured through

a sieve which allowed the N. brasiliensis eggs in the solution to pass through but which retained the larger faecal matter. The solution which contained the eggs was collected into a bowl, well stirred to prevent the N. brasiliensis eggs from settling to the bottom of the bowl, and 15 ml of the mixture was immediately poured into a 15 ml labelled test tube. The mixture was again stirred and another 15 ml labelled test tube was filled. Thus two aliquots were taken from each sample.

The labelled test tubes containing the faecal samples were placed in a centrifuge, and spun for 5 minutes at 2000 r.p.m.

The supernatant fluid was then discarded and a super-saturated solution of sodium chloride was added to the test tube until the tube was full. The test tube contents were mixed by repeated inversion of the test tube, with a thumb blocking the entrance. Following inversion a pipette was quickly placed in the solution and the solution was rapidly transferred on to one half of a McMaster slide. The test tube was inverted again and another sample was placed in a similar manner on the second half of the same McMaster slide. Consequently four separate readings were made for each faecal sample and a mean value was calculated for each.

The N. brasiliensis eggs could be readily seen using the 10 x lens of a binocular microscope. The number of eggs within the marked squares was counted. This number was multiplied by 100 to give the number of eggs in one gram of faeces.

B. METABOLIC UPTAKE MEASUREMENTS

1. Labelled phosphate solution

Sodium dihydrogenorthophosphate (^{32}P) (Radiochemical Centre, Ltd., Amersham) was diluted with isotonic saline to give a dilution of 20 uCi/ml. The pH was adjusted to 7.4.

2. Injection procedure

The experimentally infected rats were weighed and each rat was marked with a number at the base of the tail with a 'magic marker'. The volume of ^{32}P to be administered was assessed using 1 ml of ^{32}P solution per 150 g rat total body weight. After being lightly anaesthetised in a jar containing 'Trilene' the tail of each rat was bathed for one minute in a 50°C hot water bath. This procedure ensured good visible dilation of the tail veins. Using a 2 ml disposable syringe and a 25 gauge, 1.5 cm long needle the ^{32}P solution was injected into the vein. Access to the vein was easier at the distal end of the tail as the vein was nearer to the surface at this level. A small beaker containing a Trilene soaked swab was placed near to the rat's nose to ensure continued anaesthesia and to reduce the movements of the rat. The rats were returned to their cages and killed four hours after receiving the isotope injection.

3. Radioactivity measurements

The labelled, weighed counting bottles containing the various samples of surface-dried N. brasiliensis worms were placed in order in an automatic liquid scintillation counter. Empty bottles were placed in front and behind of the sequence of sample bottles to measure the background count. The ^{32}P activity in the samples was assessed using the Cerenkov counting method (Parker and Elrick, 1966).

In the first experiment, the results were not corrected for quenching, and were expressed directly. In later experiments, it was appreciated that a certain amount of quenching was occurring. Therefore, the quench correction was applied using either an experimentally prepared graph or by the internal standard method. For the internal standard method the worm samples were dissolved in 2 ml 'Soluene-100'(Packard Instruments, Ltd., Caversham, Berks.), with heating where necessary, and the radioactivity determined. The standards were treated with 2 ml 'Soluene-100'. After counting each sample was treated with a known amount of labelled inorganic phosphate and recounted.

C. VAGINAL SMEAR TECHNIQUE

A drop of distilled water was held in a short blunt pipette. The female rat was held on its back by holding the rat by the loose skin of the neck. The pipette was inserted into the vagina and the distilled water was squirted out and then drawn back into the syringe. Vaginal cells were held in solution. These were observed under a microscope using a magnification of 10 x and the stage of the oestrous cycle assessed from the prevalence of the various cell types present each day.

D. SOLUTIONS

(a) Mammalian Ringers solution

Stock A	NaCl	-	81.80 litre
	KCl	-	4.10 "
	$NaHCO_3$	-	4.70 "
Stock B	$CaCl_2\ 6H_2O$	-	4.38 "

Working solution

100 ml	Stock A
800 ml	Distilled water
100 ml	Stock B

(b) Physiological saline - 0.85%

Stock solution

170.0g NaCl dissolved in

2 litres of distilled water.

Working solution

Dilute the stock solution x 10.

(c) Glucose/Mammalian Ringers Solution

1 litre Ringer solution

1 g glucose.

OBSERVATION OF A PRIMARY INFECTION OF *Nippostrongylus brasiliensis* IN BOTH SEXES OF TWO STRAINS OF RATS

INTRODUCTION

The course of a primary infection of N. brasiliensis has been studied by many workers using various parameters. Faecal egg counts and worm burdens during the course of an experimental infection have been quantitively assessed by Jarrett et al. (1968). The sudden reduction in faecal egg counts towards the end of infection always occurred about two days earlier than the reduction in adult worm burdens. Furthermore, worm expulsion in female rats was found to occur two days earlier and at a greater rate than in male rats (Murray et al., 1971).

The objective of the present experiment was to see if the depression in metabolic uptake also occurred earlier in female than in male rats, i.e. is there a consistent correlation between depressed metabolite uptake and expulsion?

During the course of the initial experiment, using rats supplied by the London supplier, it was noticed that the rats were infected with a number of Hymenolepis diminuta. Apart from the difficulty in separating these tapeworms from the N. brasiliensis adult worms, and the possible inaccuracies in the worm wet weight results, the tapeworm could also have interfered with the ^{32}P uptake results. In an attempt to eliminate these inaccuracies rats were ordered from a different animal supplier. Subsequently, Hooded Lister rats from the Rowett Research Institute, Aberdeen were used and found to be tapeworm-free. Thus, the course of a primary experimental infection was studied in two different strains of rats.

This led to the following observations which showed that there was a difference in the time of expulsion of N. brasiliensis from the two different strains of rat.

MATERIALS AND METHODS

Fifty male and fifty female Hooded Lister rats from Animal Suppliers, London ('London' strain) and the same number of rats of both sexes from the Rowett Research Institute, Aberdeen ('Aberdeen' strain) were experimentally infected with 4,000 N. brasiliensis larvae as described earlier in materials and methods I.

Four days after experimental infection faecal pellets were collected and faecal egg counts were assessed daily from day 4 to day 14 post infection, using the McMaster slide technique (Gordon and Whitlock, 1939).

Each day five rats from each group were injected intravenously in the tail vein with 1 ml (20 uCi) ^{32}P-sodium dihydrogenorthophosphate per 150g body weight of rat as described in materials and methods I. Four hours later the rats were killed and the N. brasiliensis adult worm burden was recovered from each animal. The worms were washed three times in ice-cold saline, surface dried and placed in a labelled pre-weighed counting bottle and reweighed.

After the experiment had been completed all the counting bottles containing the N. brasiliensis adult worms were placed in a liquid scintillator counter and the radioactivity measured using the Cerenkov method (Parker and Elrick, 1966). The results were expressed as counts per minute per mg

worm wet weight, and these values were expressed as a percentage of the maximum metabolic activity observed during each infection.

RESULTS

Faecal egg counts

The results of the daily faecal egg counts are recorded in Table 1. Faecal egg counts became positive on day 6 when all groups except the Aberdeen strain of female rats showed relatively high egg counts.

Self-cure, as judged by the reduction in faecal egg counts to zero, occurred earliest in the female Aberdeen strain of rats on day 11, followed by self-cure in male Aberdeen strain of rats on day 12. Self-cure occurred in the London strain of rats in the female group on day 13 and in the male group on day 14.

These results clearly demonstrate a significant difference between the two strains of rats on the time of expulsion of the parasite. Within each strain the already recognised male/female differences also occurred.

N. brasiliensis adult worm burdens

The adult worm burdens were assessed by wet weights and are recorded in Table 2 and Tables A and B in the Appendix. 'Self-cure', as assessed by a decrease in worm burdens to less than 1mg wet weight, occurred in the female Aberdeen strain of rats on day 11, the male Aberdeen strain on day 12 and in male London strain sometimes after day 13.

TABLE 1

Faecal eggs per gram of faeces observed in both sexes of two strains of Hooded Lister rats experimentally infected with 4,000 N. brasiliensis larvae

Days after infection	London strain		Aberdeen strain	
	Male rats	Female rats	Male rats	Female rats
5	0	0	0	0
6	52 500	30 200	8 400	0
7	46 500	45 000	10 300	6 500
8	64 500	42 300	22 800	23 150
9	50 000	30 600	7 300	5 650
10	30 000	29 400	4 800	500
11	28 500	6 000	200	0
12	6 600	100	0	0
13	1 000	0	0	0
14	0	0	0	0

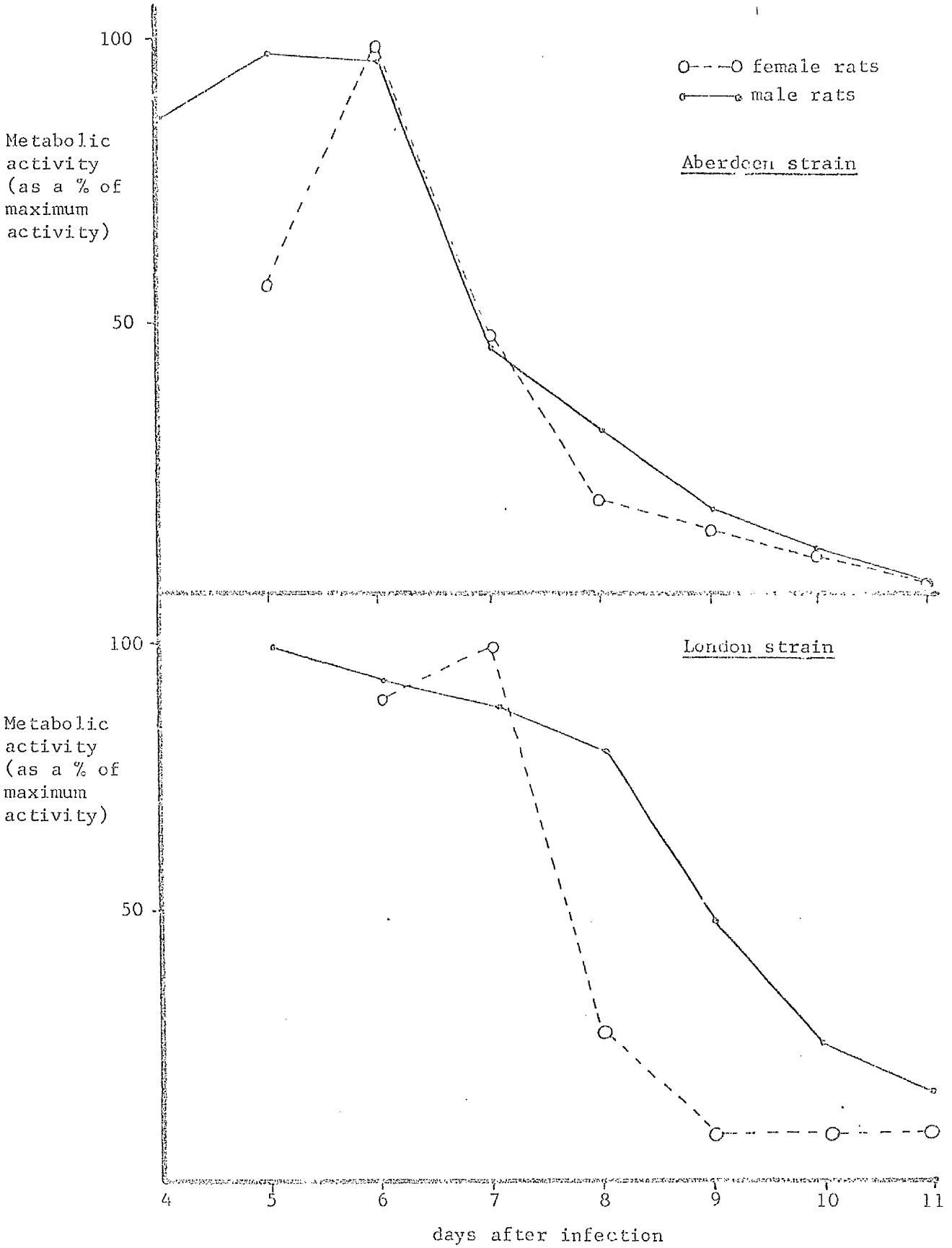
TABLE 2

Wet weight of adult worm burdens (mg) in both sexes of two strains of Hooded Lister rats experimentally infected with 4,000 *N. Brasiliensis* larvae

Days after infection	London strain				Aberdeen strain			
	Male rats		Female rats		Male rats		Female rats	
4	0	NS	0	<.01	2.7 ± 0.8	<.01	0	
5	54.0 ± 16.3	<.01	0	<.01	22.7 ± 5.3	NS	25.5 ± 16.5	
6	62.1 ± 15.2	<.05	13.8 ± 3.0	<.05	25.7 ± 2.7	NS	47.5 ± 18.5	
7	79.5 ± 19.6	<.05	26.8 ± 2.9	NS	22.4 ± 3.4	NS	33.9 ± 20.1	
8	49.8 ± 13.6	NS	54.8 ± 4.0	<.01	23.6 ± 5.5	NS	25.5 ± 16.3	
9	54.5 ± 12.4	NS	47.9 ± 5.5	<.001	12.4 ± 3.9	NS	29.7 ± 16.3	
10	89.6 ± 23.1	<.05	22.8 ± 2.2	NS	16.1 ± 3.9	NS	21.9 ± 12.9	
11	49.2 ± 20.6	NS	19.2 ± 2.5	<.001	1.7 ± 1.3	<.01	0	
12	31.8 ± 5.4	<.01	13.3 ± 1.3	<.001	0.2 ± 0.1	<0.01	0	
13	15.2 ± 12.6	NS	0.2 ± 1.3	NS	0	NS	0	

Figure 1

Metabolic activity expressed as a percentage of the maximum activity in both sexes of Aberdeen and London strains of Hooded Lister rats infected with N. brasiliensis



Metabolic uptake

The results of Henney et al. (1971) for the metabolic uptake by the N. brasiliensis in female rats of London strain are incorporated into this section. Experimentation on the four groups of rats was not conducted simultaneously. Therefore the actual isotopic counts between the groups cannot be compared quantitatively, hence only the relative difference in uptake pattern can be contrasted. The metabolic activity was thus expressed as a percentage of the maximum activity and is shown in Figure 1 and Table A in the Appendix.

The general pattern of ^{32}P uptake appeared to be very similar in each group of rats, i.e. a few days of high ^{32}P uptake were followed by a sudden and continual decrease in uptake. However the onset of the sudden decline in isotope uptake was different in each group, and occurred about four days after the start of worm expulsion, i.e. it occurred earlier in the Aberdeen than in the London strain, and in the London strain about one day earlier in the female (after day 7) than in the male rats (after day 8). In this experiment there was no significant difference in the rate of worm expulsion in the male and female Aberdeen rats (after day 6).

DISCUSSION

The course of N. brasiliensis infection in rats can be divided into four phases, namely Loss Phase 1, the Plateau Phase, Loss Phase 2, and the Threshold Phase (Jarrett et al., 1968a). Not all the larvae injected into the rat are able

to complete the migration to the lungs and finally to the small intestine. A number are lost en route, or destroyed. This constitutes Loss Phase 1. The Plateau Phase is formed by the surviving larvae which have matured to adults in the small intestine of the host. The N. brasiliensis adult worms utilise the metabolites of the host, copulate and the females produce eggs which are released into the lumen. Suddenly (around 10 - 12 days post-infection) there is an exponential expulsion of the majority of worms in which the female worms are removed somewhat earlier than the males. This constitutes the Loss Phase 2 or self-cure (Mulligan et al., 1965). A small number of residual worms are left in the small intestine and these constitute the Threshold Phase.

The results of this experiment confirm the findings of earlier workers regarding the expulsion of N. brasiliensis infection in the rat and provide further insight into its possible mechanism. Faecal egg counts became negative before worm burdens decline (Jarrett et al., 1968a). As observed by Murray et al. (1971) worm expulsion, as determined by faecal egg counts and worm burdens, occurred about a day earlier in female rats than in males. On the basis of the same parameters this applied to both strains of rat.

These observations were extended however by the findings that, as assessed by faecal egg counts and worm burdens, there was an even more marked difference in the onset of 'self-cure' between the two strains of rats. Thus, although the London strain of rats showed 'self-cure' from day 13 and 14 in female

and males respectively, this occurred two days earlier in each sex respectively, in the Aberdeen strain of rats.

The '^{32}P uptake' technique of Henney et al., (1971) provided evidence of immunological worm damage some days before their expulsion, and the results of this experiment confirm these initial observations. They also provided an excellent opportunity to see if worm expulsion was consistently preceded by a drop in metabolic uptake by the worms. The decline in ^{32}P uptake from day 6 after infection onwards was thought to be related to the onset of immunological damage. The results have shown that in both sexes of the London strain of rats immunological damage as monitored by reduction in '^{32}P uptake' began six days prior to complete worm expulsion. In the Aberdeen strain of rats, however, the onset of immunological damage commenced on the same day (after day 6) in both sexes of rat, i.e. 1 and 2 days earlier than the female and male London strain of rats respectively.

One possible explanation for this anomaly is that the more efficient worm expulsion of the Aberdeen strain may have surpressed the exhibition of the differences usually observed between the two sexes. Thus immunological damage in the Aberdeen strain commenced six days after infection which is possibly the minimum period required for protective antibodies to be stimulated, produced, mobilised and become effective. Under these circumstances the factor responsible for the accelerated worm expulsion in female rats may not have had time to operate and to become effective.

In summary, therefore, female rats were able to expel their worm burdens earlier and at a greater rate than male rats. The decline in metabolic activity occurred earlier in the Aberdeen strain than in the London strain. It also occurred in female rats earlier than in male rats of London strain, but simultaneously in both sexes of the Aberdeen strain. The results indicate that good correlation existed between the decline in metabolic activity, indicative of immunological damage, and the timing of self-cure.

It has been suggested that the difference observed in the onset of worm expulsion between male and female rats may be related to the influence of sex hormones (Waddell et al., 1971). From the results presented here it was evident that there was a larger variation in the wet weights of worms recovered from the female rats than those recovered from male rats. This suggested to the author that a relationship might exist between the oestrous cycle of the rat and the course of infection of N. brasiliensis. This was the subject of further investigations which are described later.

Although the observation on strain difference arose by chance there is clearly scope for further study of this phenomenon. Breed difference in response to parasitic infections is well documented, e.g. the dwarf N'dama and Maturu cattle of West Africa are more resistant to trypanosomiasis than Zebu cattle (Mason, 1951; Weitz, 1970; Roberts and Gray, 1972, 1973; Murray and Murray - personal communication). Much less is known about strain differences. Perhaps the Nippostrongylus/rat

system could serve as a 'model' for studying some of these complex genetic aspects of host/parasite interactions. This theme will be elaborated in more detail in the final discussion at the end of this section.

OBSERVATION OF A PRIMARY INFECTION OF *Nippostrongylus brasiliensis* IN YOUNG RATS

INTRODUCTION

Jarrett et al., (1966) and Kassai and Aitken (1967) found that neonatal rats infected with N. brasiliensis do not expel a primary infection of the parasite as efficiently as adult rats. The full response of the rats to the parasites does not occur until the rats are 6 - 7 weeks of age (Jarrett et al., 1966), as the immune response is neither effective nor efficient in young rats (Good and Papermaster, 1964).

The degree of effectiveness of the immune response appears to be related to the infection dose. Thus a worm population of N. brasiliensis will persist for several months in a young rat which had been infected with a small dose of 500 larvae whereas a larger infection of 2000 larvae will result in worm expulsion by young rats although this is delayed for a few days and the efficiency of expulsion is also reduced (Jarrett, et al., 1966, 1968b).

In the previous experiment the metabolic activity was assessed during the course of an experimental infection in two strains of both sexes of Hooded Lister rats. High ^{32}P uptake or high metabolic activity suddenly decreased early in the infection. It was believed that this metabolic embarrassment was related to the occurrence of immunological damage by the host to the parasites. The results also indicated that there was a direct correlation between the decline in metabolic activity and the onset of self cure.

The following experiment was designed to investigate the worm burdens and metabolic activity of the adult worms during the course of an experimental infection of 2000 N. brasiliensis larvae in young rats. This again provided an excellent opportunity to determine whether worm expulsion was consistently preceded by a drop in metabolic uptake by the worms.

MATERIALS AND METHODS

Twenty five 4 - 5 week old (80 - 100 g total body weight) male Hooded Lister rats of the Aberdeen strain were infected subcutaneously each with 2000 N. brasiliensis larvae.

On various days after day 6 of infection five rats were injected intravenously in the tail vein with 1 ml (20 uCi) ^{32}P-sodium dihydrogenorthophosphate per 100 g of body weight. Four hours later the rats were killed and the N. brasiliensis worms were collected and washed three times in ice-cold saline and surface dried. The parasites were then placed in pre-weighed labelled counting bottles and reweighed.

At the end of the experiment all the counting bottles containing the worms were placed in a liquid scintillation counter, and the radioactivity of the worms calculated. The results were corrected for quenching as described earlier in materials and methods I and were expressed as corrected counts per minute per worm wet weight (mg). These values were expressed as a percentage of the maximum metabolic activity observed during the infection.

RESULTS

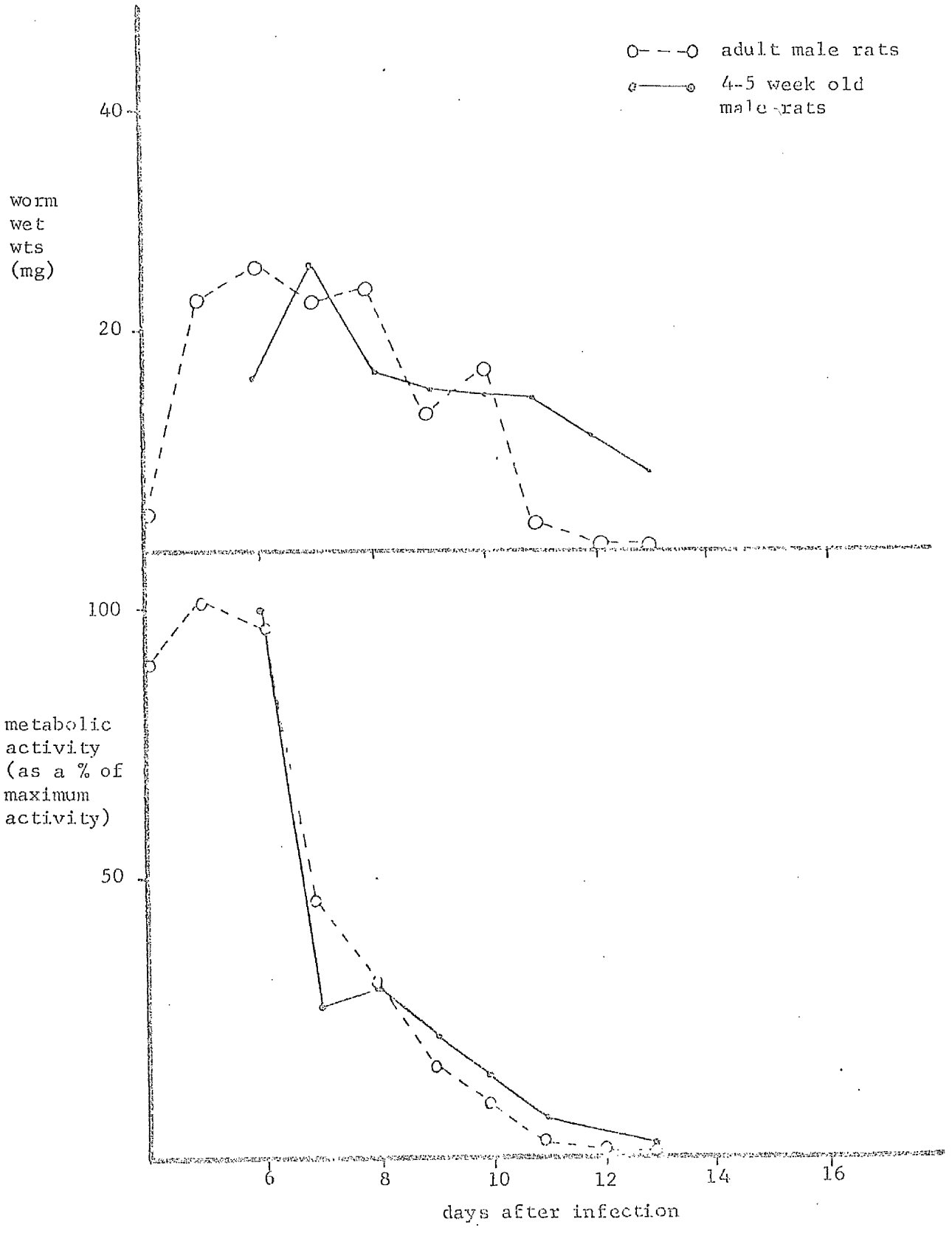
The results of the adult worm wet weights and the corrected counts per minute per milligram of worm wet weight are recorded in Figure 2 and in Table B in the Appendix. In Figure 2 the results are compared with those of adult male Hooded Lister rats of Aberdeen strain from the previous experiment and the metabolic activity is expressed as a percentage of the maximum activity.

It is evident from these results that expulsion of the adult worms occurred more efficiently in some young rats than in others, especially from day 11 onwards. The mean results indicate that complete expulsion has not occurred at the end of the experiment on day 13.

The size of the worm burden was similar in both young and adult rats even though the young rat had been given an infection of 2000 larvae and the adult rat one of 4000 larvae. However, in the young rats the worm burdens on day 11 were still maintained at 15 mg followed by a gradual reduction, whereas in the same day the worm burdens in the adult rats suddenly declined to 5 mg followed by near to total 'self-cure'. After day 6 the ^{32}P uptake declined rapidly and similarly to the ^{32}P uptake curve of adult male Hooded Lister rats of Aberdeen strain, although the variation within the groups was greater in the young rats compared to the adults.

Figure 2

Worm burdens expressed as worm wet weights and metabolic activity expressed as a percentage of the maximum activity in 4-5 week old male rats and in adult male rats of Aberdeen strain infected with N. brasiliensis



DISCUSSION

These results show that the decline in metabolic activity in the *N. brasiliensis* adult worms occurred at the same stage of infection in the young Hooded Lister rats as in adult rats of the same sex and strain. Although the adult worms were expelled from the young rats this expulsion took longer to occur in some rats than others and was generally slower to occur than in the older rats. Furthermore the size of the worm burdens were comparable to those in adult rats even though the young rats had been infected with 2000 larvae whilst the adult rats had been infected with 4000 larvae.

It appears that more larvae were able to complete the migration to the lungs and thence to the small intestine in the young rats compared with the adult rats i.e. Loss Phase I was smaller in young rats compared to adult rats. Hence a greater proportion of the infected larvae matured in young rats, perhaps due to reduced immunological efficiency of the young host.

Although Ogilvie and Hockley (1968), using the 'worm transfer technique', found that worms in neonatally infected rats were damaged later than in adult rats, Jarrett *et al.* (1966, 1968b) observed that worms recovered from young rats were structurally damaged by immunity to the same extent as worms which were damaged in adult rats. This experiment using the '^{32}P uptake technique' substantiates this latter observation.

Metabolic activity, indicative of immunological damage, was found to occur early in the infection after day 6 and occurred simultaneously with the onset of immunological damage in adult rats of the same sex and strain. However, in the young rats worm expulsion was evidently delayed, indicating that retention of the worms by the young rats, or in other words the delay in 'self-cure', may be related to a relative inefficiency in the mechanism of the second stage of worm expulsion.

It would be interesting to give a lower infection of N. brasiliensis to even younger rats in which the adult worms might be expected to be retained for a longer period and to monitor the metabolic activity of the worms. However, in order to use the present technique efficiently and accurately, the rats have to be of sufficient size to permit injection of isotope into the tail vein to be made. In addition to this the size of the worm burden has to be sufficient to allow for the collection, washing and drying procedures to be conducted accurately.

The results of this experiment indicate that the ^{32}P-uptake technique appears to have distinguished the two stages of 'self-cure' described from other parameters by various workers (Ogilvie, 1965; Jones and Ogilvie, 1971; Murray et al., 1971; Kelly and Dineen, 1972). It appears therefore that young rats retain their worm burdens longer than adult rats despite the fact that the worms have been immunologically damaged at the same stage of the infection.

Further experiments on the use of this technique as a 'tool' to separate these two different stages of the 'self-cure' process are described and discussed further in a later section of this thesis.

MEASUREMENTS OF THE RATE OF PASSAGE OF FOOD IN RATS UNDERGOING A PRIMARY INFECTION OF *Nippostrongylus brasiliensis*

INTRODUCTION

In the previous experiments the uptake of ^{32}P by the N. brasiliensis worms in the rat duodenum was shown to decrease abruptly about 5 - 6 days prior to the sudden decline in the number of eggs counted in the faeces. Since the faeces have to pass the length of the alimentary canal before the faecal egg count can be assessed, there may be a significant delay in observation of self cure as assessed by this parameter. Thus the true timing of reduced egg laying by the female parasites does not precisely coincide with the fall in faecal egg counts. Could the true timing of reduced egg laying capacity by the female parasites coincide with the earlier metabolic embarrassment of the N. brasiliensis worms?

The following experiment was designed to estimate the rate of passage of food from the duodenum to the time of defecation in a rat during an experimental infection with N. brasiliensis. The rate of the passage of food has been assessed in many animals by various workers using iron oxide (Bergeim, 1926), chromium sesquioxide (Moor and Winter, 1934) and phenol red (Reynell and Spray, 1956). In the present experiment an inert green indicator dye was used.

MATERIALS AND METHODS

Ten female Hooded Lister rats of the Aberdeen strain were experimentally infected with 4,000 N. brasiliensis by

the method described earlier in materials and methods I. All ten rats were caged together and were given food pellets and water *ad libitum*. On day 7 of infection, ordinary food pellets were removed and replaced by pellets which had been coated in a green inert dye (Michrome No. 240 light green S.F. yellowish, Edward Gurr Ltd., London, S.W.14).

All the rats continued to feed. Rats were killed individually at various intervals and autopsied. The alimentary canal was exposed and the position of the green dye in the alimentary tract was recorded. The length of time for the dye to pass from the duodenum at the site of infection to the defecated faecal pellets was noted.

RESULTS

Table 3

Record of the length of time which elapsed before dyed food taken orally by the rats was observed as green faecal pellets in rats experimentally infected with *N. brasiliensis* larvae

Time	Description of the rate of passage of dyed food
0	Dyed food pellets given
1 hour	Dark green colouration with food in stomach and up to 3 inches from the caecum. Duodenum flaccid and watery.
1.5 hours	Green dye passed into caecum and large intestine, and newly formed pellets in upper colon.
3 hours	Green dye in the faecal pellets up to a distance of three faecal pellets from the anus.
3.5 hours	All faecal pellets green in rectum.
4.5 hours	Green faecal pellets defecated.

DISCUSSION

The rats began to eat the dyed food pellets almost immediately. Observations showed that the dyed food reached the stomach within one hour and that the passage through the duodenum was very fast, possibly due to the flabby state of the duodenal walls and its liquid contents.

The length of time taken for the dyed food to be eaten and defecated was only four and a half hours. Consequently the faecal egg counts performed on fresh faecal matter would give a very accurate insight into the female parasites' egg-laying capacities about four hours previously.

Thus, the four day interval between the fall in metabolic activity and the fall in faecal egg count cannot be related to the time taken to eliminate the faecal matter. Furthermore metabolic embarrassment does not appear to produce any immediate effect on the ability of the female parasites to lay their eggs.

Although relatively empirical these results contribute towards an understanding of the sequence of events which occurs during infection. It would be interesting to repeat the experiment each day during the course of an experimental infection to assess if towards the end of infection the duodenal contents solidify and whether there is any delay in the length of time taken for food to pass through the alimentary canal. Hence, the optimum frequency of sampling of faecal egg counts and subsequent delay factor could be determined and more accurate results obtained.

INVESTIGATIONS INTO THE EFFECT OF THE OESTROUS CYCLE OF THE RAT ON THE COURSE OF A PRIMARY INFECTION OF *Nippostrongylus brasiliensis*

INTRODUCTION

It has been shown that female rats infected with N. brasiliensis expel their worm burden earlier than male rats (Murray et al., 1971) and that lactating and parturient female rats retain their worm burden longer than non-lactating rats (Connan, 1970). Lymphocytes in infected lactating rats are less effective than in non-lactating rats and immunological damage cannot therefore occur until later (Dineen and Kelly, 1972). It would appear that this suppression of the immunological response may be related in some way to the effects of the circulating hormones which are associated with the later stages of pregnancy and lactation, since in nulliparous rats a more apparent immunological response occurs.

In the earlier experiment using a primary infection of N. brasiliensis in both sexes of two strains of rats it was evident that the individual variation in the daily worm burdens was greater in the female rats than those in the male rats as manifested by differences in standard error. During the course of the oestrous cycle in female rats there is a change in the hormonal status in the blood stream so that the effect of different hormones in varying concentrations or by their interactions might, in some way, affect the immune responsiveness of the host.

The female reproductive system undergoes cyclic changes (Ebling and Highnam, 1969). During the oestrous cycle in the rat marked changes take place in the wall

of the vagina as well as in the uterus and ovaries. As a consequence of these changes cells are shed in sequence into the vaginal lumen.

Proestrus is characterised in a vaginal smear by groups of rhomboidal nucleated cells. About 12 hours later oestrus begins and is indicated by the appearance of cornified cells in the smear. Some nucleated epithelial cells reappear at 30 hours and by 45 hours polymorphonuclear granulocytes appear in large numbers producing a very thick smear. This stage is called metoestrus. Dioestrus occurs when some nucleated epithelial cells and a few leucocytes are present at 60 - 72 hours. At 96 hours the leucocytes disappear and proestrus starts again. Thus proestrus lasts for about 12 hours, oestrus about 33 hours and metoestrus and dioestrus together about 50 hours (Figure 4).

The principal hormones which are secreted during ovulation i.e. during oestrus, are the oestrogens. The corpus luteum, formed after ovulation, secretes progesterone and high concentrations of progesterone are found in the blood just prior to oestrus during proestrus. If fertilisation does not occur the corpus luteum regresses and progesterone secretion stops.

In this experiment the course of an experimental infection of N. brasiliensis was observed in three groups of female rats, each group in a different stage of the oestrus cycle.

MATERIALS AND METHODS

One hundred female Hooded Lister rats of Aberdeen strain were infected with 4,000 N. brasiliensis larvae by the methods described earlier in materials and methods I. On each day following infection rats were inspected and vaginal smears performed to determine which day of the oestrous cycle the rats had reached and those at the same stage of the cycle were placed together in the same cages.

On each successive day of infection from day 5 to day 10 five rats from each of the three groups, in either oestrus, proestrus or dioestrus, were intravenously injected with ^{32}P. Four hours later the worms were removed, washed, killed and surface-dried as described in materials and methods I. The adult worms were placed in pre-weighed labelled counting bottles and the bottles and worms were reweighed. At the end of the experiment all the counting bottles containing the adult worms were assayed for radioactivity in a liquid scintillation counter. The results were corrected for quenching as described in materials and methods I and expressed as corrected counts per minute per mg of wet weight of worms.

Vaginal Smears

A short narrow pipette containing a little distilled water was inserted into the rat's vagina and some of the contents was removed. This sample was placed on a microscope slide and the cells were examined using 10 x magnification under a binocular microscope.

RESULTS

The daily adult worm wet weights and corrected counts per minute per worm wet weight for each group are recorded in Table C in the Appendix and in Figure 3.

The total mean results for daily worm burdens followed the same general pattern as observed in the earlier experiment for the same sex and strain of rat, i.e. initial high worm burdens of about 50 mg weight followed by a decline after day 8, but the course of infection in rats divided into oestrus, proestrus and dioestrus showed marked differences. In the proestrus group the normal pattern was observed, i.e. the same pattern was observed as the mean results obtained in the 'strain experiment' with female rats of the same strain not grouped according to period of oestrus. The worm burdens in the dioestrus group were much lower and remained constant until day 8 after which a similar sudden expulsion occurred. In the oestrus group the worm burden, although initially higher on day 5, was expelled at a greater rate than in either of the other two groups. After day 6 the worm wet weights were reduced by two thirds and by day 8 the weight of worms recovered was only 3 mg. The difference in worm burdens between the proestrus and oestrus groups was significant.

In all three groups (i.e. oestrus, proestrus and dioestrus) the ^{32}P uptake by the adult worms declined after day 6 in the same way as previously observed in the strain experiment. Similar ^{32}P uptake curves were observed and there was no significant differences between the dioestrus, oestrous and proestrus groups (Figure 3).

Figure 3

Worm burdens expressed as worm wet weights and metabolic activity expressed as a percentage of the maximum activity of female Hooded Lister rats of Aberdeen strain infected with N. brasiliensis and experiencing proestrus, oestrus and dioestrus

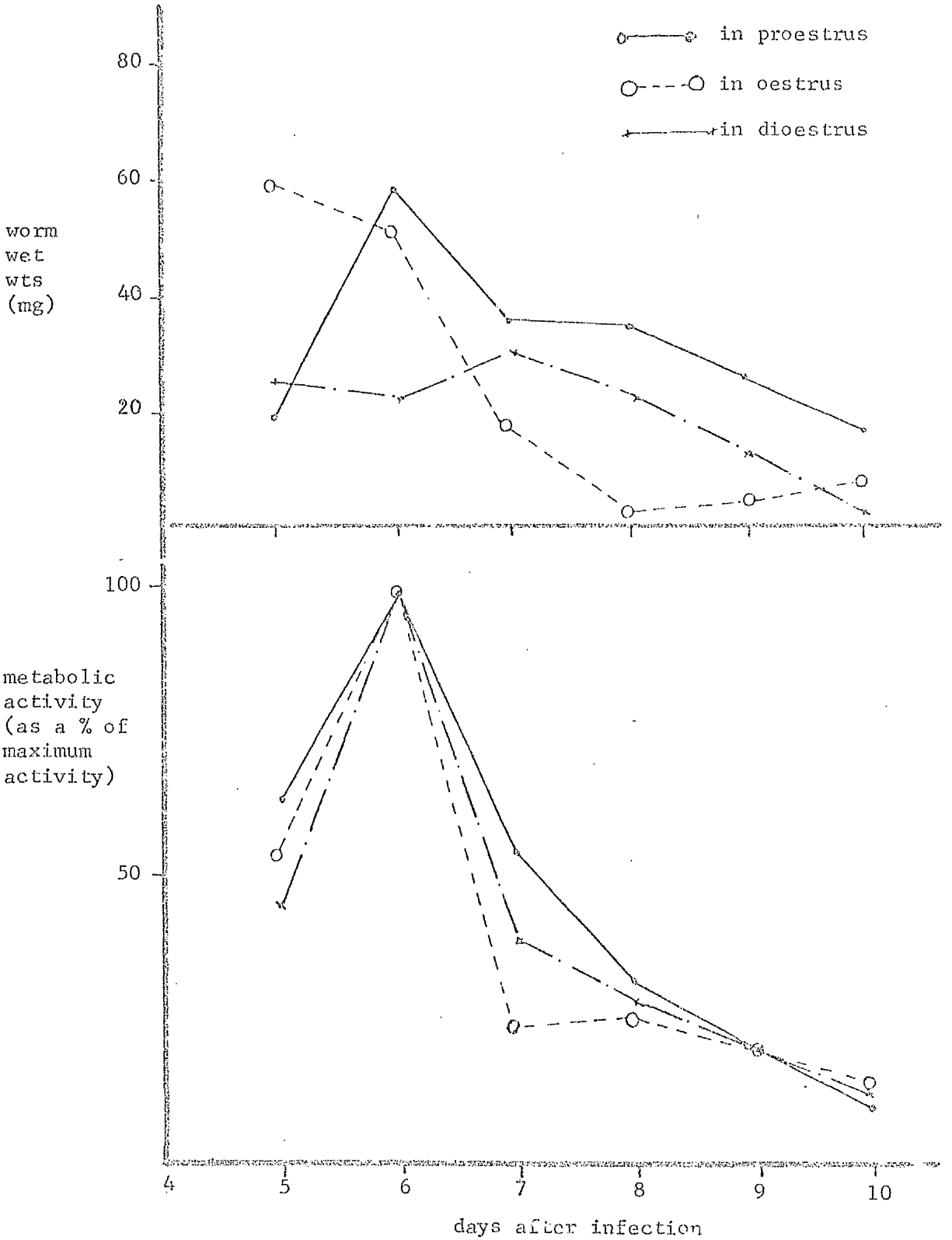
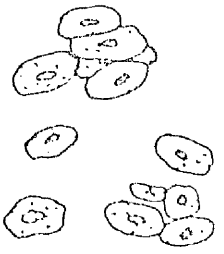


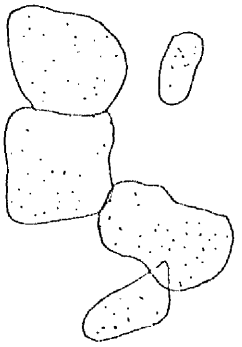
Figure 4

The successive changes in vaginal epithelial cell types in rats at each stage of the oestrus cycle

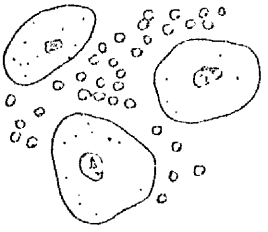
(a) proestrus



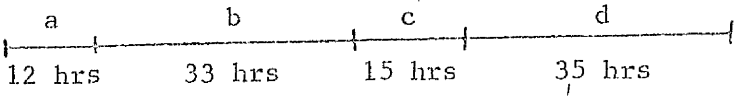
(b) oestrus



(c) metoestrus



(d) dioestrus



DISCUSSION

The results indicate that although the same pattern of events for both worm burdens and ^{32}P uptake occurred as was seen for the same sex and strain of rat in the experiment on strain described earlier, differences were apparent according to the particular stage of the oestrous cycle.

As judged from the results of ^{32}P uptake the metabolic activity of N. brasiliensis adult worms does not appear to be affected by changes in the oestrous cycle.

The difference in worm burden and worm expulsion between the three groups was more marked as judged by the results of the mean worm wet weights. While the rats in proestrus showed the normal pattern of worm burdens and expulsion, i.e. similar to the mean in the first experiment, those in dioestrus had relatively low worm burdens with normal time of expulsion. Those rats in oestrus showed higher initial worm burdens and a significantly higher rate of expulsion. In the oestrus, proestrus and dioestrus groups worm expulsion could be said to have started on days 5, 6 and 7 respectively.

These findings help to explain the results of earlier experiments in which there was greater individual variation of worm burdens amongst female rats than in males. The explanation for this phenomenon remains conjectural however, and only tentative suggestions can be made at this stage.

In those rats in proestrus worm expulsion was delayed significantly compared to the worm burdens in the oestrus group of rats. This may be related to the high concentrations

of progesterone which are present during proestrus. Progesterone is known to act as a smooth muscle relaxant and may result in reduced peristalsis of the gut thereby affecting worm expulsion. In a later experiment rats were treated with depoprovera, a long acting progesterone, to observe if expulsion could in fact be retarded by progesterone. It would appear from these results that hormonal factors only influence stage II of worm expulsion as immunological damage occurred simultaneously and early on in infections in all three groups.

THE EFFECT OF 'DEPOPROVERA' (medroxyprogesteroneacetate) ON THE COURSE OF A PRIMARY INFECTION OF *Nippostrongylus brasiliensis* IN BOTH SEXES OF RATS

INTRODUCTION

The precise mechanism of immune expulsion of N. brasiliensis from the small intestine of the rat is still the subject of some controversy (see reviews by Jarrett and Urquhart, 1971; Ogilvie and Jones, 1971; Murray, 1972).

The concept of a two stage process is accepted by many workers in the field (Urquhart, Mulligan, Eadie and Jennings, 1965; Jones and Ogilvie, 1971; Murray, Jarrett and Jennings, 1971; Kelly and Dineen, 1972), and it is now clear that significant immunological damage to the parasite occurs early in the infection, i.e. during the hypothetical Stage I (Ogilvie and Hockley, 1968; Lee, 1969; Henney, Maclean and Mulligan, 1971). So far no generally accepted description of Stage II has been put forward. It could represent an intensification and culmination of the immunological processes which are involved in Stage I or it could be a separate and perhaps relatively non-specific process involved in removing worms already damaged by specific immunity (Jones and Ogilvie, 1971). A clearer understanding of expulsion could come from a study of factors which inhibit expulsion, particularly if a factor could be found which acts selectively on either Stage I or Stage II. There is evidence to suggest that the two stages can be separated as examplified by the experiment described earlier using young rats.

The hormone status of the host can influence immunity to parasites and the most marked changes occur in the female

in the post-parturient phase, i.e. during lactation (Connan, 1970). Because of the great complexity of hormonal changes occurring around the time of parturition there is a good case for investigations where possible, of the effect of specific hormones. Obvious hormonal differences between peri-parturient and normal rats are increased levels of progesterone until 2 - 3 days before parturition (Uchida, Kadowaki, Nomura, Miyata and Miyake, 1970) and increased levels of prolactin during late pregnancy (Bast and Melampy, 1972). During lactation prolactin levels are high and initially 20 hydroxypregn-4-on-3one and later progesterone levels are also elevated (Ochida, 1970). Some suppression of rejection of N. brasiliensis in rats treated with ovine prolactin has recently been described by Kelly and Dineen (1973).

In a previous experiment in this thesis it was noticed that in female rats infected with N. brasiliensis the standard differences of the worm burdens were greater than the standard differences in male rats. It was thought that the oestrous cycle of the host may have influenced the course of infection in some way. To investigate this observation the oestrous cycle of rats was monitored concurrantly with a primary infection of N. brasiliensis. During the course of infection the worm burdens of the rats experiencing proestrous were higher than rats in oestrous and dioestrous and the decline in metabolic activity slightly less severe (Figure 3). During proestrous higher titres of progesterone are released into the body of the host.

The present series of experiments describes the effect of medroxyprogesteroneacetate (Depoprovera) on the expulsion of N. brasiliensis from the rat. Again, the opportunity arose to test the validity of the 'metabolite uptake' method. In this case, this method was used as a 'tool' to separate the end of Stage I i.e. the timing of immunological damage, and the beginning of worm expulsion, Stage II.

MATERIALS AND METHODS

A. The effect of Depoprovera on the expulsion of N. brasiliensis from the male and female hooded Lister rats (London strain)

Six groups of male and 6 of female rats of normally 5 animals per group were infected with 4,000 N. brasiliensis larvae per rat on day 0 of the experiment. Depoprovera (1 mg per injection) was given to one group of males and one group of females on day 9, and to further groups of males and females on day 11. Faecal egg counts were performed daily from day 6 - 14 and representative groups were killed on day 15 and day 17 and the worms recovered and counted. The results of the egg counts are shown in Table 4, and worm burdens in Table 5.

Depoprovera Administration

In Experiment A rats (london strain) were injected intramuscularly with 1 mg of Depoprovera either on day 9 or day 11 of the infection. In Experiment B involving 'Aberdeen' rats Depoprovera was injected on day 7 of the infection, since the onset of parasite expulsion occurs two days earlier in the Aberdeen strain.

RESULTS

It is clear from the results that the administration of Depoprovera to male rats on day 9 of the infection interfered with the normal expulsion of the parasites as indicated by the numbers of worms recovered on days 15 and 17. Even when Depoprovera administration was delayed until day 11 significant worm retention was demonstrable on day 15. Depoprovera similarly administered to female rats was without effect (Table 5).

In the light of these findings it is very interesting to note (Table 4) that Depoprovera had no effect on the faecal egg counts in either male or female rats.

The situation seen in the treated male rats where some retention of parasites was not accompanied by prolongation of egg output implies that the retained worms were nevertheless damaged. For this reason it was felt desirable to repeat the experiment on male rats and to introduce some additional measurement of damage or metabolic impairment of the parasites (Experiment B).

TABLE 4

Faecal egg counts (e.p.g) of male and female rats injected with Depoprovera on day 9 or day 11 of a N. brasiliensis infection

days after infection	Male rats			Female rats		
	Control	Injected with DP on day 9	on day 11	Control	Injected with DP on day 9	on day 11
6	52 000	40 000	40 000	30 200	26 200	25 000
7	47 000	53 000	64 000	45 000	57 000	50 000
8	64 000	46 000	42 000	42 000	120 000	55 300
9	50 000	32 000	32 000	30 600	66 400	48 200
10	30 000	25 000	19 000	29 400	14 400	20 500
11	28 000	23 000	21 000	6 000	13 800	9 900
12	6 600	8 000	5 500	100	100	900
13	1 000	1 200	300	0	0	100
14	0	1 200	0	0	0	0

TABLE 5

Mean worm burdens on days 15 and 17 of male and female rats injected with Depoprovera (DP) on day 9 or 11 of an *N. brasiliensis* infection

		Worms recovered on day 15	Worms recovered on day 17
Male rats	Control	30 ± 18	21 ± 5
	Injected with DP on day 9	430 ± 147 ($<.001$)	80 ± 23 ($<.001$)
	Injected with DP on day 11	140 ± 65 ($NS<.1$)	22 ± 17 NS
Female rats	Control	20 ± 12	24.8 ± 12
	Injected with DP on day 9	37 ± 54 NS	15 ± 10 NS
	Injected with DP on day 11	3 ± 13 NS	11 ± 6 NS

B. The effect of Depoprovera on the metabolic activity and expulsion of N. brasiliensis from male hooded Lister rats of Aberdeen strain

MATERIALS AND METHODS

Seventy rats were each infected with 4,000 N. brasiliensis larvae on day 0 of the experiment. Groups of five were killed daily from day 4 onwards. ^{32}P-labelled inorganic phosphate was injected intravenously into each rat 4 hr prior to necropsy and the worms recovered, weighed and assayed for radioactivity as described under Materials and Methods I. On day 7 of the infection half of the remaining rats were injected intramuscularly with 1 mg of Depoprovera. From day 8 onwards 5 treated and 5 control rats were injected daily with labelled inorganic phosphate and killed 4 hr later for recovery and radioassay of worms. The mean weight of worms and their mean radioactivity levels are shown in Table 6, Figure 5, and in Tables D in the Appendix.

RESULTS

Allowing for alteration in 'timing' associated with the use of 'Aberdeen' as opposed to 'London' rats the results of this experiment fully confirm the observations made under Experiment A in that the administration of Depoprovera leads to some inhibition of normal immunological expulsion. This is clearly shown by the weights of worms recovered from treated and control rats

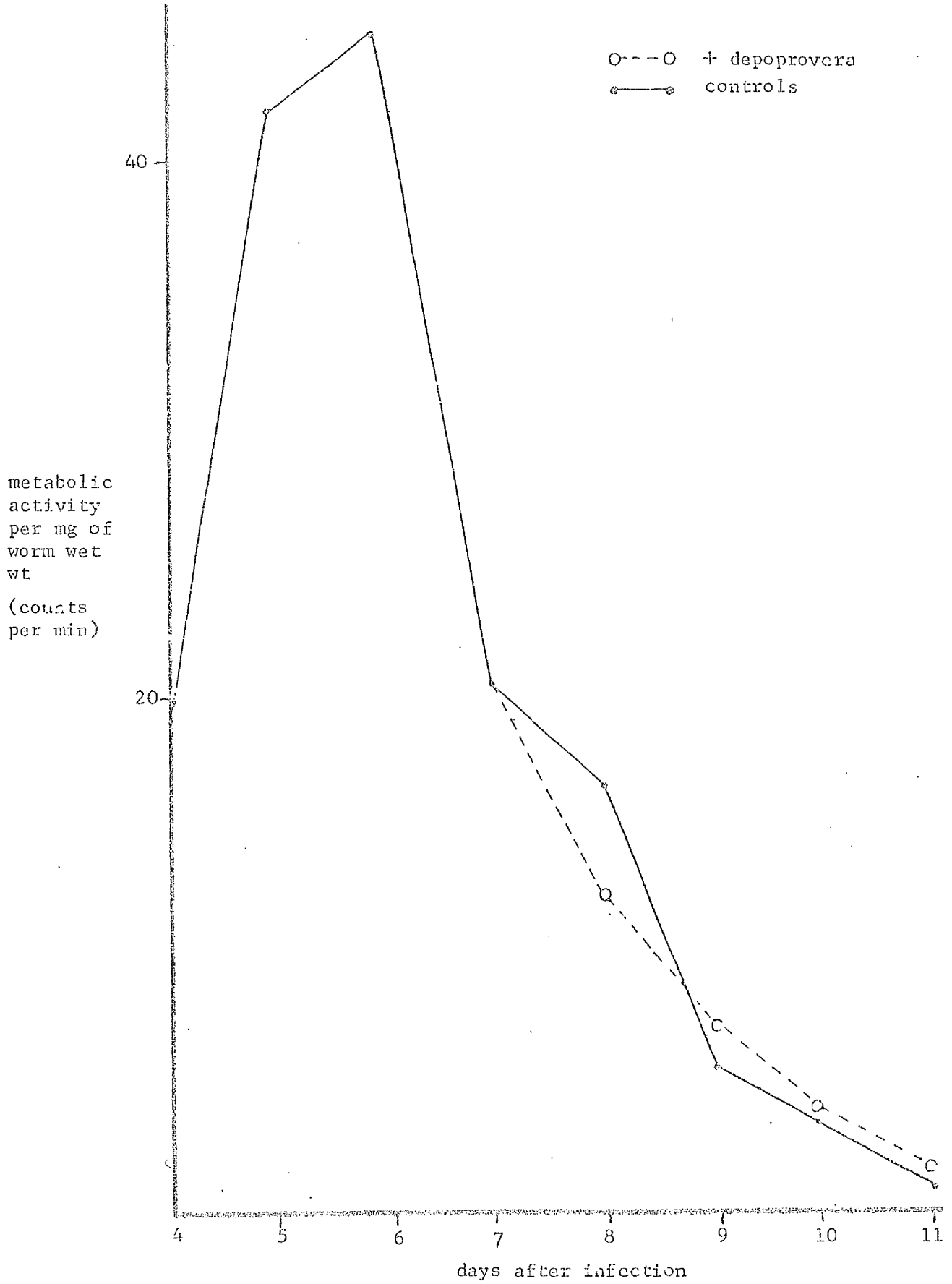
TABLE 6

Worm burdens (mg worm wet wt) of male rats of Aberdeen strain injected with Depoprovera on day 7 of *N. brasiliensis* infection

Days after infection	Control		Injected with DP on day 7
4	2.68 ± 0.8		-
5	22.60 ± 5.3		-
6	25.70 ± 6.1		-
7	22.40 ± 3.4		-
8	23.60 ± 5.5	NS	26.2 ± 4.7
9	12.4 ± 3.9	<.05	25.2 ± 3.1
10	16.1 ± 3.9	NS	25.3 ± 4.4
11	1.23 ± 3.4	<.02	9.6 ± 2.6
12	0.17 ± 0.06	<.05	11.2 ± 8.3

Figure 5

The effect of Depoprovera administered to male Hoodel Lister rats of Aberdeen strain on the uptake of (^{32}P) inorganic phosphate by N. brasiliensis worms



on days 10, 11 and 12 (Table 6). On the other hand there is no interference by Depoprovera with immunological damage to the worms as measured by phosphate uptake (Figure 5). This is again in line with the results of Experiment A where worm damage was manifested by the depression of egg output.

DISCUSSION

These results indicate that in the male rat Depoprovera interferes with the expulsion of N. brasiliensis rather than with the immunological damage which the parasite suffers due to the development of host immunity. In this respect it differs significantly from cortisone which interferes with the expulsion of the parasite (Ogilvie, 1965; Urquhart et al., 1965) but also inhibits immunological damage because egg output is maintained beyond the time when it would have fallen in untreated rats and the transfer of metabolites from the host is likewise maintained (Henney et al., 1971). Prolactin has recently been shown to prolong the infection in castrated male rats (Kelly and Dineen, 1973) and here again the effect is observable, both in terms of worm burden and egg output.

It seems possible therefore that the action of Depoprovera is related to its properties as a smooth muscle relaxant. It has been suggested by Ogilvie and Jones (1971) that adult worms secure their position in the gut through the secretion of acetylcholinesterases which produce a local paralysis in the host's gut and that production of antibodies by the host against these enzymes releases the gut from its imposed local paralysis. A good deal of attention has been given to the role of histamine and 5HT as mediators of increased gut permeability to macromolecules and hence antibodies (Murray, 1972), and it has also been emphasised that increased release of these mediators occurs around the time of expulsion. However both histamine and 5HT have stimulating effects on

peristalsis and it is this which may be important in the expulsion of worms.

In the above circumstances the relaxing effect of Depoprovera would tend to oppose the stimuli leading to increased gut motility and hence to delay the expulsion. In this connection it is interesting that transit time through the small intestine is increased during pregnancy, i.e. when progesterone levels are highest (Pany, et al., 1970).

It is not possible at the present stage to explain the relative ineffectiveness of Depoprovera to interfere with the expulsion of N. brasiliensis from the female rat. It may be that the female which will have previously encountered considerable amounts of progesterone has a capability, possibly through enzyme induction, to rapidly inactivate excess amounts of progestational agents. Alternatively the cells in the female, for example the smooth muscle of the gut and/or mucosal cells, may be somewhat less responsive to progesterone-like agents than those in the male because of a familiarization effect with progesterone-like hormones.

DISCUSSIONS AND CONCLUSIONS

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The extent to which Nippostrongylus brasiliensis worms become immunologically damaged in the rat host has been assessed from the structural damage to the worms (Jarrett et al., 1966, 1968b; Ogilvie and Hockley, 1968; Lee, 1969), from the effect of acquired immunity on the migration development and expulsion of a challenge infection (Sarles and Taliaferro, 1936; Taliaferro and Sarles, 1939, 1942; Chandler, 1932, 1935, 1936a, 1937, 1938; Spindler, 1936; Thorsen, 1953; Jackson, 1960; Ogilvie, 1965b; Jarrett et al., 1968a; Ogilvie and Hockley, 1968; Ogilvie, 1969), and from the ability of worms to recover when transferred to noninfected rats (Sarles and Taliaferro, 1936; Chandler, 1936a; Mulligan et al., 1965; Ogilvie and Jones, 1967; Neilson, 1969; Henney et al., 1971, 1973).

More recently attempts have been made to monitor the metabolic activity of the parasite in its host and to assess how this is altered by the development of immunity by the host, i.e. to measure immunological damage in terms of altered function of the worm. Since adult worms obtain nutrients by extra corporeal digestion, and absorption of metabolites from the tissue fluids of the host (Rogers and Lazarus, 1949), Henney et al., (1971, 1973) tried to devise a technique for measuring the ability of the adults to absorb labelled phosphate from the host rats which had been intravenously injected with the isotope. This method does not of course measure 'net transfer' of phosphate from the host tissue fluid to the parasite

but it gives some indication of the rate of equilibration of a metabolite between host and parasite which probably reflects to some extent the metabolic activity of the latter.

During an experimental primary infection the capacity of N. brasiliensis adult worms to take up radioactive inorganic phosphate from their host was significantly reduced preceeding actual worm expulsion. This reduction in the uptake of labelled phosphate was thought to be related to the onset of immunological damage in the parasites (Henney et al., 1971, 1973).

This novel approach of Henney et al. (1971, 1973) was used in the series of experiments described in Section I to assess the value of the ^{32}P-uptake method as an index of impaired metabolic activity of the parasite in the immune host. To do this, situations were chosen in which worm expulsion was known to vary or was altered artificially to determine whether a corresponding alteration in the timing of metabolic uptake could be observed.

For example since Murray et al. (1971) had found that female rats 'self-cured' earlier than male rats it was decided to follow the ^{32}P-uptake by the worms in both sexes of rats to determine whether a correlation existed between metabolic upset and worm expulsion. Furthermore during the course of the study it was observed that rats from two animal suppliers 'self-cured' at a different time after infection. Hence metabolic damage was monitored in male and female rats of two different strains given a primary infection of N. brasiliensis. The results of these experiments indicated that immunological damage to the worms occurred earlier in the females than in the male rats of the London strain, although this separation between the sexes

was not apparent in the Aberdeen strain of rats. There was generally a good correlation between the onset of immunological damage, as judged from a decline in ^{32}P-uptake, and adult worm expulsion. The same period elapsed between the decline in metabolic activity as measured by ^{32}P-uptake, reduced egg production and adult worm expulsion, regardless of time of onset of immunological damage. This strengthens the idea that a reduction in metabolic uptake is some index of immunological damage.

There were some situations in which the change in timing of metabolic upset did not correspond with actual expulsion of worms. For example young rats, or female rats experiencing proestrus or in male rats treated with depoprovera all had worms which persisted longer than normal although their metabolic uptake was apparently depressed at the normal time. It seems reasonable to interpret this as the occurrence of immunological damage which was then not followed by the usual degree of worm expulsion. This theory is further supported by the faecal egg count results of the male rats treated with depoprovera. It would seem therefore that the '^{32}P-uptake' method was of value in separating the now recognised two-stage mechanism of worm expulsion.

The mechanism of immunity resulting in the expulsion of adult N. brasiliensis worms from the intestine of the rat has caused much controversy. The mechanism is believed to involve two separate steps (Barth et al., 1966; W.F.H. Jarrett et al., 1968). Initially the first stage in the expulsion was regarded as the development of a hypersensitivity in the gut which gave rise to a local anaphylactic reaction with increased permeability to plasma

macromolecules at the sites occupied by the worms. Major worm damage and expulsion was thought to result from the action of anti-worm antibodies on the parasites at this time. More recent results are difficult to reconcile with this simple hypothesis, e.g. significant immunological damage to the parasite appears to occur quite early in the infection (Ogilvie and Hockley, 1968; Lee, 1969; Henney et al., 1971, 1973), i.e. before the major macromolecular leak occurs.

This information has led to a new hypothesis on worm expulsion. In the first step the adult worms are believed to be irreversibly damaged by protective antibodies (Ogilvie and Hockley, 1968; Jones and Ogilvie, 1971; Ogilvie and Love, 1974). This first step is followed by a second step in which sensitised lymphocytes (Keller and Keist, 1972; Dineen et al., 1973) or mast cells (Murray, 1972) are thought to act on the damaged worms and cause their expulsion.

The protective antibodies produced by the host rat in the first step of worm expulsion appear to interfere with the metabolism of the parasite by inhibiting feeding, possibly by preventing the release of digestive enzymes (Ogilvie and Jones, 1973). The inhibition of the worms' metabolism was detected by Henney et al. (1971, 1973) as early as day 8 after infection, when the capacity for the worms to take up inorganic phosphate dramatically and irrevocably decreased. The results in the present thesis indicate that in some strains of rats the entire period of infection was of significantly shorter duration. As a consequence, or probably a cause of this, the metabolic upset of the worm evidently also occurred earlier than normal. Similarly an experimental

infection in female rats was of shorter duration than in male rats and metabolic upset was found to occur correspondingly early.

It is thought that the digestive enzymes are probably mechanically prevented from being released from the worm by plugs of precipitate blocking the oral and excretory openings (Sarles, 1938; Taliaferro and Sarles, 1939). These plugs of precipitate can be detected in the excretory pore of the worms after the tenth day of infection and almost certainly represent antibody-antigen complexes. The antigens are probably enzymes secreted by the worm.

One possible enzyme to act as an antigen could be acetylcholinesterase (Lee, 1970; Edwards et al., 1971). The function of this enzyme is unclear, but it is thought to aid the worm maintain its position in the host by acting as a 'biological holdfast'. As immunity to the worm develops the amount of acetylcholinesterase in the worm increases, and the proportion of its isoenzymes alter. These changes in concentration of acetylcholinesterase appear to be induced by the formation of anti-acetylcholinesterase antibodies (IgG) (Jones and Ogilvie, 1972). As anti-acetylcholinesterase production increases so the enzyme production by the worms increases and conversely as the amount of antibodies decrease so the amount of enzyme produced by the worms decrease. Presumably the adult worms are attempting to secrete more enzyme to help maintain their position in the gut and to compensate for the amount complexed with anti-acetylcholinesterase antibodies. When unable to do this further, alterations

in the antigen by isomer formation possibly defeats the immune response of the host for a short time before the antibodies eventually interfere with the 'biological holdfast' forcing the worms to leave their position in intimate contact with the mucosa.

The inhibition of feeding of the worm presumably due to the antigen-antibody plugs of precipitate blocking the worm's oral and excretory openings possibly results in malnutrition. The malnutrition itself is assumed to cause damage to the gut of the worm (Jones and Ogilvie, 1971). Severe cytoplasmic changes in the large gut cells of the worms could be detected by the tenth day of infection when cytoplasmic ribosomes were replaced by large vacuoles (Ogilvie and Hockley, 1968; Lee, 1969).

This metabolic damage by antibody action and the structural changes in the gut of the worms are not however sufficient to result *per se* in efficient worm expulsion from the host. Once the worms are damaged the second step, the actual physical expulsion of the worms, begins. There has been much discussion and continuing controversy as to the effector mechanism of worm expulsion.

Urquhart *et al.* (1965) suggested that elimination of adult *N. brasiliensis* worms was induced by an anaphylactic reaction. A number of workers have found that the number of eosinophils (Wells, 1962) globular leukocytes (Whur, 1966) and mast cells (Taliaferro and Sarles, 1939; Jarrett *et al.*, 1968; Murray, *et al.*, 1971; Ottolenghi *et al.*, 1975) in the intestinal mucosa at the time of worm expulsion increased dramatically.

However other workers have observed that the increase in mast cells occurred after the worms were expelled (Wells, 1962; Kelly and Ogilvie, 1972). These opposing observations may of

course be related to the effectiveness of the techniques for detecting mast cells, probably dependant on the method of fixation, (Enerback, 1966a; Murray et al., 1968).

A greater understanding of the two stages of worm expulsion has arisen from the fact that worms damaged by antibody action are not necessarily expelled from the host rat. For example, expulsion of N. brasiliensis adult worms was delayed in neonatally infected rats as compared to adult rats (Jarrett et al., 1966, 1968b; Jarrett, 1971; Connan, 1970). However, a similar number of mast cells per unit area were found in the intestine of infected young rats as there were in adult rats (Jarrett et al., 1969). Also anaphylaxis is known to be fully developed in young infected rats by four weeks (Jarrett and Urquhart, 1969) although their capacity to eliminate worms did not fully mature until they were seven weeks old (Binaghi et al., 1966; Jones and Ogilvie, 1967b). This suggested to Ogilvie and Jones (1971) that the presence of mast cells was not essential for anaphylaxis to occur locally. Using the worm transfer technique Jones and Ogilvie (1971) found that the worms from youngrats were expelled normally when transferred to adult rats, indicating that the worms had been previously damaged in the young rats. This was confirmed in the present study when, using the '^{32}P-uptake' technique, the metabolic damage to the worms occurred at the same time in young as in adult rats, yet in young rats there was a delay in worm expulsion.

Similarly, in lactacting rats, although damage occurred to the worms at the same time as in non-lactating controls (Kelly and Ogilvie, 1972) the worms were not expelled (Connan, 1973;

Dineen and Kelly, 1972) although the intestinal mucosa of lactating rats had been shown to be infiltrated with large numbers of mast cells at the same time and to the same extent as in non-lactating controls (Kelly and Ogilvie, 1972; Connan 1973). Worm expulsion occurred normally however in lactatating rats and neonatally infected rats which were given sensitised lymphocytes from adult donors at the same time as larval infections (Dineen and Kelly, 1972, 1974; Keller and Keist, 1972). These results suggested to Keller and Keist (1972) that functionally intact lymphocytes were a prerequisite for the second step of worm expulsion, and that prolonged infestations in lactating and neonatal rats result from a deficiency in their capacity to mount the lymphocyte-dependent immune reaction against the worms.

The second step in worm expulsion was found to be sequential to the first step (Dineen et al., 1973) as only damaged worms could be expelled effectively by giving sensitised lymphocytes to the host rat. A recent series of experiments using irradiated rats has shown that lymphocytes can only produce the expulsion of damaged worms by collaboration with another cell-type derived from the bone marrow (Jones and Ogilvie, 1971; Ogilvie et al., 1977). There is still speculation as to the nature of these bone marrow cells. The most popular suggestions are that they are either eosinophils or mast cells and it has been suggested that worm expulsion may be due to a sudden release of amines from the mast cells. This release of amine has been found to occur after the interaction of reaginic antibodies on the mast cell surface with worm allergen (Barth et al., 1966;

Jarrett et al., 1968; Murray et al., 1971; Murray, 1972). The induction of immunity is thymus dependent but it is not yet known whether effector lymphocytes are also thymus derived.

Recently Sinski and Holmes (1977) have demonstrated a close relationship between the parasitic binding capacity of local IgA and immunological damage which occurs prior to expulsion of adult worms and therefore provides useful evidence that IgA may be involved in the induction of worm expulsion. This hypothesis is supported by Murray (1972) who suggests that expulsion is achieved by the summation of several immunological events in the mucosal surfaces and in particular the local IgA potentiates the release and passage of local anti-worm antibody (IgA) or systemically produced anti-worm antibody (IgG).

How any of these factors sufficiently immobilies the worms or prevent them maintaining their position in the intestine of the rat is not understood. The results of the depoprovera experiments tend to suggest that the second stage is entirely or in part non-immunological and may be due to changes in gut mobility, and this is supported by the results of the investigations on the effect of the oestrus cycle on worm expulsion. In this case it was found that, although immunological damage occurred at the same time, irrespective of the stage of oestrus, the rate and onset of worm expulsion was delayed significantly in the rats in proestrus compared to the rats in oestrus. This delayed expulsion of worms during proestrus may be related to the presence of higher concentrations of progesterone which are present during this phase. Progesterone may perhaps be the cause of the delay in worm expulsion, in a similar manner to that found in the depoprovera

experiment, through its action as a smooth muscle relaxant. However, this theory cannot be extended to explain the delay in expulsion in young rats when the amounts of progesterone would be expected to be relatively low. Delayed worm expulsion has also been observed in rats treated with various drugs such as cortisone (Ogilvie, 1965; Urquhart et al., 1965), but a precise interpretation of these experiments is difficult to find (Keller and Ogilvie, 1972). Many of the drugs used to inhibit worm expulsion have been found to have an inhibitory effect on lymphocytes as well as on the production of amines (Kelly and Dineen, 1972b).

How progesterone effects the second stage of worm expulsion is as yet unknown. The fact that another hormone, prolactin, has also been shown to cause the host rats to retain their worm burden is of interest (Connan, 1970). A further study of the influence of the hormonal status of rats on experimental infection with N. brasiliensis would probably help to elucidate this subject.

Whilst the difference in the timing of worm expulsion occurring in female and male rats has been recognised and documented previously the fact that the strain of rat has so much influence on the timing of 'self-cure' was hitherto unknown. In view of the importance laid on each part of the infection in terms of studying 'self-cure' it is obvious that greater attention should be made to the strain of rat used. Strain difference could be investigated further in either of two ways. Firstly, to use the N. brasiliensis model to study the expulsion in more detail or secondly to look at the practical implications in an

economically important helminth infection. An opportunity arose to study strain difference in an economically important parasite, H. contortus in Kenya in three strains of sheep. This work constitutes Section II of this thesis.

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SECTION II

STUDIES ON STRAIN DIFFERENCES IN THE RESISTANCE OF SHEEP TO *Haemonchus contortus*

INTRODUCTION

Whilst it was useful to study the influence of strain on the host-parasite relationship in a laboratory model, such as the well documented N. brasiliensis-rat system, it was believed equally important to apply the information gained from these studies to an economically important helminth. The system chosen was Haemonchus contortus in sheep since this parasite is widely recognised as one of the most important of the main pathogenic nematode species in most tropical and sub-tropical areas. Furthermore, the occurrence of the 'self-cure' phenomenon in this system and recent evidence that difference in susceptibility to H. contortus may be related to the strain of the host, provided a suitable system in which to extend the work of the first section of this thesis, i.e. a study of the extent to which the strain of the host influences the host-parasite relationship and the timing of the 'self-cure' phenomenon.

H. contortus is a blood sucking nematode parasite of the Trichostrongylidae family which occurs in the abomasum of sheep, goats, cattle, buffalo and many other wild ruminants. The primary pathogenic effect of H. contortus is the anaemia caused by the blood sucking activities of both the larval and adult stages and the haemorrhage produced by those activities (Fourie, 1931; Andrews, 1942; Dargie, 1974). The extent of the anaemia is apparently governed by the number of worms present in the host and the host's own capacity to replace the lost blood (Clark et al., 1962).

The adult female Haemonchus measures about 20 - 30 mm. White ovaries spiral around the blood-filled intestine giving

the characteristic 'Barber's pole' appearance. The adult male is about half the length of the female. It has a well developed bursa with an asymmetrical dorsal lobe supported by a Y-shaped dorsal ray. Male and female adults feed on whole blood taken from the sub-mucosal capillaries. The female is often quoted as one of the most prolific egg-laying helminths and it is estimated that a single female will produce up to 10,000 eggs per day; in other words an average production of approximately one egg every ten seconds.

These eggs are passed out into the intestinal contents and are deposited with the faeces on the pasture where under optimum conditions they undergo rapid development. The first larval stage can be fully formed in less than a day if the conditions are favourable, i.e. temperature of 11 - 21^{o}C and high relative humidity (93 - 95%) (Rees, 1950; Silangwa and Todd, 1964; Waller and Donald, 1970). However, in adverse conditions the eggs can lie dormant for many weeks. Once the egg hatches the first stage larva emerges and feeds on bacteria within the faeces. Moulting gives rise to the second stage larva which also feeds on bacteria. The development to the third stage larva takes only 2 or 3 days under favourable conditions. The L_3 stage is the infective stage and is more resistant to dessication than the previous stages (Ellenby, 1968). This arises largely through the retention of the cuticle of the L_2 stage. However, the ensheathed L_3 stage larva cannot feed but remains dormant on the pasture up to 6 - 12 months (depending on the conditions) until grazed by the definitive host (Allonby, 1974; Onar, 1974;

Waller, 1974; Levine et al., 1974). After being swallowed by the host the infective larvae lose their protective sheath in the host's rumen and then find their way into the abomasum. Here they burrow into the mucosa and become covered with mucus. The third moult is followed by a period of growth during which the L_4 larvae feed on whole blood (Clark et al., 1962; Dargie and Allonby, 1975). After a further ten days the L_4 larvae emerge from within the mucosa and move about freely on the surface of the mucosa. They then moult for the last time, become sexually mature and begin their voracious blood-sucking activities. After copulalation the females begin egg laying and these can be observed in the faeces of the host between 18 to 24 days after infection.

The blood loss caused by the worms results in anaemia, which if severe enough, results in lethargy, loss in condition and even death. In hyperacute haemonchosis sudden death, which is often the first sign of the disease, can occur within one or two days of infection (Soulsby, 1965; Allonby, 1974). In acute cases there is sudden lethargy and progressive weakness often terminating in death (Fourie, 1931; Baker et al., 1959; Baker and Douglas, 1966). In chronic haemonchosis long term weakness and emaciation results in stunted growth and poor wool yield considerably reducing the economic viability of the host (Allonby, 1974).

The occurrence of the 'self-cure' phenomenon or the periodic expulsion of the entire adult worm population in H. contortus in sheep was one of the earliest reports of possible immunity to a helminth infection (Stoll, 1929). It is known to occur periodically in situations where sheep are left untreated and is

apparently the principle way in which acute haemonchosis is limited. The timing of self-cure of naturally acquired H. contortus infections has been shown to be associated with periods of significant rainfall although it is not clear why this relationship exists (Gordon, 1948, 1967; Stewart, 1950a, b, 1953). The rainfall is thought to stimulate both the hatching and development of Haemonchus to the infective stage and the growth of new grass. Whether the antigenic stimulus of the larvae causing a local hypersensitivity response (Stewart, 1953; Soulsby and Stewart, 1960) is responsible for self-cure or whether newg grass contains anthelmintic properties (Allonby and Urquhart, 1973) has yet to be clarified. In an experimental infection conducted indoors Altaif (1975) investigated both these stimuli and found that self-cure occurred equally well when sheep were given either infective larvae or fresh grass. An apparent anomaly to the immunological theory regarding self-cure in the field is that it apparently does not confer any protection against rapid reinfection (Gordon, 1948; Lopez and Urquhart, 1967; Allonby and Urquhart, 1973), and in fact worm burdens following reinfection are generally higher than the pre-expulsion levels.

Despite the widespread occurrence of this pathogenic nematode in tropical and subtropical areas the control of the disease has proved to be problematical due to a number of reasons. Although strategic drenching with anthelmintic therapeutics, combined with good management procedures can prevent the occurrence of acute disease, this is an expensive exercise which rarely achieves complete control of the disease. In most areas reinfection is

rapid following anthelmintic treatment and most animals are continuously affected with low to moderate worm burdens throughout their lives.

A further and ever increasing problem is the continual development of resistance by parasites to anthelmintics (Theccorides, 1974; Colglazier et al., 1974). Strains of H. contortus have become resistant to most of the modern anthelmintics, for example, phenothiazone (Drudge et al., 1957), thiabendazole (Drudge et al., 1964; Conway, 1964; Smeal et al., 1968; Colglazier et al., 1974), morantel (Le Jambre et al., 1974) and parabendazole (Berger, 1975).

Another possible form of control is the development of methods of immunological control. Whilst there have been various attempts to immunise sheep against H. contortus the success of vaccination evidently depends on several factors such as the age of the host at vaccination and the animal's earlier experience of infection. These factors are also known to vary with the breed of sheep involved. For example, vaccination trials with 7 - 17 month worm-free Scottish Blackface sheep using attenuated larvae have been consistently successful (Jarrett et al., 1961; Urquhart et al., 1962; Bitakaramire, 1966). However, in attempting to vaccinate 7 month old Merino sheep, Mulligan et al., (1961) were successful in immunising only 6 out of the 10 animals used. Lopez and Urquhart, (1967) also failed to protect 7 month-old Merino lambs. Thus there appear to be fundamental differences in the immunological competence of different breeds of sheep.

Recent work in Kenya has shown that some breeds of sheep have a greater innate resistance to H. contortus than other breeds. For example, Masai sheep were found to be much more resistant to H. contortus than Merino sheep in Kenya (Preston and Allonby, 1977; Allonby and Preston, 1977) and Scottish Blackface sheep were more resistant than Dorest horn sheep in Britain (Altaif and Dargie, 1976). However, within each breed it was observed that there were marked individual differences in susceptibility to H. contortus. These individual differences in susceptibility to H. contortus within each breed appear to be associated with the strain of the host.

Biochemical polymorphism has been used recently as a means of distinguishing strains of animals. An easily detected polymorphism and one which has been used as a means of distinguishing strains of sheep is haemoglobin polymorphism. Two alleles (A and B) and three phenotypes (A, AB and B) are commonly found (Harris and Warren, 1955; Evans et al., 1956; Helm et al., 1957; Huisman et al., 1958a, b). There is some evidence that sheep with haemoglobin A (HbA) are more resistant to H. contortus than sheep with haemoglobin B (HbB), (Evans et al., 1963; Loggins et al., 1973; Evans and Whitlock, 1964; Jilek and Bradley, 1969). The practical application of this experimental finding has been suggested by Allonby and Urquhart (1976) who observed that in Merino sheep reared for two years on H. contortus-endemic pasture that HbA sheep had higher PCV values, 'self-cured' more often and, more important in terms of production, maintained consistently higher total body weights than sheep with HbB.

It seems surprising in view of the persistent difficulties in achieving control of haemonchosis by the use of anthelmintics and of the difficulties in developing immunisation techniques that differences in the strain of the host have not been pursued as a possible aid in controlling helminthiasis. The possible utilisation of haemoglobin polymorphism for selecting genetically resistant strains of sheep to H. contortus i.e. 'haemoncho-tolerant' animals, appeared to justify further investigation and these studies are described in the next section of this thesis.

GENERAL MATERIALS AND METHODS II

SECTION II

MATERIALS AND METHODS

INDEX

A. HAEMATOLOGICAL TECHNIQUES

Blood samples were collected from the jugular vein of each sheep using E.D.T.A. as anticoagulant, by means of an evacuated venoject blood collecting tube, a two-way needle and a needle holder (Terumo Corporation, Tokyo, Japan).

Packed cell volume percentages (PCV) were determined by the microhaematocrit method of Fisher (1962). Capillary tubes containing the blood samples were sealed at one end by the heat from the flame of a bunsen burner and centrifuged for 5 minutes in a microhaematocrit centrifuge (Hawksley and Sons Ltd., London, England). The percentage PCV was determined from the scale on a Hawksley Microhaematocrit Reader.

B. HAEMOGLOBIN TYPING

Haemoglobin typing was performed by electrophoresis on cellulose acetate paper using a multi microband electrophoresis apparatus, (Shandon Scientific Co.). This method allows for a clear and rapid separation of the bands, and was found to be just as effective as the conventional starch-gel procedure used by Smithies (1955) and later modified by Huisman (1963).

SOLUTIONS

Buffer

16.5g of barbitone acetate buffer (Oxoid Ltd., London) were dissolved in 500ml of warm distilled water, cooled and made up to one litre with distilled water.

Prebuffer

25.2g Tris (hydroxymethyl-aminomethane), 2.5g E.D.T.A.' (ethylenediamine tetra-acetic acid) and 1.9g boric acid were dissolved in 1000 ml distilled water.

All compartments were filled with enough buffer to cover the metal electrodes. Care was taken that both sides of the compartments contained the same volume of buffer. Buffer-moistened filter papers (Whatman No. 3 No. 3MM.23 x 4.5cm) were placed on both shoulder pieces. The cellulose acetate papers ('Celagram', Shandon Scientific Co.), size 78 x 150mm were soaked in prebuffer taking care that each part of the paper made contact with the prebuffer instantaneously so that no air bubbles occurred. The cellulose acetate paper was carefully blotted between filter paper and placed across the bridge of the tank.

A drop of each blood sample was placed on a multi applicator plate (Shandon Scientific Co.), and haemolysed by the addition of a drop of distilled water to each.

Using a multi applicator (Shandon Scientific Co.) 10 samples of haemolysed blood were tested simultaneously. The applicator was carefully dipped into the haemolysed blood samples and any excess blood was removed with filter paper.

The samples were carefully applied to the middle of the cellulose acetate paper. The lid was replaced and 200 volts were applied at constant current. After 20 mins it was possible to see a good separation of the haemoglobin bands.

The cellulose acetate paper was then placed in a 0.2% solution of Ponceau S dye in 3% trichloracetic acid for about 30 mins. The paper was removed, washed in 5% acetic acid, blotted, dried and read.

Reading of haemoglobin types

Haemoglobin type AA migrated towards the anode faster from the base line than haemoglobin type BB. Two lines could be seen for haemoglobin type AB.

C. INDOOR EXPERIMENTS

1. Experimental animals

A description of the breed, age, sex, treatment, numbers of sheep and their origins is given at the beginning of each experiment.

2. Housing and maintenance

Sheep were separately confined to standard sheep metabolism cages. Each sheep was fitted with a leather harness to which a canvas faecal bag was attached and tied in position around the anus. Inside the canvas bag was placed a removable polythene bag into which the faeces were collected and this was changed every 24 hours.

Urine passed through a metal grid at the base of the metabolism cage and was collected in a wide-necked bowl which was placed under the cage.

Food and water was provided in a compartment at the front of the cage.

A description of the diets of the sheep is given at the beginning of each experiment.

D. PARASITOLOGICAL TECHNIQUES

1. Culture of Haemonchus contortus

A pure strain of H. contortus was obtained from the Veterinary Research Laboratories, Kabete. Kenya. Here the H. contortus were passaged through adult Merino sheep reared and maintained under worm-free conditions indoors. Faeces were collected daily using a faecal bag and cultured by half filling 500 ml jars with faeces and incubating the jars for 14 days at 22°C in darkness.

Infective L_3 stage larvae were recovered from faecal culture by a method similar to that described by Roberts and O'Sullivan (1950). The jars were filled with warm water and allowed to stand for several hours. The coarse debris was removed using a 250 mesh/cm seive and the suspension was filtered through filter paper under pressure using a Buchner Funnel attached to a water pump. The filter papers were then placed larval side down on a fine seive (Mesh No. 10) in a Baermann apparatus filled with warm water and left for 2 - 3 hours to allow the larvae to migrate from the filter paper to the base of the funnel. The larvae were then released into a measuring cylinder.

2. Experimental infection

The number of H. contortus infective larvae in the suspension was assessed by removing 0.025 ml of the suspension using an autozero pipette. The number of larvae contained in this volume were counted, and this value was multiplied by 40, thus assessing the number of H. contortus larvae in 1 ml. Five of these readings were performed and an average figure was

taken to be the number of larvae present. However this method was only employed if more than 200 larvae were counted in each sample otherwise the larval suspension was concentrated further. The larval suspension was then diluted or concentrated by the removal or the addition of physiological saline until the required concentration was achieved.

The number of larvae used is described in each experiment. In all experiments the larval dose administered was related to the body weight of the sheep. The larvae were administered orally using a disposable syringe.

3. Faecal egg counts

A faecal sample was collected daily from each sheep. The faecal egg counts were performed by a modified McMaster technique (Gordon and Whitlock, 1939; Whitlock, 1948) and as described previously in general materials and methods I.

4. Abomasal worm burdens

The sheep were stunned with a captive bolt pistol and exsanguinated from the jugular vein. The abdomen was opened and the abomasum and omasum removed together and placed on to a metal tray.

The abomasum was separated from the omasum, and incised along its greater curvature. The contents of the abomasum and tray were then washed out into a plastic bucket with physiological saline. A series of aliquots of the thoroughly mixed diluted abomasum contents were removed and placed in a Petri dish, and the numbers of worms in each aliquot were counted. By summation the numbers of worms in the whole sample were counted and recorded. The entire abomasal mucosa was scraped off

with a sharp knife, chopped finely and 200g lots placed into separate jars. The jars were filled with pepsin-hydrochloric acid mixture and incubated at 42°C for 6 hours. The digests were formalinised, diluted to 2 litres and two samples, each of 200ml, were removed and retained for later examination. The number of larval stages present in each aliquot were counted under a binocular microscope at 10 x magnification and the total worm burden calculated.

The pepsin - HCl mixture was similar to that described by Herlich (1956), i.e. 10g of 1:2500 pepsin powder was dissolved in 600 ml of water and acidified with 30 ml of conc. HCl.

E. RADIOISOTOPIC TECHNIQUES

1. Labelling of erythrocytes with ^{51}Cr

On day 0 of each experiment blood samples were collected from the jugular vein of each sheep using E.D.T.A. as anti-coagulant, by means of evacuated venoject blood collecting tubes, a two way multi-sampler needle and needle holder. The PCV was determined for each blood sample. The method used for labelling the erythrocytes with ^{51}Cr was that of Holmes et al (1968).

After centrifuging at 1200 r.p.m. for ten minutes the plasma from each blood sample was separated and retained. The red blood cell samples were placed in labelled universal bottles, stoppered with cotton wool, and were incubated for 30 min at 37°C with ^{51}Cr in the form of sodium chromate Radiochemical Centre, Ltd., Amersham, England). The blood

samples were gently mixed from time to time. About 200 uCi ^{51}Cr were added per ml of packed red cells.

The ^{51}Cr-labelled red cells were washed three times by the addition of physiological saline (37°C) mixing and centrifugation as described above. The supernatant containing the unbound ^{51}Cr was disposed of after each washing using a Pasteur pipette attached to a vacuum pump.

The ^{51}Cr labelled red blood samples were then re-constituted with their respective plasma and about 1 ml of each sample was placed in a 1 ml syringe for use as a standard. The remaining blood was drawn into a 20ml disposable syringe for injection. In all cases each sheep received its own erythrocytes and plasma. The syringes and blood samples were each weighed before and after injection so that the weight of ^{51}Cr-labelled blood injected into each sheep and used for each standard could be calculated.

^{51}Cr standards

The ^{51}Cr-labelled blood samples contained in the 1 ml syringes were separately dispensed into 200ml volumetric flasks and diluted to 200 ml with 0.05% NaOH.

2. Labelling of sheep albumin with ^{125}I

The method employed was described by Vogel (1951).

Three days before injection into the sheep the following solutions were made up.

Buffer A Nine ml M.glycine in M/4 Nacl + 1 ml N - NaOH. This buffer was used to convert iodine monochloride to hypoiodite (pH 8.5).

Buffer B Eight ml M. glycine in M/4 Nacl + 2 ml N - NaOH. This buffer was used to dissolve the protein (pH 9.0).

Preparation of a stock solution of iodine monochloride

Five g KI and 3.22 g KIO_3 were dissolved in 37.5 ml distilled water. 37.5 ml conc. HCl and 5 ml CCl_4 were added to the solution. This stock solution contained approximately 147 mg I/ml as iodine monochloride (ICl).

The mixture was shaken vigorously and a slight pink colour was noted.

Solution 1

In order to prepare a 2% solution 600 gm of commercial sheep albumin were dissolved in 30 ml of distilled water in a conical flask. Fifteen ml of glycine Buffer B was mixed with this protein solution.

Solution 2

The stock iodine monochloride solution was diluted 350 times with saline so as to contain 0.42 mg I/ml. 4 ml of this diluted solution was placed in a conical flask and to it 10 MC; carried free Na ^{125}I was added.

15 ml of glycine Buffer A were quickly mixed with solution 2, then added immediately to solution 1.

The labelled albumin was transferred to a dialysis sac using a filter funnel. The dialysis sac contained 2.5 gm of 'carrier' bovine serum albumin. Carrier protein was used to reduce the specific activity of the labelled albumin to less than 5 uCi/mg thereby reducing the possibility of radiation decomposition (Freeman, 1961; Berson, Yalow, Schreiber and Pest, 1953). The labelled albumin was dialysed overnight in a cold room ($5^{o}C$) in about 20 litres of distilled water to remove the unbound iodide. The following day the dialysis sac was transferred to 20 litres of a solution of 0.85% NaCl for another 24 hours.

The labelled albumin was then placed into a centrifuge tube and centrifuged for 30 mins at 1200 r.p.m. It was then drawn into 20 ml disposable syringes for injection into sheep and into two 1 ml syringes for use as standards.

The 20 ml and 1 ml syringes containing the ^{125}I-labelled albumin were weighed both before and after injection so that the weight of ^{125}I-labelled albumin injected into each sheep and that used for the standards could be calculated.

^{125}I Standards

The ^{125}I-labelled albumin contained in two 1 ml syringes were separately dispensed into two 250 ml volumetric flasks and diluted to 250 ml with 0.05% NaOH.

3. Injection of labelled materials into sheep

A wide bore catheter needle was inserted into the right jugular vein of each sheep and a plastic jugular catheter was fed into the vein.

The syringe containing the labelled red cells was placed in one of the entrances of a three-way tap. When labelled albumin was used in the experiment the syringe containing the protein was inserted into the entrance of the second tap.

The ^{51}Cr-labelled red cells were injected into the vein of the sheep followed by the ^{125}I-labelled albumin. The catheter was then flushed out with saline to make sure all the labelled material had entered the sheep.

The time was noted when both the labelled samples had been injected into the sheep. Ten minutes after the injection a blood sample was removed from the left jugular vein using E.D.T.A. as anticoagulant. The ten minutes time

lapse before the first sample was withdrawn was to ensure the complete mixing of the ^{51}Cr-labelled red cells within the circulation.

4. Daily Procedure

Blood samples

Every 24 hours a blood sample of about 3 ml was removed from the jugular vein of each sheep using E.D.T.A. as anti-coagulant and a PCV determination made. One ml of each blood sample was pipetted into counting bottles labelled with the day of the experiment and the sheep number. When pipetting care was taken that all the red cells were removed from the pipette by releasing the blood very slowly. Nine ml of 0.05% NaOH was added to each 1 ml blood sample as a diluent.

For determination of plasma ^{125}I activity the blood samples were centrifuged for 10 mins at 2000 r.p.m., the plasma was removed and 1 ml of each sample was transferred into a counting bottle and diluted with 9 ml of 0.05% NaOH.

Faecal samples

The 24 hour faecal sample was collected at the same time each day and weighed. A 10 g sample of the faeces from each sheep was weighed and pressed into counting bottles. Additionally a 3 g sample of faeces was taken daily from the faecal sample and a faecal egg count estimation was performed.

Urine samples

A total urine collection was made daily and the volume of urine produced by each sheep was recorded. A 10 ml sample of urine from each sheep was transferred into labelled counting bottles.

Radioactivity measurements

Radioactivity measurements were performed using an automatic well-type gamma scintillation spectrometer.

The counting bottles containing the blood, plasma, urine and faeces samples were measured at the end of the experiment for radioactivity. One ml of the standard solutions of ^{51}Cr and ^{125}I were diluted with 9 ml of 0.05% NaOH in separate counting bottles and used to calculate the total amount of injected radioactivity.

A SURVEY OF HAEMOGLOBIN TYPES OF SHEEP IN KENYA

INTRODUCTION

Haemoglobin polymorphism has been used recently as a means of distinguishing strains of animals and has been studied in man, sheep, goat. cattle, horse, donkey, mule, dog, cat, chickens and also in some laboratory animals (Evans et al., 1956; Rodnan and Ebraugh, 1957; Ogden, 1961; Schmid, 1962a, b; Jones et al., 1972). Sheep, goats, mules, cattle and horses show a clear polymorphism with two alleles, and three possible phenotypes, namely AA, AB and BB (Harris and Warren, 1955; Evans et al., 1956; Helm et al., 1957; Huisman et al., 1958a, b). The pig, dog and cat are thought to have a single haemoglobin type; whereas the fowl has heterogenous haemoglobins, the synthesis of which are believed to be controlled by at least three genes (Rodnan and Ebraugh, 1957).

Many authors have attempted to explore the possible use of these biochemical polymorphic traits as a tool for the determination of parentage (Osterhoff, 1964), the investigation of the genetic background of a breed (Huisman et al., 1958b; Evans et al., 1958a), and the genetic relationship between breeds. An interesting speculation as to this latter point was made by Bangham and Blumberg (1953) who postulated that the Jersey breed of cattle were descended from cattle of the ancient Indus valley civilisation. Furthermore, the intimate ecological association between human groups and their domestic animals has led Evans et al. (1958a) to believe that a study of gene frequencies in animal breeds may enable human migration to be traced.

The gene frequencies for haemoglobin type have been recorded for over 120 breeds of sheep from many countries by numerous

workers. (See Review Agar et al., 1972.) The distribution of the haemoglobin types varies markedly between breeds of sheep (Evans et al., 1958a; Meyer, 1963). Since haemoglobin A has been shown to have a higher affinity for oxygen than haemoglobin B, it has been postulated that natural and human selection would favour haemoglobin A type sheep at high altitudes and haemoglobin B type sheep at sea level (Huisman etal., 1958; Dawson and Evans, 1962; Naughton et al., 1963, Sirs, 1966). Evans et al. (1958a, b) found that there appeared to be a tendency for haemoglobin A type sheep to be associated with mountainous and relatively cold areas and for haemoglobin type B sheep to be found at sea level. However, there was no difference in distribution of the haemoglobin types in sheep inhabiting mountain, hill and lowland areas in Sardinia (Dassat and Sartore, 1968). Haemoglobin B appeared to be common in sheep living in the Cuneo Province of Italy (Dassat, 1964); in a number of breeds of sheep from the Peruvian Andes (Chan, 1968); and in sheep from the himalayan region (Agar, et al., 1968; Agar and Seth, 1971). It appears, therefore, that other factors in addition to the possible association with altitude and/or temperature must influence the natural selection of haemoglobin type in each particular location.

More recently, it has been found that a relationship exists between biochemical polymorphism and reproductive performance and other parameters of production, particularly in sheep. The possible association between haemoglobin type and reproductive performance in the Merino ewe was studied by Evans and Turner (1965). They showed that HbA ewes produced and reared fewer lambs than HbAB or HbB sheep. Similar observations were also made by Dooley (1965)

and in other breeds in different countries, i.e. Langhe ewes in Italy (Bernoco, 1967, 1968; Dassat and Bernaco, 1968), Marwari ewes in India (Arora et al., 1971) and Awassi x East Fresian ewes in Asrael (Reshef, 1965). However, HbA Blackheaded Mutton sheep in Northern Germany were more fertile than HbB ewes (Meyer et al., 1967). Consequently Obst and Evans (1970) suggested that HbA had better reproductive performance under conditions of a cold harsh environment because of their enhanced productive performance whereas HbB predominated in warm environments.

An interesting observation was made by Blunt and Evans (1961) who showed that the gene frequencies of the haemoglobin types of Romney Marsh and South Down sheep in Australia were predominantly HbA whereas the original stock in their native British environment were predominantly HbB. This suggested that HbA might be in some way associated with an adaptation to the Australian environment. One possible basis for this adaptation in Australia was suggested by Evans et al., (1963) who noticed some correlation between haemoglobin type in sheep experimentally infected with H. contortus, in that the HbA sheep had higher haematocrits and lower faecal egg counts. This indicated that haemoglobin type might be linked with resistance towards this blood-sucking abomasal parasite.

In an attempt to elucidate the relationship between haemoglobin polymorphism and the strains of sheep breeds in various environments it was decided to conduct a survey of haemoglobin types of some exotic and indigenous sheep breeds where both co-existed in a country of great topographical diversity. Kenya was chosen as in its 582,644 km^2, ranging in altitude from

sea level to over 4,600 metres, it contains six of the seven eco-climatic zones described by Pratt et al., (1966); sheep being represented in five of these zones. H. contortus is also endemic in Kenya, but the size of the challenge is related to rainfall; hence in areas of high rainfall it would be expected that there would be a high H. contortus challenge, whilst only a spasmodic challenge would occur in the arid areas. Sheep of seven different breeds or their crosses were sampled from all the eco-climatic zones where sheep are found and their frequency of haemoglobin types was determined for the sheep strain in each zone. Apart from defining the variation in haemoglobin types between sheep breeds it was hoped that, should any clear pattern of distribution emerge, this would prove useful in understanding the population genetics of these characters and perhaps throw some light on their possible relationship to the distribution and prevalence of H. contortus infection.

MATERIALS AND METHODS

Sheep were sampled from the five eco-climatic zones of Kenya in which sheep were represented. The flocks of sheep sampled belonged largely to local herdsmen. In most cases each herdsman maintained a flock of between 10 to 15 animals, although in a few cases larger flocks of 100 sheep or more were encountered.

Most of the smallstock owners had never treated their animals with anthelmintics, nor had they sprayed or dipped them

with acaricide. Continuous mating and non-castration were usual and hence the 'gene pool' could be regarded as reflecting the rigorous conditions of traditional management with greatest emphasis on natural selection.

Care was taken to make a random selection of animals in as many locations within each ecological region as possible. This was done by driving through an area observing sheep in each village or settlement. If a flock of more than ten sheep was observed, permission was asked to sample five sheep. After sampling these sheep the next flock was sampled from a co-operative farmer of the next village. In this way a number of flocks of sheep could be sampled in one day. After a sampling period of 2 - 3 days all samples were taken to Nairobi for processing. In some areas local prejudices about taking blood were not overcome particularly in areas where drinking water was scarce, and blood and milk from their animals were the source of the family's main liquid supply.

Sampling technique

Samples of whole blood were collected from the jugular vein of the sheep using evacuated heparanised venoject tubes and a two-way needle. In the laboratory the blood samples were tested for haemoglobin type as described in general materials and methods II.

RESULTS

The map of Kenya showing the six eco-climatic zones of Pratt et al., (1966) is presented in Figure 6, and the distribution of sheep in Kenya is found in Figure 7. The eco-climatic zones and record of the Provinces and Districts from where the samples were taken is found in Table 6. The incidence of the three haemoglobin phenotypes in each breed from the five eco-climatic zones in which sheep are represented are recorded in Table 7.

It is apparent that the great majority of these breeds are polymorphic with respect to the three haemoglobin types and that there is quite a wide variation in the gene frequencies from breed to breed. However in the majority of cases there was a greater frequency of the B gene compared to the A gene. Only in one district in eco-climatic zone II was the frequency for A (0.98) greater than that for B (0.02).

The Masai sheep in zone II had a higher proportion of A gene (0.29) compared to those in zone III (0.25) which had higher A frequencies than the Masai sheep in zone IV (0.09) and zone V (0.06). Of the exotic sheep a similar trend was observed. For example, Dorper sheep in zone II showed a gene frequency for A of 0.98, and in zone III, 0.69; and 0.33 in Merino sheep in zone III whilst in zone IV the frequency for A was 0.23.

These results indicate that the incidence of A gene decreased, in both indigenous and exotic breeds, in sheep in the drier areas. Conversely the incidence of A genes increased in wetter areas.

Figure 7

A map of eco-climatic zones of Kenya

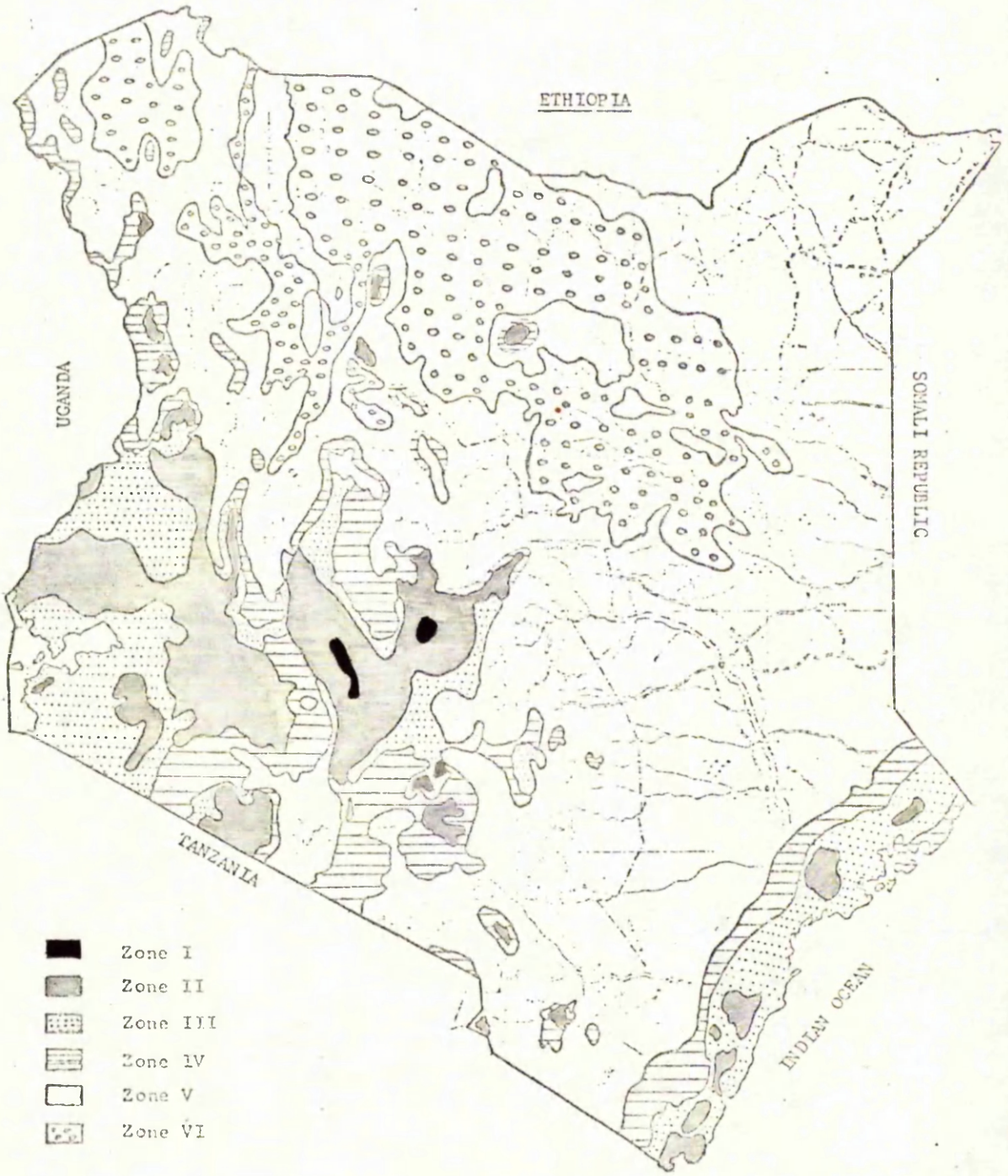

Figure 8

A map of Kenya showing sheep distribution

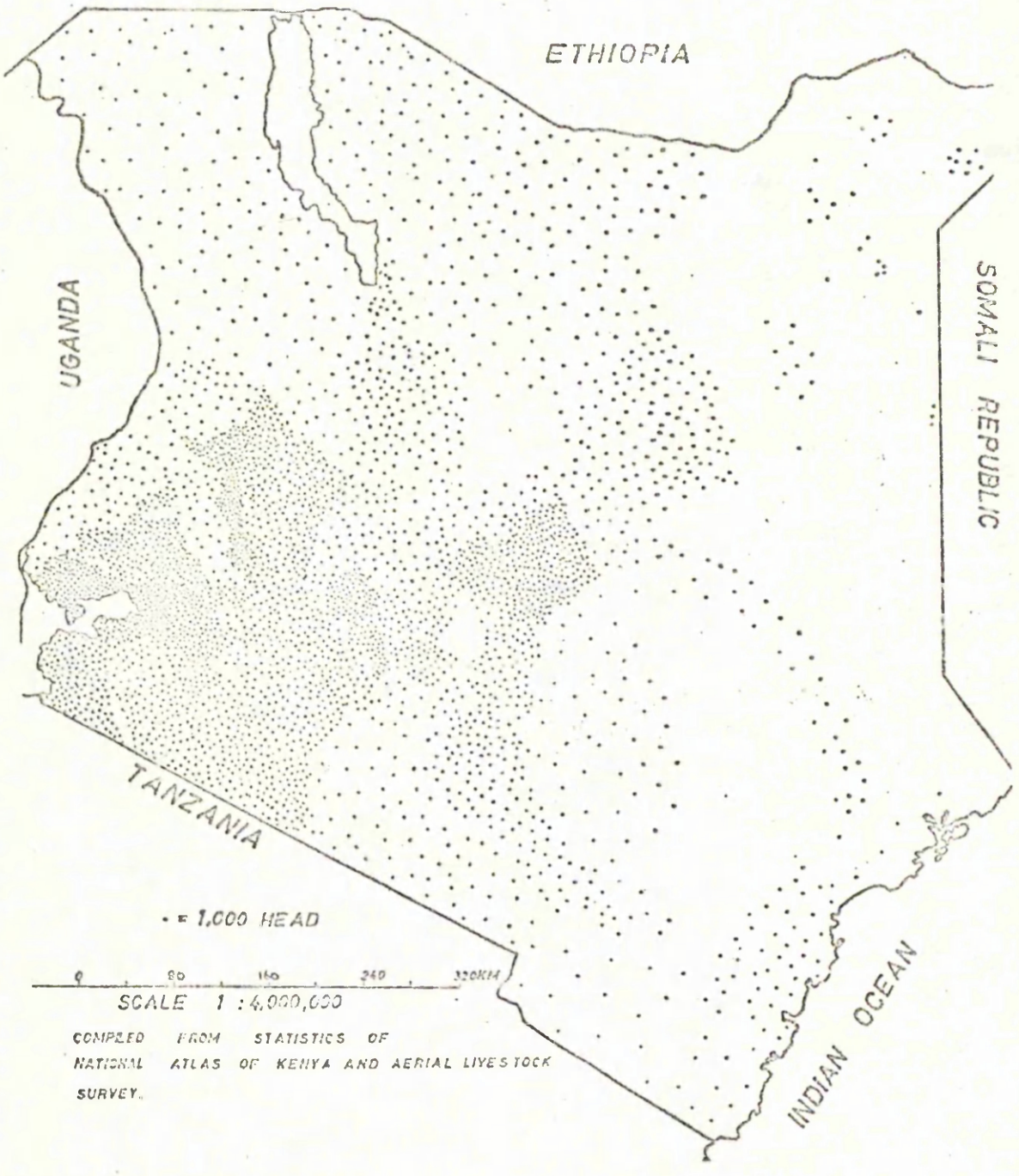

TABLE 6

Provinces and Districts sampled in each eco-climatic zone during the survey of Kenya

Eco-climatic Zone		Moisture Index	Provinces sampled	Districts sampled
I	Afro-alpine climate	governed by latitude not moisture	*	*
II	Equatorial climate	-10	Western	Bungoma
			Rift Valley	Laikipia
III	Dry sub-humid to semi-arid	-10 to -30	Rift Valley	Nakuru
			Coast	Kilifi
IV	Semi-arid	-30 to -40	Eastern	Machakos
			Rift Valley	Narok
V	Arid	-42 to -51	Eastern	Machakos
			North-Eastern	Garissa
			Coast	Taita
			Rift Valley	Baringo
VI	Very arid	-51 to -57	Eastern	Marsabit
VII	True desert (does not occur in Kenya)	-57 to -67	-	-

* sheep not represented in this zone

TABLE 7

Frequency of haemoglobin types in different breeds of Kenyan sheep

Eco-climatic Zone	District	Breed	No. of animals	Gene Frequency A	Gene Frequency B
II	Bungoma	Dorper	70	0.98	0.02
		Fat-tailed	102	0.02	0.96
II	Laikipia	Corriedale	195	0.09	0.91
		Masai	80	0.29	0.71
III	Nakuru	Merino	129	0.33	0.67
		Masai	100	0.25	0.76
		Dorper x Merino	36	0.17	0.83
		Hampshire x Merino	28	0.29	0.79
III	Kilifi	Dorper	23	0.02	0.98
IV	Machakos	Merino	71	0.23	0.75
IV	Narok	Masai	16	0.09	0.91
V	Garissa	Blackhead Persian -type	42	0.17	0.83
V	Taita	Blackhead Persian	16	0.00	1.00
V	Baringo	Masai	31	0.06	0.94
VI	Marsabit	Blackhead Persian	20	0.00	1.00

DISCUSSION

The results of this survey show that although all three haemoglobin phenotypes are represented in the sheep breeds in Kenya there was little difference between the gene frequencies of the breeds except in the case of the Dorper sheep tested from zone II, in which a significantly greater frequency of A genes was noted compared to all the other groups. The results suggest therefore that within each sheep breed in the higher areas of Kenya there is generally a tendency for a higher frequency of gene A and conversely in the drier zone the frequency of B genes increases within each breed. This trend was observed in both exotic and indigenous breeds of sheep.

These results extend the observations of Evans et al., (1958a) who found that Blackhead Persian and Masai sheep had only HbB type of haemoglobin; and those of Khattab (1968) who found exactly equal numbers of genes for haemoglobin A and B in Merino sheep in Sudan. The location of these animals within each country was unfortunately not reported.

The reasons for this divergence of haemoglobin type is not clear but as early as 1957, Evans demonstrated differences in fluid intake in sheep, which were related to sheep with a low potassium (LK) potassium-level in their red cells. By inference haemoglobin B sheep had a significantly lower fluid intake compared to sheep with high potassium intake, i.e. HbA sheep. Evans suggested that this may be a significant factor in selection. However, not until 1975 were further investigations made (Michell, 1975), and although direct examination of water uptake was not made a 'Na^+ preference' test was conducted on

Cheviot sheep in Britain. Again by inference it was suggested that haemoglobin B sheep required less water than HbA sheep.

In the arid regions of the world it is quite likely that the presence or absence of water per se is the major factor which influences the natural selection of the haemoglobin genes. Since most of Kenya comprises of zones IV and V, i.e. semi-arid and arid, it is possible that gene selection has favoured haemoglobin B, i.e. as HbB sheep possibly require less water they have been naturally selected in the drier areas of Kenya. This does not, however, explain the apparent advantage of HbA in the wetter areas.

It was impossible to identify any direct correlation between altitude and the A gene. However, since the diversity of altitude in Kenya is so extreme it would be expected that if the advantage of A gene in its relation to oxygen affinity were important then a much higher frequency of the A gene should have emerged by natural selection.

A possible reason for this gradient of haemoglobin types between the wet and dry areas of Kenya could be related to the findings of Evans et al., (1963) whose observations indicated that HbA sheep were possibly more resistant to H. contortus. This nematode is endemic in Kenya (Round, 1962) but since a relative humidity of 93 - 95% is required for its survival, it is only in the wetter areas of Kenya where haemonchosis is a continual problem.

Since such a correlation between haemoglobin type and resistance to H. contortus could be of practical importance

to the survival of different sheep strains, it was decided to investigate this phenomenon in a more controlled environment in sheep grazing continuously on H. contortus-endemic pasture.

OBSERVATIONS OF THE EPIDEMIOLOGY OF HAEMONCHOSIS IN MERINO SHEEP OF DIFFERENT HAEMOGLOBIN TYPES GRAZING H. contortus-ENDEMIC PASTURE AT A FARM NEAR MACHAKOS, KENYA

INTRODUCTION

The results of the haemoglobin survey of sheep in the different eco-climatic zones of Kenya indicated that, although the B gene for haemoglobin type was generally more prevelant in all the breeds sampled, there was a tendancy for the frequency of the A gene to increase in the wetter areas. In these regions of high rainfall, it would be expected that there would be a high H. contortus challenge, whereas only a spasmodic challenge would normally occur in the arid areas. Hence resistance to haemonchosis would appear to be a likely selection factor for the A gene in Kenya where H. contortus is endemic.

Evans et al. (1963) infected HbA and HbAB sheep with H. contortus and found that sheep with HbA had lower faecal egg counts than HbAB sheep. They suggested that this was due to the fact that HbA sheep were more resistant to the establishment of H. contortus adults. However, Jilek and Bradley (1969) and also Radhakrishnan et al. (1972) studying Florida Native sheep found no such relationship, i.e. there was no indication that HbA sheep were less susceptible to haemonchosis than HbB sheep, nevertheless HbA sheep appeared to 'self-cure' more readily. This readiness to 'self-cure' in HbA sheep was also observed in a preliminary study by Allonby and Urquhart (1976) in Merino sheep reared on endemic pasture in Kenya. Sheep with HbA appeared to undergo 'self-cure' more frequently and effectively than HbAB sheep which themselves 'self-cured' more readily than HbB sheep. If such a relationship between Hb type and response to H. contortus infection exists then this could have practical

application in the selection and development of more haemomcho-tolerant strains of sheep in Kenya. This is especially important in a country in which traditional management methods do not advocate the use of anthelmintics and 'self-cure' is the only way in which the worm burden of the sheep is periodically eliminated.

Hence it was decided to perform an epidemiological study in order to clarify whether any relationship existed between the three strains of sheep and their response to a natural infection with H. contortus. From the results of the survey it was apparent that there was a greater likelihood of finding sheep with all three phenotypes in a flock of exotic rather than indigenous sheep. A suitable source of Merino sheep, with all three phenotypes present was found as a flock already grazing on H. contortus-endemic pastures at a farm near Machakos. An epidemiological study of haemonchosis in the three strains of Merino sheep was performed throughout a two year period. Faecal egg counts were monitored every three weeks and later every two weeks and at the end of the experimental period six sheep of each homozygous type, i.e. HbA and HbB were autopsied and their worm burdens were examined.

MATERIALS AND METHODS

A flock of 71 Merino sheep, consisting largely of castrates between 1 and 4 years old were grazed on H. contortus-endemic pasture at a farm near Machakos, which is situated

about 50 km east of Nairobi in an area of Acacia-savannah grassland, which is characteristic of ecological zone IV.

At the beginning of the experiment blood samples in EDTA were taken from the jugular vein of each sheep. The haemoglobin type for each sheep was determined by paper acetate electrophoresis using the method described in general materials and methods II.

Faecal samples were taken from each sheep every three weeks during the first 98 weeks, then every two weeks thereafter. Faecal egg counts were performed on the samples by the McMaster technique as described in general materials and methods I.

During the 108th week of the study twelve sheep, six of HbA type and six of HbB were autopsied and the numbers of gastro-intestinal nematodes in each sheep were identified and counted.

The rainfall figures were recorded and kindly supplied by the Machakos Meterological Station, situated about 5 km from the farm.

RESULTS

Haemoglobin phenotypes

Sheep with HbB were the predominant type of sheep in this flock (45 sheep), the HbA sheep were the fewest represented (7 sheep) whilst the HbAB sheep were intermediate (19 sheep).

No. of animals	Gene frequencies	
	A	B
71	0.23	0.77

Faecal egg counts

The mean feecal egg count results for each group of sheep are recorded in Table E in the Appendix. The results for HbA and HbB sheep are illustrated in Figure 6.

The results show that in general the faecal egg counts of sheep with HbAB were intermediary between HbA and B sheep, and therefore for the sake of clarity the description is made with reference only to the two homozygous groups.

At the beginning of the experimental period, i.e. August, 1974 the mean faecal egg counts of HbA and B sheep were similar (2,500 epg). However at the next sampling period the number of eggs decreased in both groups to about 200 epg. After this time the results diverged.

The mean egg count results for HbB sheep gradually but with fluctuations increased to about 1,500 epg by January, 1975. Then followed a sudden reduction in egg count to about 400 epg. The egg counts increased steadily reaching about 1,500 epg followed by another sudden reduction in March to 400 epg after which the counts again rose to reach about 200 epg in August.

After diverging from the HbB group the egg counts of the HbA sheep rose slightly during October, 1974 then decreased suddenly in January, 1975 as 'self-cure' occurred (as judged by a fall in egg counts of more than 80%). After a slight increase in egg counts the HbA group 'self-cured' again and except for one month when the egg counts increased to about 1,500 epg the HbA group remained very low from March to July. In August the egg count rose rapidly to around 1,000 epg.

During the second year of sampling the egg counts of both groups remained fairly static from August, 1975 until January, 1976; HbA egg counts were about 1,000 epg, whilst HbB results were higher at about 1,500 epg.

In January, 1976 the egg counts for the HbB groups decreased to about 300 epg by March then increased steadily to above 3,500 epg by May. By mid-June the egg counts decreased again and the sheep 'self-cured' for the first time followed by a steady increase to about 300 epg. The counts for HbA sheep followed a similar trend but 'self-cure' occurred during both January, 1976 and June. Between these latter periods of 'self-cure' the counts increased to a mean of only about 700 epg.

Faecal egg counts of HbA and HbB sheep periodically converged, i.e. August, 1974 (2,525 and 2,600 respectively), September, 1975 (1,300 and 1,653 respectively) and October, 1975 (1,033 and 1,540 respectively). From the size of the egg count this seems to be related to periods of high challenge.

Faecal egg counts in relation to rainfall

The rainfall figures for the Machakos area are illustrated in Figure 6. Two main periods of heavy rains occurred each year from about March to May and from October to late December. Each of the four periods of heavy rainfall during the experimental period was found to coincide with reduced faecal egg counts in both groups. By defining 'self-cure' as a drop in egg count of 80% or more within a period of two weeks, 'self-cure' occurred in the HbA sheep after each of the four periods of rainfall.

However 'self-cure' occurred on only one occasion in the HbB sheep, i.e. May, 1976, and was both less marked and less persistent than in the HbA sheep.

On three occasions, i.e. August, 1974, September and October, 1975, the faecal egg count results for both groups were similar and high. This may be related to a time of heavy challenge which obliterated the strain differences. The rainfall was heavy in September/October, 1975, but not so in August, 1974. During the latter month isolated rain showers and high humidity may have been sufficient stimulus to cause an accelerated development of H. contortus larvae.

Worm burdens

The results of the examination of the gastrointestinal nematode burdens showed that H. contortus represented 97% or more of the total worm burden and that in all twelve sheep autopsied it was the only nematode species present in the abomasum. The other intestinal nematodes present were Oesophagostomum columbianum and Trichostrongylus species but since so few were present they are not considered further.

The H. contortus worm burden of six sheep of HbA and six of HbB autopsied during the 108th week are recorded in Table 8. The results show that sheep with HbB had significantly higher (< 0.01) worm burdens than sheep with HbA, i.e. a mean of 1,062 worms compared to a mean of 301 worms in the HbA sheep. There was no significant difference between the HbA and HbB groups in either the male/female worm ratio (0.98 and 0.90 respectively) or the egg laying capacity of the female worms (11.02 and 9.59 eggs per day respectively).

Figure 6

The mean faecal strongyle egg counts of HbA and HbB Merino sheep grazing H. contortus-endemic pastures for two years at a farm near Machakos and the daily rainfall during the same period

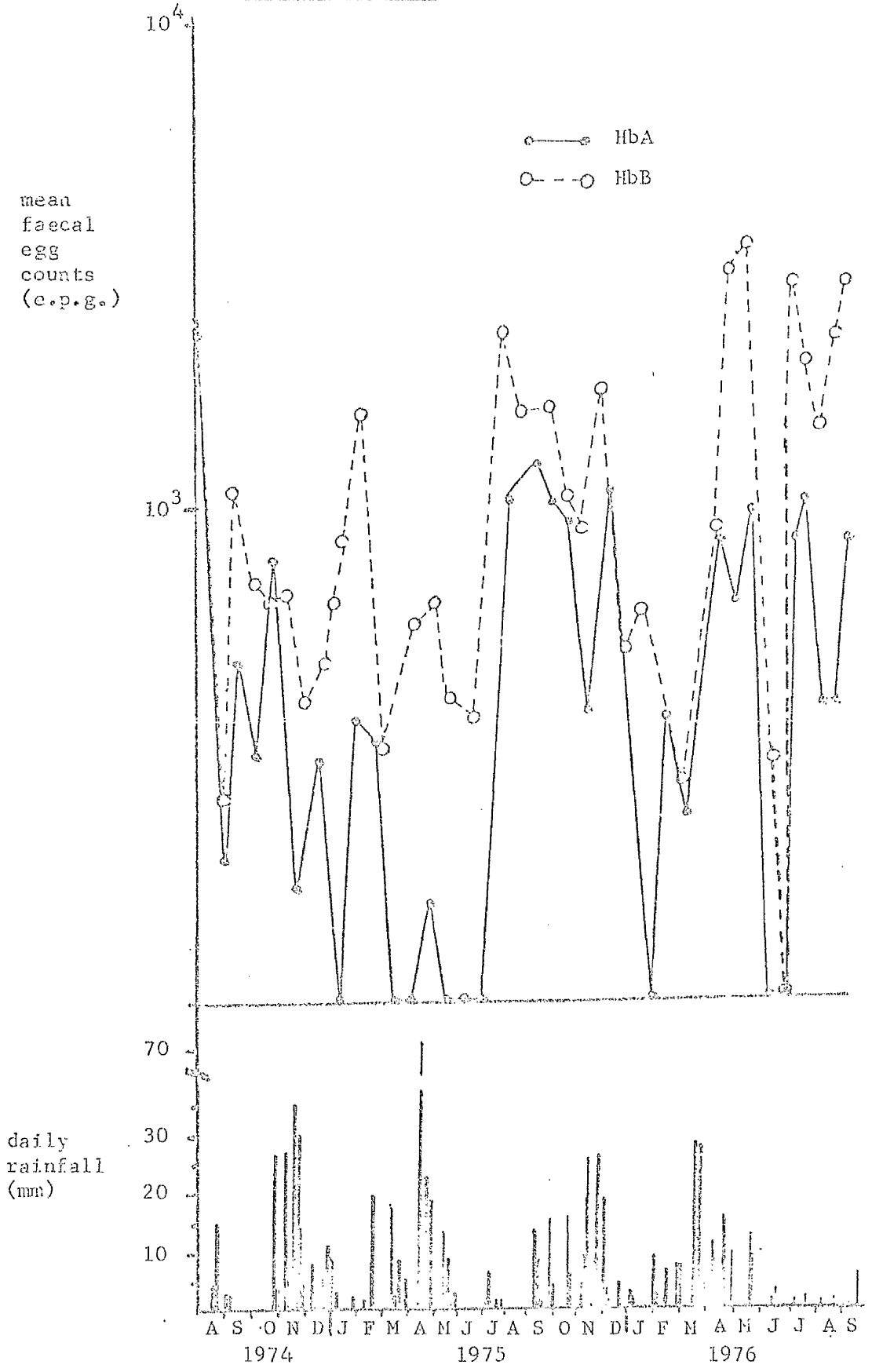

TABLE 8

Worm burden data at autopsy of six Merino sheep of HbA and six of HbB after being reared on H. contortus endemic pasture at a farm near Machakos for two years

Sheep No.	Total No. of worms	male/female	epg/female
HbA			
W5	177	0.84	5.21
G8	144	1.09	10.15
G93	241	0.72	4.15
G99	518	0.99	9.96
W94	495	1.32	13.15
W82	231	0.94	23.53
Mean	301.00 ± 66.65	0.98 ± 0.08	11.02 ± 2.85
HbB			
W70	609	0.82	6.87
W50	477	0.63	8.87
G80	1254	1.31	9.58
W88	1883	0.93	5.63
G12	803	0.71	12.31
G9	1347	1.01	14.29
Mean	1062.17 ± 216.54	0.90 ± 0.09	9.59 ± 1.33
P	<0.01	NS	NS

DISCUSSION

The results of this field experiment indicate that HbA type sheep are more able to survive in H. contortus-endemic environments and confirms what has already been suggested about the relationship between haemoglobin type and resistance to H. contortus (Evans et al., 1963; Evans and Whitlock, 1964; Loggins et al., 1973; Allonby, 1974; Altaif and Dargie, 1976). Generally the mean faecal egg counts of HbA sheep during the study period were lower than those of the HbB sheep. However this difference disappeared at certain times throughout the grazing season. These times appeared to correspond to periods of high rainfall or higher challenge. Therefore it appears that the difference in resistance between the haemoglobin types is not absolute but can perhaps vary with the size of the challenge. After periods of rainfall the HbA sheep 'self-cured' and this occurred four times altogether during the study period. However 'self-cure' occurred on only one occasion in the HbB sheep and was both less marked and less persistant than in the HbA sheep.

These results substantiate the findings of Radhakrishnan et al. (1972) and Allonby and Urquhart (1976) who observed that HbA type sheep showed 'self-cure' more frequently and effectively than those with HbB and HbAB. The much lower mean egg counts for HbA sheep compared to the HbB sheep also augments the results of Evans et al. (1963), who experimentally infected both HbA and HbAB sheep with H. contortus and found that the trend in

faecal egg counts and worm burdens at autopsy suggested that HbA sheep were more resistant to the establishment of adult infections. However this experiment extends these previous findings in that there is some evidence that the relative resistance of the three haemoglobin types can vary with the size of the infection.

However the survey of haemoglobin frequencies of sheep breeds in Kenya indicated that the majority of sheep are HbB and consequently are relatively susceptible to haemonchosis. In addition to resistance to haemonchosis three other factors could perhaps influence the selection of a particular haemoglobin type. The first factor is that natural selection might favour the A gene in sheep reared at high altitudes due to the greater oxygen affinity of HbA blood (Huisman et al., 1958; Dawson and Evans, 1962; Naughton et al., 1963; Sirs, 1966). The second factor is that the A gene might be of advantage due to the better reproductive performance in HbA sheep in cold weather (Obst and Evans, 1970) although the B gene has been shown to predominate in warm environments where the reproductive performance of HbB sheep is better (Evans and Turner, 1965; Dooley, 1965; Bernoco, 1967, 1968). The third factor which might influence the selection of a particular haemoglobin gene is that there is evidence that HbB sheep require less water; therefore, the B gene would be of selectional advantage in areas of drought (Evans et al., 1958; Michell, 1975).

In Kenya the wetter areas, in which there was a tendency for the A gene to increase in frequency, generally coincided

with the cooler areas of higher altitude and where H. contortus incidence was higher, thus it was not possible to distinguish the most important 'selection factors' from the survey results. However, tentative suggestions can be made.

Since much of Kenya is prone to prolonged periods of water shortage and warm climatic conditions at breeding times, the 'selection factors' towards the B gene may be related to drought resistance and possibly better reproductive performance. However, since altitude changes in Kenya are so diverse, ranging from 0 to 4,500 metres, it would be expected that if the advantage of the A gene in its relation to greater oxygen affinity at high altitude were important then a much higher frequency of the A gene would have emerged. Furthermore since the conditions even in the high altitude areas of Kenya are not nearly as severe as those in temperate climates the recorded advantage of the A gene in terms of its improved breeding performance in regions with 'cold and severe weather' would not necessarily apply. Hence resistance to haemonchosis would appear to be the most likely and important selection factor for selection in favour of the A gene.

The way in which this resistance to haemonchosis is expressed may perhaps occur in one or two ways. The first may be related to the ability of the sheep to withstand the pathogenic effects of H. contortus by homeostatic mechanisms. This form of resistance has been described as 'resilience' by Riffkin and Dobson (1977), and may be related to the host's

ability to replace blood and protein. However, resistance may also imply an antagonistic response by the host against the development and presence of the parasites. This immunological competence of the host could be manifested in a number of ways, i.e. suppression of worm establishment, larval development, egg production and the amount of blood ingested by the parasites.

The results of the field experiment indicated however that, apart from periods which were apparently associated with high larval challenge, faecal egg counts were generally lower in the HbA sheep compared with the HbB sheep also that worm establishment was suppressed and 'self-cure' occurred more often and effectively in HbA sheep than HbB sheep. It was therefore decided to investigate further the degree and type of resistance to haemonchosis expressed by the different strains of sheep and to study their relative response to various levels of experimental infection and to experimentally induced 'self-cure'.

A STUDY OF THE PATHOPHYSIOLOGY OF PRIMARY AND SECONDARY EXPERIMENTAL INFECTIONS OF H. contortus (300L/kg) IN MERINO SHEEP OF DIFFERENT HAEMOGLOTIN TYPES

INTRODUCTION

The results of the study of naturally acquired haemonchosis confirmed earlier suggestions that a relationship existed between haemoglobin type and resistance to haemonchosis i.e. HbA Merino sheep had a generally lower mean faecal egg count than the HbB sheep and on the single occasion when autopsies were performed, had a significantly lower worm burden. However from the relationship between faecal egg counts and rainfall it was evident that during periods of high rainfall, i.e. high larval challenge, that there was less disparity between the egg counts of the groups than at other times. Furthermore, the HbA sheep showed a more frequent and effective 'self-cure' than the HbB sheep.

These findings confirmed and extended the observations of various authors (Evans et al., 1963; Evans and Whitlock, 1964; Radhakrishnan, 1972; Loggins et al., 1973; Allonby and Urquhart, 1976) whose work was also largely based on the results of faecal egg counts and PCV values. Whilst these parameters can often given some indication of the degree of infection neither of these measurements can be used as a precise index of resistance or indeed the extent of any pathogenic effects.

For example, whilst a low PCV value is indicative of anaemia it is known that an animal is able to compensate for erythrocyte loss by accelerated erythropoiesis and in this way maintain a normal PCV value (Dargie and Allonby, 1976). This is provided that the nutritional value of the feed is

adequate and that no incipient iron, or other mineral deficiency, is present. Once feed quality or quantity is insufficient to support accelerated erythropoiesis the PCV inevitably begins to decline and leads to the symptoms of clinical anaemia, lethargy and progressive loss of weight which are so characteristic of the chronic disease.

Faecal egg counts can also be misrepresentative of pathogenesis since they are not directly proportional to either the number of worms present (Allonby, 1974) or to the amount of blood loss. This discrepancy arises largely from differences in the size and egg-laying capacity of the adult worms and from the haematophagic habits of the 4th and 5th larval stages before the production of eggs commences.

In investigating the mechanism of resistance to haemonchosis as either being related to immunological or other factors affecting the establishment or development of the parasites orbeing related to the host's own physiological ability to compensate for the infection it was therefore necessary to employ more precise monitoring techniques. For this reason it was decided to study the influence of sheep strain on the pathophysiology of experimental H. contortus infections using isotopically labelled erythrocytes and plasma proteins. In this way it would be possible to quantitate various parameters such as daily red cell loss and daily plasma protein loss and production in relation to total blood volume, as well as the conventional parameters.

Nine sheep were withdrawn from the field experiment described in the last section, i.e. three sheep with HbA, three

with HbAB and three with HbB. All the experimental animals had, therefore, been exposed to a natural field challenge of H. contortus prior to this study. The sheep were treated with thiabendazole (100 mg/kg) twice at monthly intervals and confined to metabolism cages. Red cells and albumin were labelled using chromium (^{51}Cr) and iodine (^{125}I) respectively and blood volume, blood and protein loss were monitored during the pre-infection phase and during a primary infection with H. contortus. In order to relate the pathogenic effects of the infection to unit body size it was decided to give a larval dose which was proportional to the total body weight of each sheep. An infective dose of 300 larvae per kg was used since this resulted in a total dose of about 10,000 larvae which has been used by many other workers as a standard rate of infection for producing a non-fatal acute syndrome.

Since the HbA sheep had shown a more frequent and effective 'self-cure' under field conditions it was also thought worth while to study the response of each haemoglobin type to experimentally induced 'self-cure' as produced by a secondary and superimposed challenge infection (Stewart, 1953; Dargie and Allonby, 1976). Following completion of the planned experiment and since equivocal results were obtained the experiment was repeated (as Phase 2) once the sheep had been maintained indoors 'worm-free' for six months after the end of the first experiment (see Figure 9).

Thus, the aims of this experiment were first to compare resistance of Merino sheep of different haemoglobin types

in pathophysiological as well as parasitological terms to experimental *H. contortus* infection and second to study their response, to experimentally induced 'self-cure' and possible differences in the development of acquired resistance.

MATERIALS AND METHODS

Experimental animals

The animals used in this experiment were nine one-year old Merino castrates which were withdrawn from the field experiment at a farm near Machakos, as described in the last experiment. Three of each haemoglobin type i.e. HbA, HbAB and HbB were selected and prior to the beginning of the experiment each sheep was treated twice with thiabendazole* at a dosage rate of 100 mg/kg.

Diet

All the sheep were given a daily ration of about 200gm of hay and 300gm of commercial sheep pellets (Kenya Farmers Association). KI (0.005) and NaCl (0.47) was added to the drinking water four days prior to the beginning and throughout the experiment in order to block the thyroid and thus ensure rapid excretion of ^{125}I.

Experimental design

A diagram of the experimental plan is found in Figure 9.

Nine Merino castrates, confined to standard sheep metabolism cages, were each injected intravenously with autologous ^{51}Cr labelled red cells and with ^{125}I labelled albumin, by the methods described in general materials and methods II. Blood samples were collected daily for radio-activity determination and PCV measurements. The total faecal output and total urine output was measured for every 24 hour period.

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* Thibenzole: Merck, Sharp and Dohme Ltd., Nairobi, Kenya.

Radioisotopic, parasitological and haematological techniques were as described in general materials and methods II. Eight days after injection of labelled cells and protein each sheep was experimentally infected orally with 300 H. contortus (L_3) larvae per kilogram of body weight. Faecal egg counts were determined from 15 days after infection. Radioisotope sampling ended 21 days after the infection with H. contortus, although the primary infection continued. The sheep were then reinfected after a further 34 days with labelled red cells and plasma protein. Sixtythree days after the primary infection the sheep were given a second oral challenge of 300 H. contortus (L_3) larvae per kilogram of body weight. Radioisotopic, parasitological and haematological sampling continued until day 14 of the second infection. This part of the experiment constitutes phase 1. The sheep were then given treatment with thiabendazole (at 100 mg/kg) twice at monthly intervals, and maintained indoors under worm-free conditions for a further four months.

Phase 2 was essentially a repetition of phase 1. The sheep were confined to metabolism cages and subjected to thiabendazole treatment. One month later autologous ^{51}Cr-labelled red cells and ^{125}I labelled albumin were injected into each sheep. Eight days later the sheep were experimentally infected with 300 H. contortus (L_3) larvae per kilogram of body weight. Radioisotopic, parasitological and haematological sampling ended 21 days after infection. Fortynine days after the primary infection labelled red cells and plasma proteins were readministered. Twelve days later a second infection

Figure 9

Diagramatic representation of experimental programme

Phase 1

- day 8	day 0	day 21
Labelled rbc's	H.contortus	end of sampling
and plasma albumin	primary	period
injected.	infection	

day 55	day 63	day 77
Labelled rbc's	H.contortus	end of sampling
and plasma albumin	secondary	period
injected.	infection	

WORM FREE PERIOD OF 150 DAYS

Phase 2

- day 8	day 0	day 21
Labelled rbc's	H.contortus	end of sampling
and plasma albumin	primary	period
injected.	infection	

day 49	day 61	day 71
Labelled rbc's	H.contortus	end of sampling
and plasma albumin	secondary	period
injected.	infection	

of 300 H. controtus (L_3) larvae per kilogram of body weight was experimentally introduced into each sheep. Radioisotope sampling continued for ten days. Faecal egg counts were monitored throughout the experiment and the sheep were weighed at weekly intervals throughout the experiment.

At the end of phase 2 the sheep were autopsied. The numbers of adult H. contortus in the abomasum of each sheep were counted and each abomasal mucosa was digested as described earlier in general materials and methods and the number of H. contortus larval stages was determined.

Calculation of results

Whole blood, red cell volumes and plasma volumes

The whole blood volumes were calculated by dividing the radioactivity per ml of the blood samples collected ten minutes post injection into the total injected radioactivity.

Circulating red cell volumes were calculated by using the PCV of the 10 min sample and using the following equation:

$$\frac{\text{Total injected activity cts/s/ml}}{\text{blood activity cts/s/ml} \times \frac{100}{\text{PCV}}}$$

Plasma volumes were calculated by dividing the radioactivity per ml of the plasma sample taken 10 minutes after injection of labelled albumin into the total injected radioactivity.

To enable comparison between animals of different weights all volumes were expressed on a body weight basis.

Faecal clearance of whole blood and red cells

Gastrointestinal blood loss was estimated by the use of ^{51}Cr-labelled erythrocytes. Abomasal bleeding was estimated as a daily faecal 'clearance' of whole blood and red cells. These results were calculated for each 24 hour period by dividing the total daily faecal activity by the activity per ml of whole blood and red cells respectively taken at the beginning of each 24 hour faecal collection period.

Red cell clearance

The persistence of labelled red cell circulating in the bloodstream can be assessed using the 'apparent red cell half-life' ($t\frac{1}{2}$). This method is not quantitative but has proved to be very useful for comparative purposes. The $t\frac{1}{2}$ values are obtained by converting the radioactivity of each blood sample to activity per ml of red cells and expressed as a percentage of the 10 min post-injection value. This percentage of red cells can be plotted against time on semilogarithmic paper.

Albumin turnover

Plasma proteins were lost into the gut as a result of the blood sucking activities of H. contortus and the degradation of this protein by digestive enzymes. This catabolism can be quantitated by the use of plasma proteins labelled with a radioisotope of iodine. Catabolic rate was measured using ^{125}I-labelled albumin. Breakdown of labelled albumin leads to the liberation of radioisotope as iodide which is rapidly and quantitatively excreted provided that the thyroid of the sheep is blocked by an excess of normal iodide.

The rate of albumin degradation can be expressed as a fractional catabolic rate F(CA), which is the fraction of the intravascular pool catabolised each day.

The amount of ^{125}I excreted in urine and faeces over a 24 hour period divided by the total plasma radioactivity at that time, gives the fractional catabolic rate F(CA).

RESULTS

Phase 1

For the sake of clarity, and since the results for HbAB sheep almost always occupied an intermediary position, only the results of the HbA and HbB sheep have been shown in the graphs and only those types are referred to in the results and discussion.

Faecal egg counts

The fluctuations in the faecal egg counts of each sheep following experimental infection with 500 H. contortus larvae were recorded in Table M in the Appendix; the mean results for haemoglobin groups HbA and HbB are illustrated in Figure 10.

Eggs were first detected in the faeces on day 20 in both groups and the egg count increased dramatically over the next few days to a level of about 15,000 epg which was maintained for about 10 days. This was followed by a gradual decline in egg count to about 6000 epg just prior to secondary infection and this general pattern was observed in both groups.

After the secondary infection with H. contortus the egg counts first increased to about 8000 epg then fell gradually in both the haemoglobin groups to a level of about 5000 epg.

Haematology

The mean daily changes in PCV for the HbA and HbB groups are illustrated in Figure 11. The individual results are recorded in Table N in the Appendix.

At the onset of the primary H. contortus infection the mean PCV value for both groups was similar i.e. about 30% and progressively declined to about 23% at 20 days after infection with no significant differences between the two groups.

Further reductions occurred in both groups so that by 55 days after infection the mean PCV values were only 15% but following the second challenge with H. contortus on day 62 the mean PCV values of both groups increased rapidly to reach about 23% by day 76.

Gastrointestinal blood and red cell losses

The mean daily red cell clearances for each group were plotted against time, and these results are illustrated in Figure 12 and represent the mean pattern and extent of abomasal haemorrhage in each group. The whole blood and red cell clearance during the period of pre-infection, primary infection and post secondary infection were calculated for each group and the mean results are illustrated in Figure 14 and Table H.

Red cell loss was about 4 - 9 ml/day in each sheep during the pre-infection period. Following the primary

infection with H. contortus faecal red cell loss was maintained at about 5 to 7 ml/day until day 9 of infection after which the losses gradually increased from 7 ml/day (on day 10) to about 15 ml/day (on day 20). However, just prior to the second infection with H. contortus the red cell loss for both groups had declined and red cell loss was maintained at about 7 ml/day in both groups.

This low red cell loss continued after the secondary infection in both groups for about 10 days. Thereafter the red cell loss of both groups increased very dramatically, i.e. from 7 ml/day (on day 70) to about 50 ml/day (on day 76) in HbB sheep, but to only about 25 ml/day on HbA sheep. The mean red cell loss from the group of the HbB sheep became significantly greater than that of the HbA sheep ($P < 0.001$).

Whole blood volumes

The whole blood volumes, circulating red cell volumes, and plasma volumes in relation to the total body weight of each sheep, as determined 8 days prior to infection and 55 days later, are illustrated in Figure 14 and Tables F and G in the Appendix.

Pre-infection, the whole blood volumes and the circulating red cell volumes were similar in both groups at about 42 ml/kg and 12 to 14 ml/kg respectively.

By 55 days after infection the whole blood volumes had increased significantly in both HbA and HbB sheep ($P < 0.05$) reaching 53 ml/kg and 51 ml/kg respectively. However, the circulating red cell volumes had decreased and were significantly ($P < 0.01$) below the pre-infection level in both groups at about 8 ml/kg.

Figure 10

Phase 1

The mean faecal egg counts of HbA and HbB Merino sheep following a primary and secondary experimental infection with H. contortus ($300L_3$/kg)

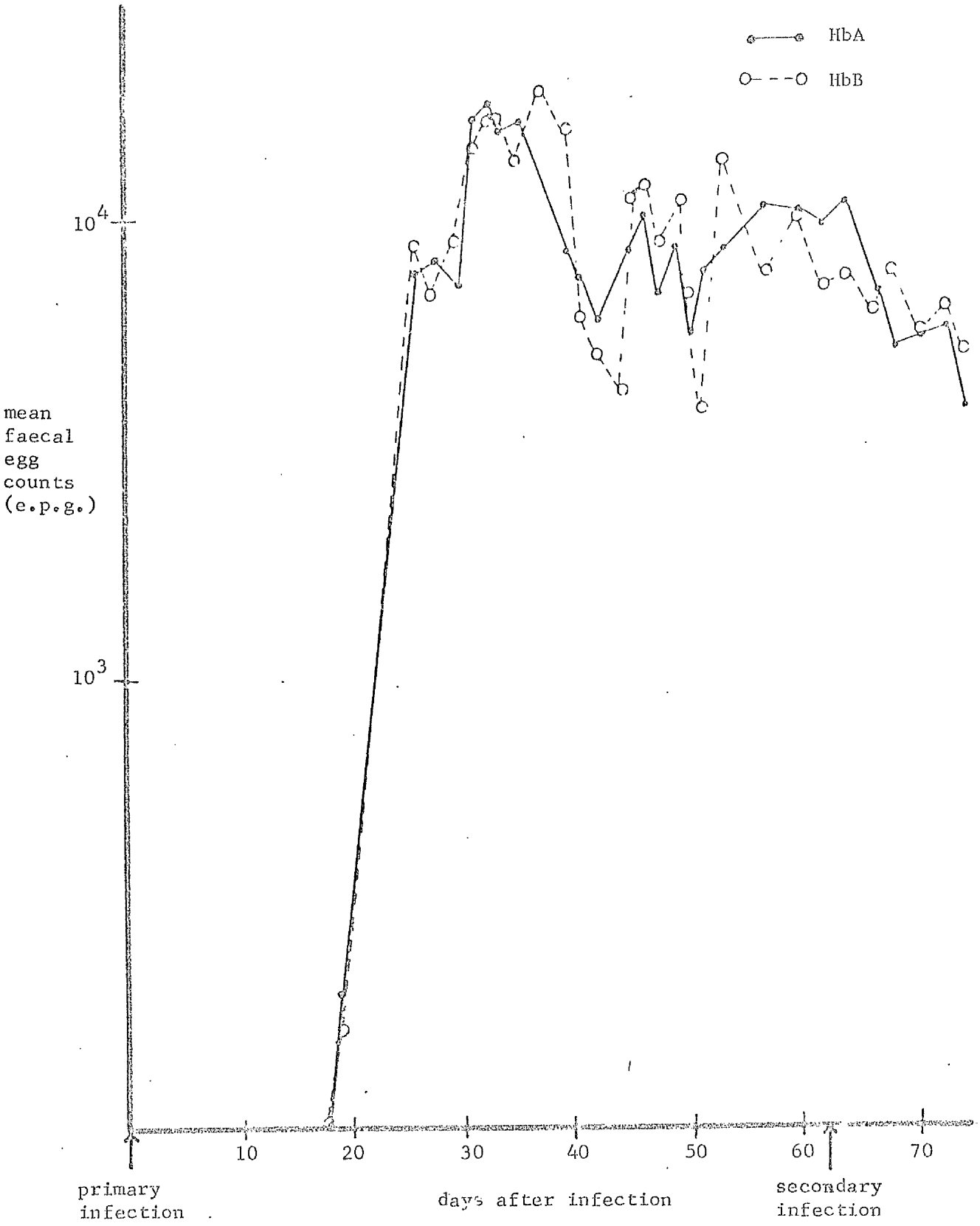


Figure 11

Phase 1

The mean PCV(%) values of HbA and HbB Merino sheep following primary and secondary experimental infection with H.contortus (300L/kg)

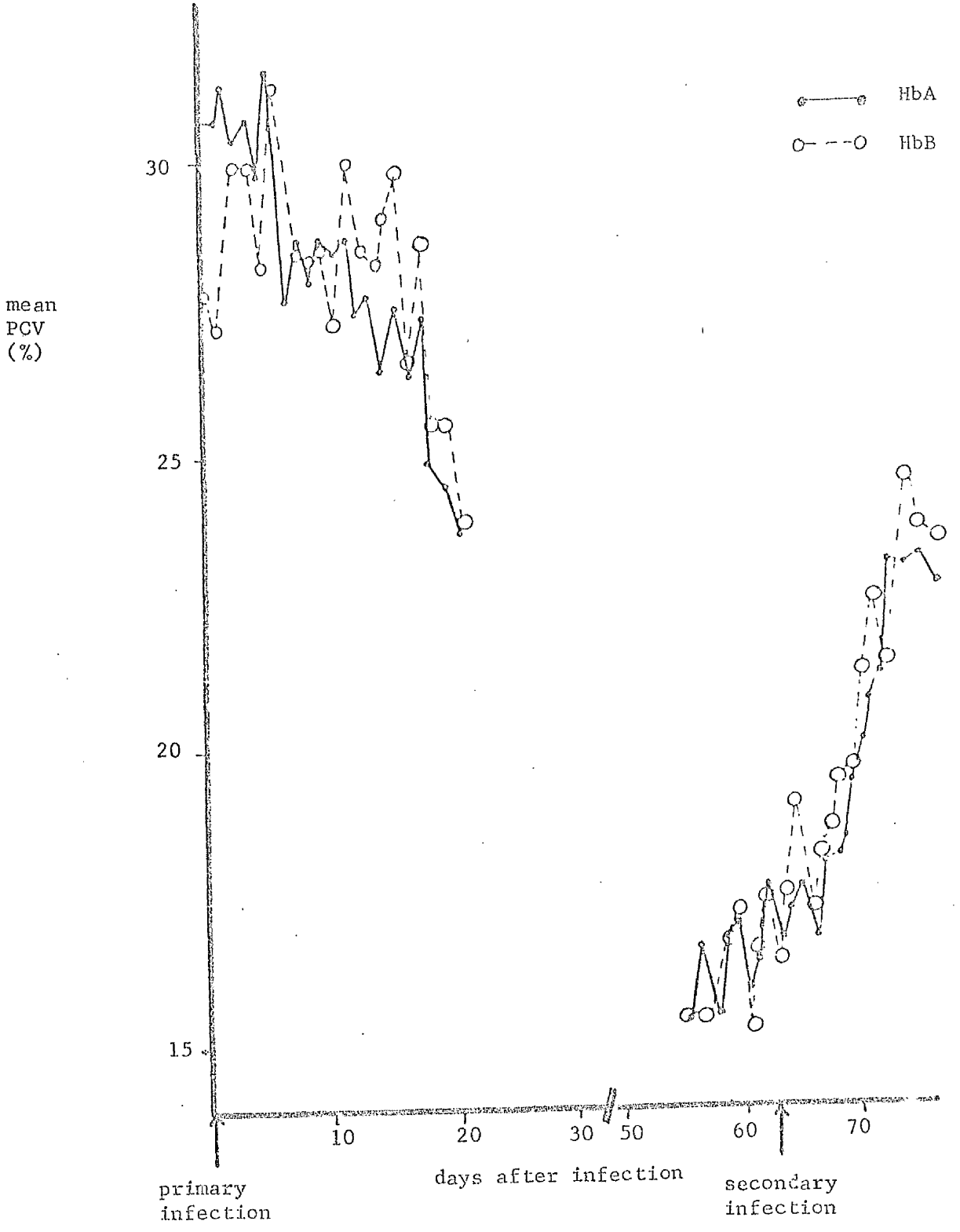


Figure 12

Phase 1

The mean daily faecal clearance of ^{51}Cr-labelled red cells in HbA and HbB Merino sheep following primary and secondary experimental infection with H.contortus ($300L_3$/kg)

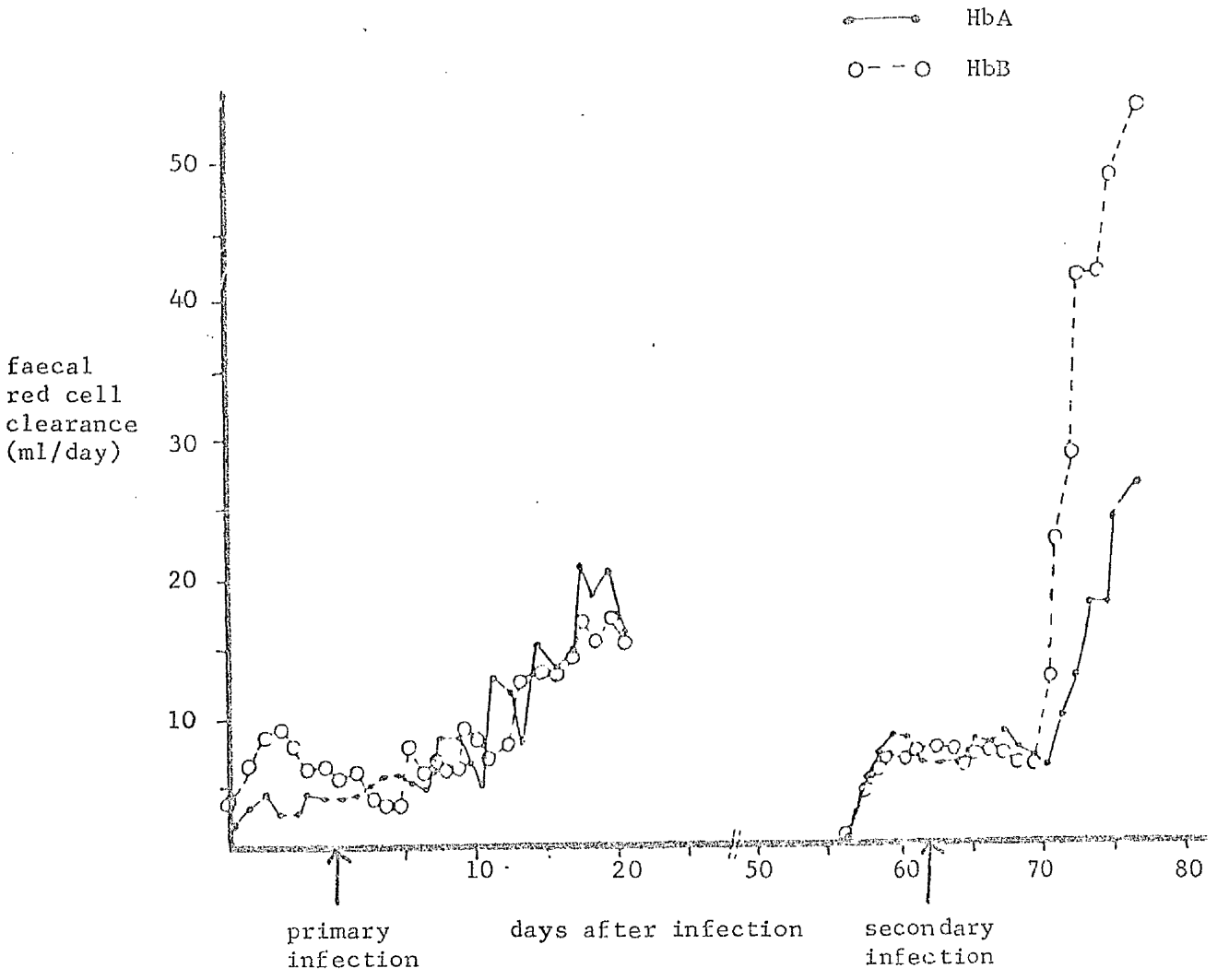


Figure 13

Phase 1

The mean daily fractional catabolic rates of labelled albumin in HbA and HbB Merino sheep following primary and secondary experimental infection with H. contortus ($300L_3/kg$).

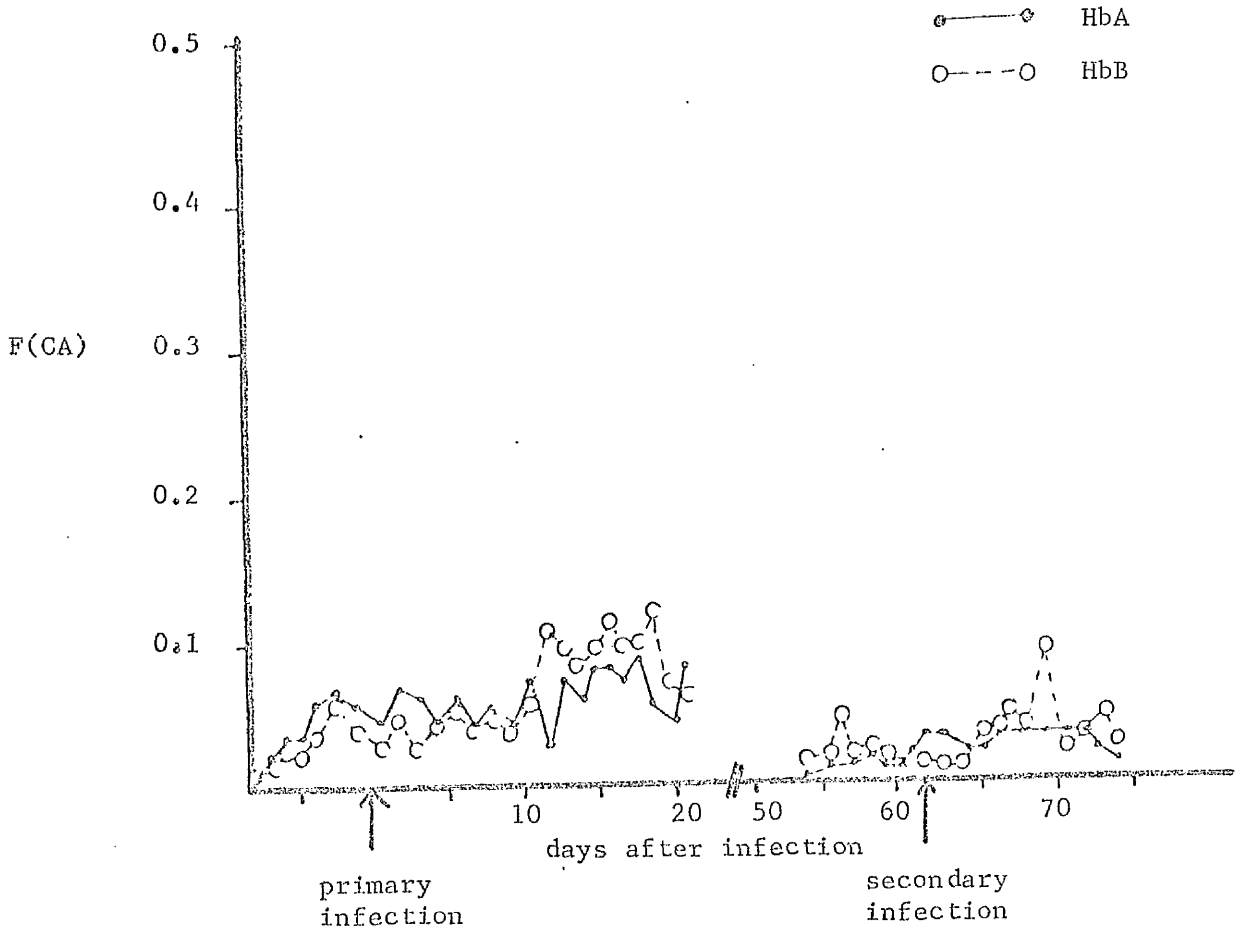
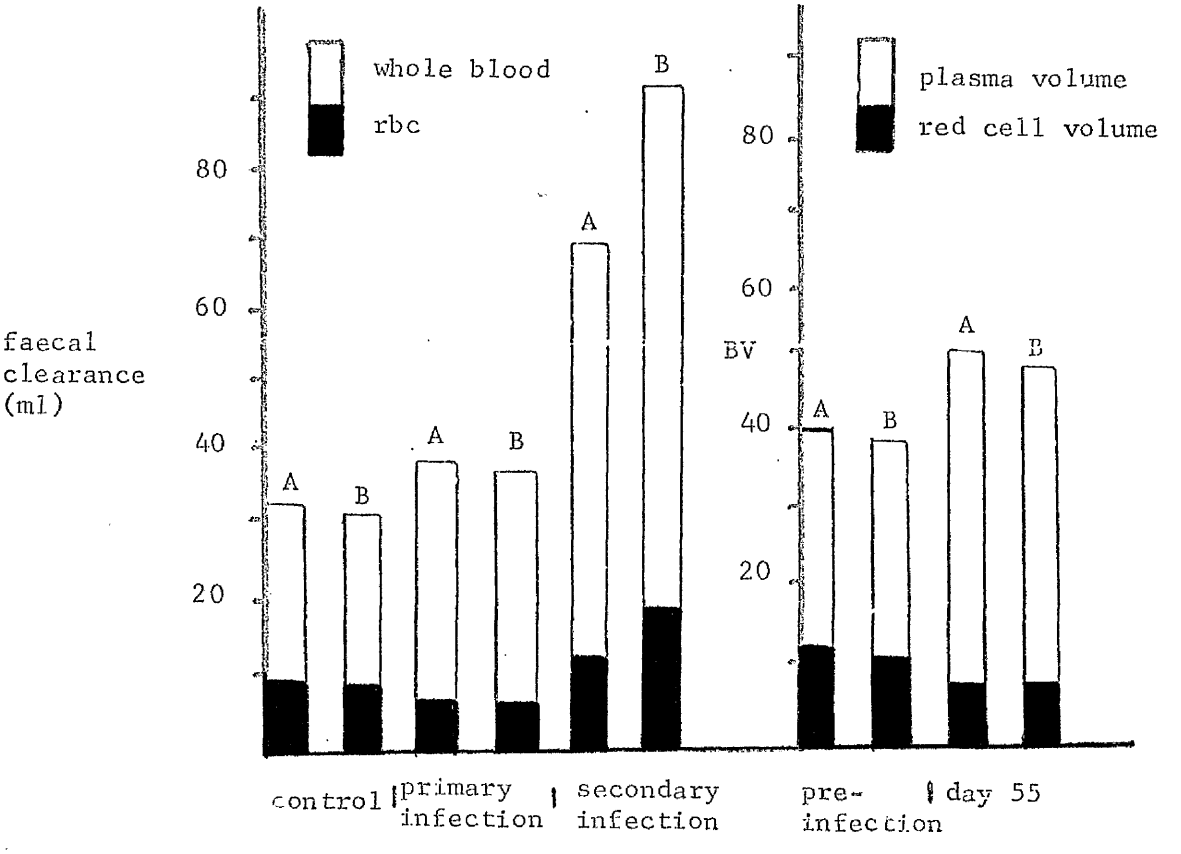


Figure 14

Phase 1

The faecal clearance of whole blood and red blood cells, red cell and plasma volumes in HbA and B type Merino sheep following primary and secondary experimental infections with H. contortus (300L/kg)



Albumin turnover

The mean daily F(CA) values are illustrated in Figure 13. The F(CA) values increased slightly during the early primary infection in both groups, i.e. from about 0.03 to 0.1, but had reduced to pre-infection values just prior to the second infection. After the secondary infection the F(CA) values increased slightly in both groups, i.e. to about 0.05. There was no significant difference between the groups during both periods.

Phase 2

Faecal egg counts

The results of the faecal egg counts following a second experimental infection at the same dose rate of 300 H. contortus larvae per kg of HbA and HbB groups of sheep are illustrated in Figure 15 and the individual results are recorded in Table M in the Appendix. There was no significant difference between the two groups and the same general pattern was observed as in Phase 1. Eggs were first detected in the faeces on day 20 in both groups after which the egg count increased dramatically to about 10,000 epg in both groups by day 40 (slightly less than in Phase 1), then fluctuated and declined to about 6000 epg by day 60. Following secondary challenge on day 61 there was no clear pattern and the mean count of both groups fluctuated between about 3000 to 10,000 epg.

Haematology

The mean daily PCV values declined in both groups during the primary infection but not as rapidly as in Phase 1 (Figures 16 and Table N in the Appendix). The mean PCV values for HbA sheep decreased from 28% to 25% just prior to the second challenge and from 26% to 20% in HbB sheep over the same period.

During the latter part of the primary infection the HbA sheep developed a significantly higher mean PCV value than the HbB sheep, i.e. about 25% and 19% respectively ($P < 0.05$). As in Phase 1 after the second challenge the PCV values for both groups increased rapidly, i.e. from 25% to 29% in HbA sheep within 10 days; and from 20% to 24% in HbB sheep during the same period.

Gastrointestinal blood and red cell losses

The mean daily red cell clearance for both HbA and HbB groups are illustrated in Figure 17. Mean daily red cell loss prior to infection was maintained at a low level in both groups, i.e. about 3 ml/day. After day 22 post primary infection the red cell loss increased slightly in both groups to about 6 ml/day.

Following the second infection red cell loss gradually increased in both groups from about 5 ml/day to about 7 ml/day, then gradually at the end of the sampling period the red cell loss had returned to pre-infection levels of about 3 ml/day. There was no significant difference between the two groups throughout the period. (Tables K and L in the Appendix and Figure 19.)

Whole blood volumes

Prior to infection the mean whole blood volumes in HbA sheep were significantly higher than HbB sheep, i.e. 53 ml/kg compared to 42 ml/kg respectively ($P < 0.05$). This difference was also reflected in the results of the red cell volumes, i.e. 15 ml/kg and 10 ml/kg respectively ($P < 0.05$), (Figure 19 and Tables I and J in the Appendix). After 49 days following primary infection the whole blood volumes had increased in both groups to 58 ml/kg in HbA sheep and 51 ml/kg in HbB sheep, i.e. this was significantly higher in the HbB sheep. Although no significant differences were observed between the groups in whole blood volumes and plasma volumes, HbB sheep had significantly lower red cell volume compared to HbA sheep, i.e. about 10 ml/kg compared to 14 ml/kg ($P < 0.05$).

Albumin turnover

The results of the daily fractional catabolic rate F(CA) are illustrated in Figure 18. The F(CA) values increased from 0.05 after day 15 to about 0.15 in both groups, but had decreased to pre-infection values just prior to the secondary infection. These values were maintained into the secondary infection until day 20 when the values increased to 0.2 in both groups. There was no significant difference between the groups during the two periods.

Worm burdens

The whole burdens at autopsy are recorded in Table 9. There was no significant difference between the groups either of the mean number of H. contortus recovered (310 worms in

Phase 2

The mean faecal egg counts of HbA and HbB Merino sheep following primary and secondary experimental infection with *H. contortus* ($300L_3$/kg)

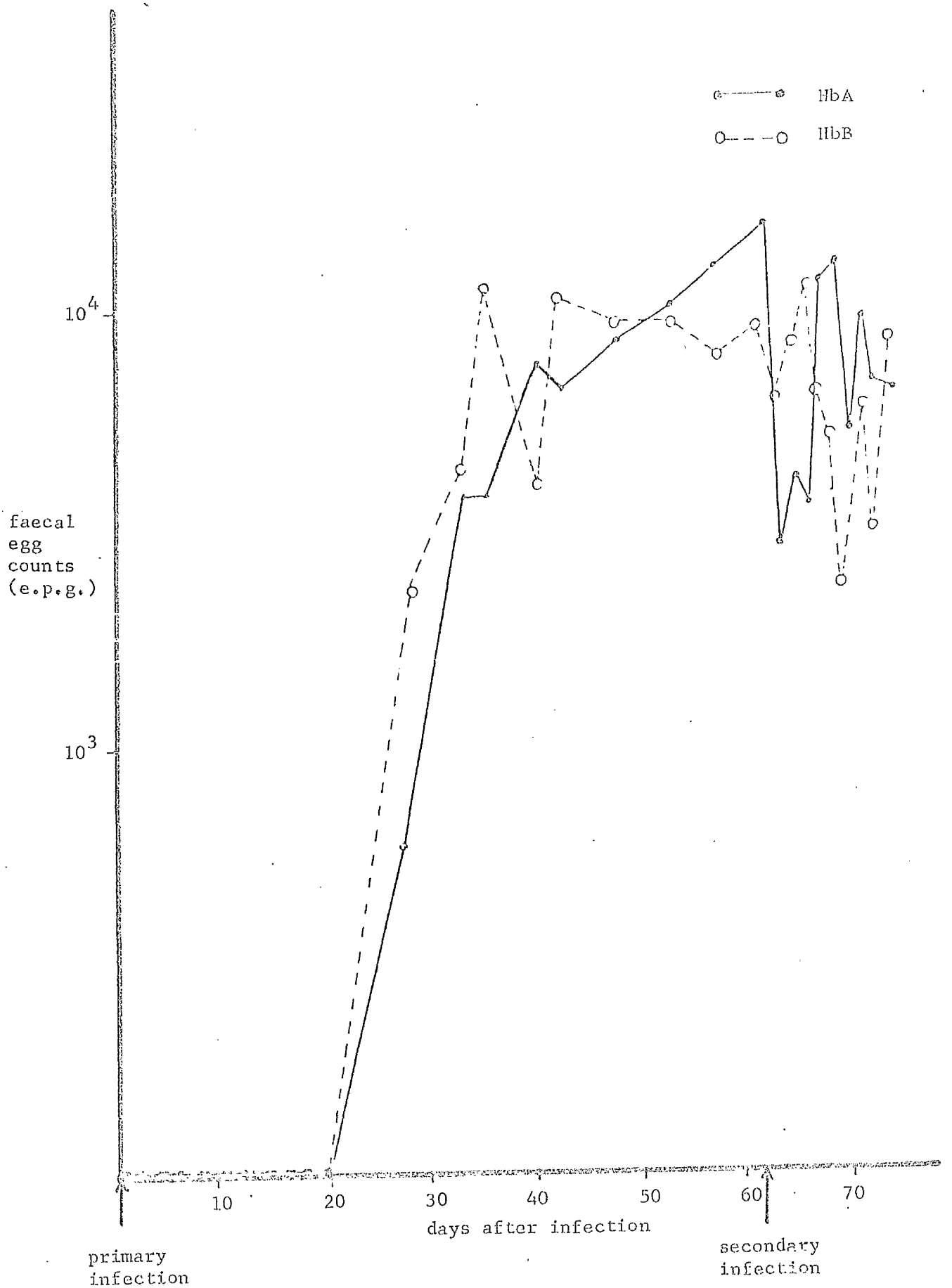


Figure 16

Phase 2

The mean PCV values of HbA and HbB type Merino sheep following primary and secondary experimental infection with H. contortus ($300L_3$/kg)

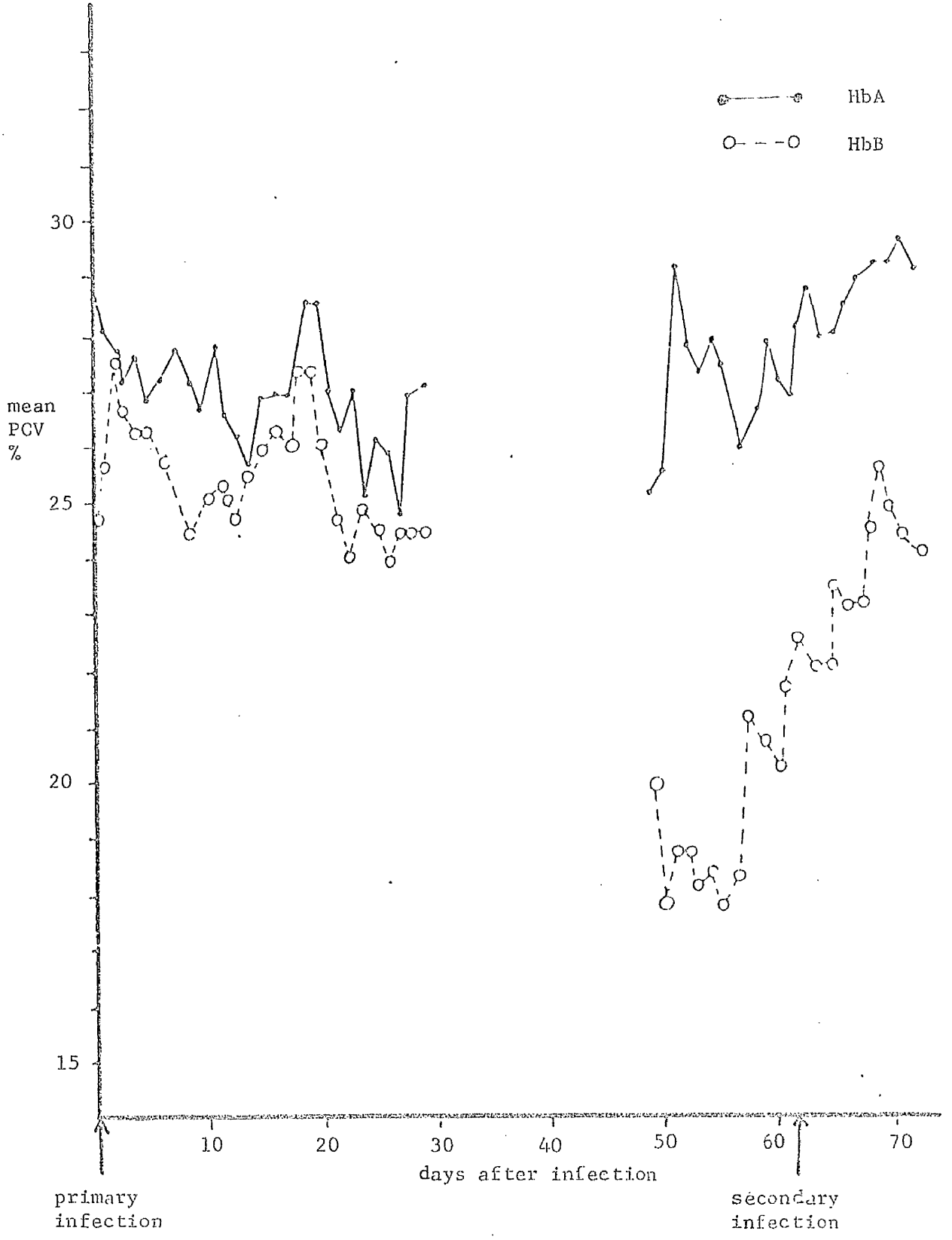


Figure 17

Phase 2

The daily mean faecal red cell clearance of HbA and HbB Merino sheep following primary and secondary experimental infection with *H. contortus* ($300L_3$/kg)

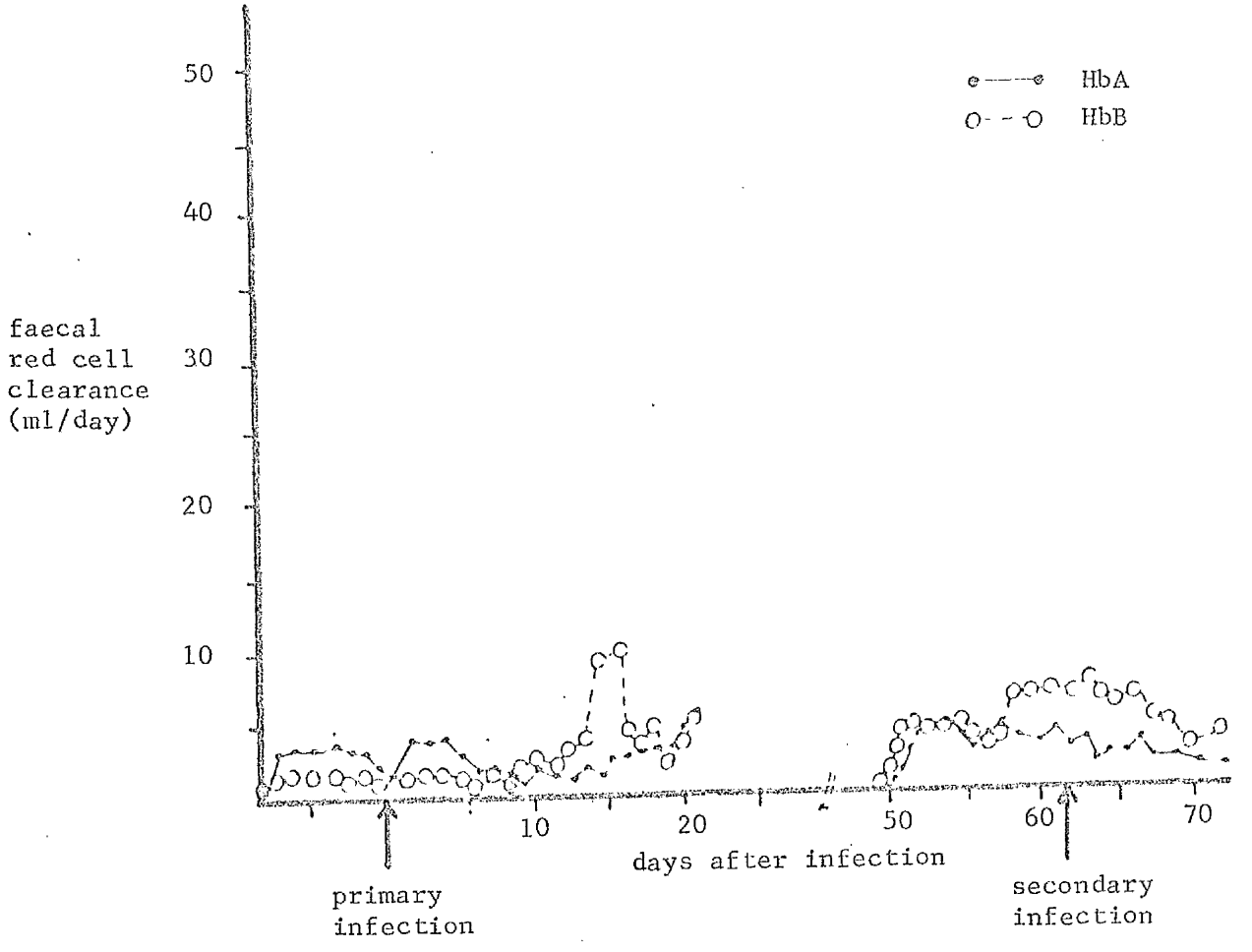


Figure 18

Phase 2

The mean fractional catabolic rates of albumin following primary and secondary experimental infection with H. contortus ($300L_3$/kg)

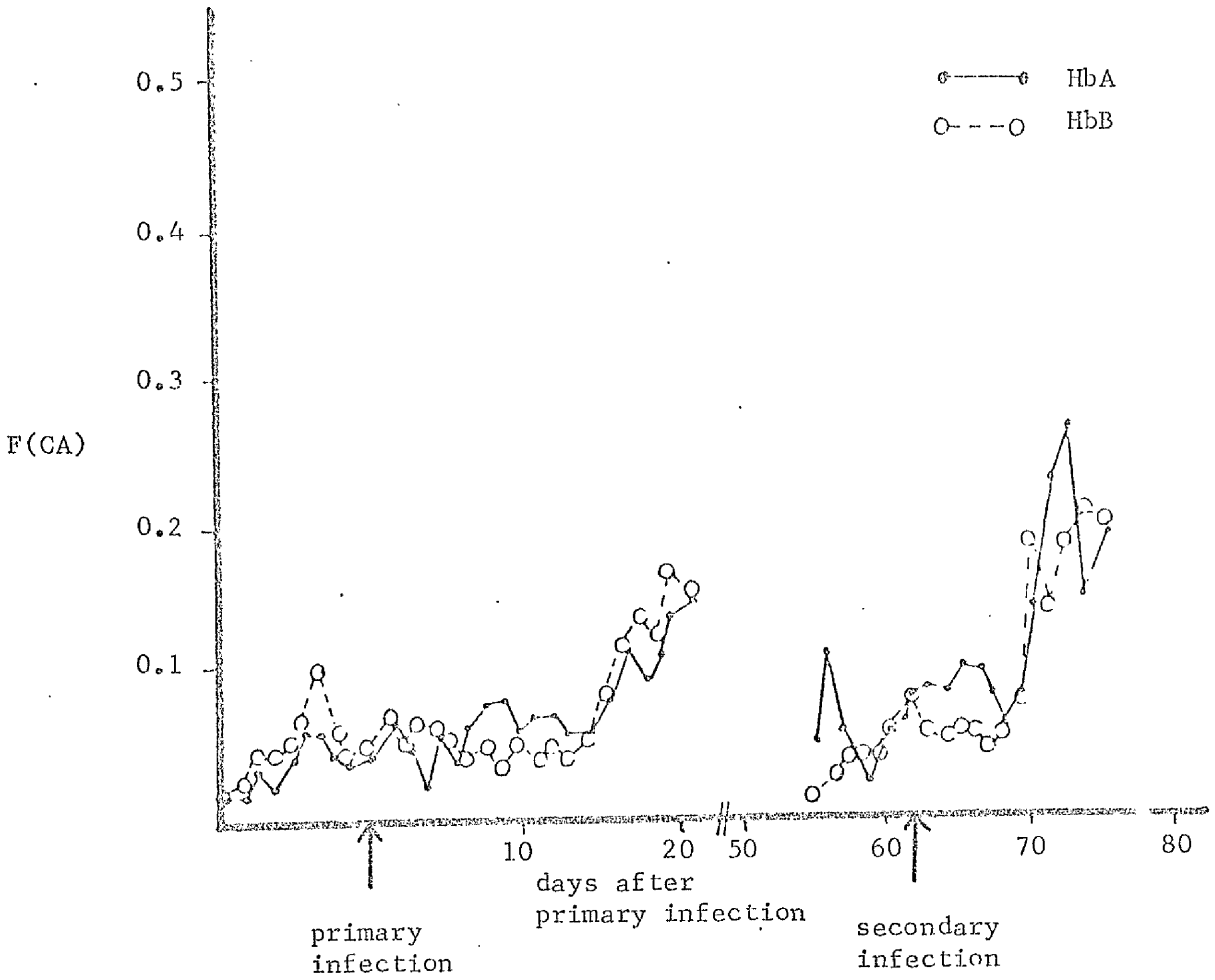


Figure 19

Phase 2

The faecal clearance of whole blood and red blood cells, red cell and plasma volumes in HbA and B type Merino sheep following primary and secondary experimental infections with H. contortus (300L/kg)

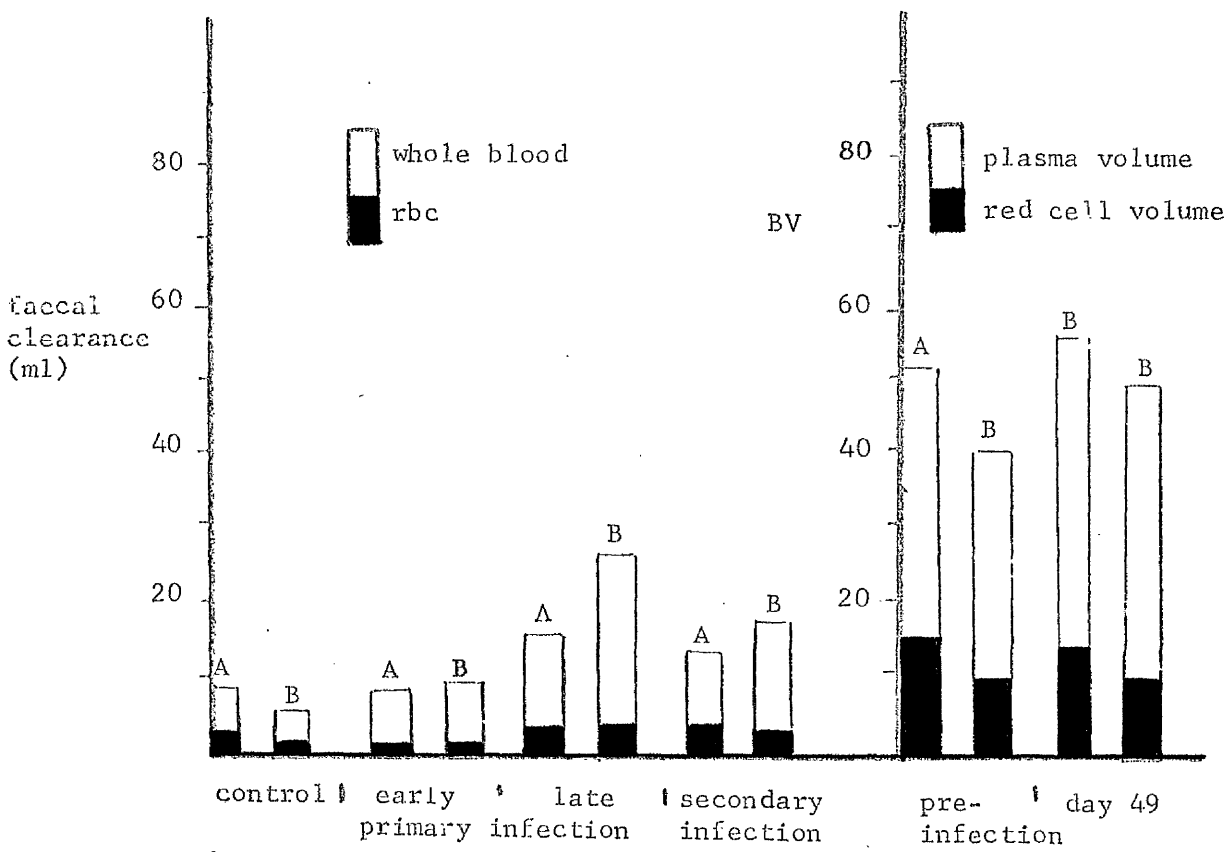


TABLE 9

Worm burden data at autopsy of Merino sheep of the three haemoglobin types experimentally infected with H. contortus (300L/kg)

HB type	Sheep No.	Total No. of worms	% recovery	rbc/worm
AA	4	0	0	-
AA	5	540	3.79	0.041
AA	6	390	2.87	0.031
Mean		310.00 ± 160.93	2.22 ± 1.14	0.036 ± 0.005
AB	3	1240	8.89	0.006
AB	7	530	3.86	0.028
AB	9	40	0.29	0.157
Mean		603.33 ± 348.35	4.35 ± 2.49	0.064 ± 0.047
BB	1	340	2.55	0.040
BB	2	510	4.10	0.021
BB	8	1320	10.14	0.021
Mean		723.33 ± 302.34	5.60 ± 2.32	0.027 ± 0.006
P		NS	NS	NS

HbA sheep and 723 worms in HbB sheep) or the amount of blood taken by each worm, i.e. 0.03 ml/day in both HbA and HbB sheep.

Total body weights of sheep

The percentage change in total body weight of sheep during the experimental periods is recorded in Table o in the Appendix. Although the sheep in both groups gained in weight during the two experimental periods, i.e. by 15.5% in HbA sheep and by 11.3% in HbB sheep, the weight gain statistically was not significantly different.

DISCUSSION

The numbers of animals involved in each group in this experiment were very small; this was also the first attempt in this department, to carry out pathophysiological measurements using isotopically labelled cells and plasma protein. Being a first attempt there was the possibility that some of the results might have been subject to some errors in the absolute sense. However, as comparative figures they were felt to be valid and useful.

The objective of this experiment was to investigate further the relationship between haemoglobin-type and the nature of resistance to haemonchosis in Merino sheep. In order to monitor more precisely the onset and development of the patho-physiological changes arising from haemonchosis in

each sheep strain, it was decided to use radioisotopically labelled red cells and albumin in conjunction with conventional parasitological and haematological techniques.

The results indicated that there was some difference between the haemoglobin groups of sheep in their susceptibility to experimental infection with H. contortus, i.e. HbA sheep developed less severe clinical and patho-physiological disturbances.

The onset and development of haemonchosis followed a similar course to that described by Dargie and Allonby (1975) and Altaif and Dargie (1976). In the first twenty days after the primary infection with H. contortus no eggs were passed in the faeces of the sheep. However, after day ten the haematocrit values decreased markedly and the amount of red blood cells lost into the gut through infection by the parasites or frank haemorrhage increased progressively throughout the period of sampling.

The faecal egg counts increased rapidly after day 20 to about 20,000 epg and were then maintained at between about 5000 and 20,000 epg for a further forty days. During which time the PCV values were reduced further as red cells lost into the gut increased. After administration of the secondary infection of H. contortus in Phase 2 , the amounts of blood and plasma protein lost into the gastrointestinal tract increased significantly and perhaps surprisingly the mean haematocrit values also showed a significant increase. A slight decrease in egg counts occurred at this time in all the groups.

The second phase was in effect a repetition of phase 1 and similar results were observed, i.e. although the trends were similar in each of the three haemoglobin groups the disease process appeared to be less severe in Phase 2, possibly due to the development of some acquired immunity. It appeared, for example, that in Phase 2 each group of sheep had H. contortus worms with a lower egg-laying capacity and reduced haematophagic habits, i.e. the mean faecal red cell clearance figures were significantly lower than those observed in Phase 1, in both the primary and secondary infections.

Following the second infection (in Phase 1 and Phase 2) there was a significant rise in haematocrit values, although this period coincided with an increase in blood loss which was quite marked from day 70. These results appeared to be difficult to reconcile. One possible explanation is that, as a result of the secondary infection, a type of anaphylactic reaction which occurred in the abomasum perhaps led to the loss of significant amounts of plasma into the gut which would have resulted in significant fall in plasma volume. Hence the PCV values, a percentage of the ratio between red blood cells and plasma would appear higher than otherwise. Unfortunately the plasma volume measurements were made prior to the second infection therefore they were unknown during this critical time. This phenomenon appears worthy of further investigation in the future and could be done by making frequent measurements of the plasma volume over the period of a secondary infection. This would help to explain this apparent anomaly of an increase in packed cell volume concurrent with an increase in blood loss.

The decreased pathogenicity of the Phase 2 infection suggests that a degree of acquired resistance had developed although a difference in pathogenicity of the H. contortus culture cannot be ruled out. Such acquired resistance, although perhaps not leading to a reduction in the numbers of worms which establish, is possibly the cause of the lower amount of blood which the parasites removed from the host in Phase 2 than was observed in the first phase. A similar phenomenon has been described by Miller (1968) in hookworm in dogs when in the immunised animal the blood loss was significantly less than in the non-immune animal.

However, the fractional catabolic rate for albumin was significantly higher in Phase 2 than in Phase 1 and since there was no corresponding increase in blood loss in Phase 2 this must represent an increased loss of plasma in some way rather than of whole blood. Again this may be a manifestation of some degree of hypersensitivity developing in the gut which caused the increased leakage of plasma.

Although the results indicated that by certain criteria the HbB sheep were slightly more susceptible to haemonchosis there was no such clear evidence of any large strain difference as was recorded recently by Altaif and Dargie (1976), who investigated this in two breeds of sheep. These workers, investigating Scottish Blackface and Finn Dorset sheep experimentally infected with H. contortus, found a significant difference between the strains of both these breeds, i.e. HbA sheep developed less severe clinical and patho-physiological

disturbances, passed fewer eggs and harboured fewer worms at necropsy than animals with HbB. The same workers also found that a breed difference existed; Scottish Blackface sheep were more resistant to H. contortus than Finn Dorset sheep. These differences between the strains and breeds were observed using an infection of 350 larvae/kg. However, when a heavier infection of 1,400 larvae/kg was used Altaif (1975) found no correlation between haemoglobin type and worm establishment. This, he suggested, was a reflection of a delayed immune response caused by exposure to excessive amounts of antigen. Since the Merino breed is generally recognised as being much more susceptible to H. contortus infection than the Scottish Blackface, it is possible that the dose rate of 300 larvae/kg, as used in this experiment, was too high for any innate differences in the susceptibility of Merino strains to be expressed.

The results reported earlier in the field studies at Machakos in which a separation of mean faecal egg counts was observed as only occurring during periods of low rainfall, i.e. low H. contortus challenge, also support this view. On the basis of these findings therefore it was decided to proceed with further experiments using experimental infections in which the level of challenge was significantly reduced below the level of 300 larvae/kg used in this experiment.

SUMMARY

In this experiment, which involved a primary and later a secondary experimental infection of H. contortus at 300 L/kg and then two further infections following an interval of being maintained worm-free, the evidence increased to show that in Merino sheep reeistance to haemonchosis is related to the difference haemoglobin types. For example during the second infection in Phase 1 the amount of blood which was lost by the HbB sheep was significantly greater than that lost by the HbA sheep. However, these differences in blood loss were much less than those reported by Altaif and Dargie (1976) in their comparable study of HbA and HbB Scottish Blackface sheep. The most likely explanation for this difference between the response of these two breeds is in the relative size of the challenge and the greater overall susceptibility of the Merino breed to H. contortus infection.

STUDIES OF PRIMARY EXPERIMENTAL INFECTIONS OF *H. contortus* IN MERINO SHEEP OF DIFFERENT HAEMOGLOBIN TYPES USING INFECTION DOSES OF 150, 50 AND 10 L_3/KG

INTRODUCTION

In the last experiment three groups of sheep of different haemoglobin types, i.e. HbA, HbAB and HbB were infected with a primary infection of 300 H. contortus larvae followed by a secondary infection of a similar sized dose nine weeks later. The results indicated that there was some evidence of differences in susceptibility to H. contortus between the haemoglobin groups, although there was no apparent difference in the occurrence of 'self-cure' or in the development of acquired immunity.

The infection dose of 300 larvae per kilogram was chosen since it was known from earlier observations that this level normally produced clinical, non-fatal haemonchosis in Merino sheep (Dargie and Allonby, 1975). This dose rate was also comparable with that used by Altaif and Dargie (1976) in Scottish Blackface sheep and Finn Dorset sheep, and resulted in a total dose of about 10,000 larvae per sheep in this experiment. This sizeof infection has been used as a standard dosage of H. contortus by many workers when an infection rate proportional to the body weights of the sheep was not required.

The observations of intermittent differences in faecal egg counts between the three groups of sheep in the Machakos field experiment indicated that during periods of high natural

challenge all groups responded equally, whereas during periods of low challenge the HbA sheep had significantly lower egg counts. Further evidence of this relationship between dose response and haemoglobin type was obtained recently from the results of Altaif and Dargie (1976) who infected two breeds of sheep, i.e. Scottish Blackface sheep and Finn Dorset sheep with 350 larvae per kilogram, and found significant differences both between the haemoglobin groups and between the breeds. However, when high dose of 14,000 larvae per kilogram was used no differences were obeerved associated with Hb type.

These observations led the author to believe that the larval dose of 300 larvae per kilogram as used in the last experiment, was perhaps too large for the haemoglobin differences in Merino sheep to become apparent. It was therefore decided to use smaller experimental infectionsof H. contortus in an attempt to distinguish more clearly the differences between the haemoglobin types and to confirm that these differences are dose dependant.

The following experiment is in three parts. Part 1 describes the course of a primary infection of H. contortus using 150 larvae per kilogram in two groups of sheep, i.e. HbA and HbB. Since the results of the previous experiments showed that HbAB sheep always held an intermediary position between HbA and HbB sheep, it was decided to investigate only the two homozygous haemoglobin types. The course of Part 1 was observed using patho-physiological, haematological and parasitological parameters.

Since in the last experiment and in other pathophysiological studies of haemonchosis in sheep the pattern of albumin catabolism tended to follow that of red blood cell loss, labelled albumin turnover was not studied in this experiment. In order to allow for any possible separation between the haemoglobin groups to develop fully it was decided to monitor pathophysiological measurements during a later period than in the previous experiments, i.e. from day 38 to 58 after a primary infection instead of from the pre-infection period to about a month after patency. In Parts 2 and 3 the sheep were infected with 50L/kg and 25L/kg respectively and haematological and parasitological measurements were performed.

MATERIALS AND METHODS

Part 1

Experimental animals

The animals used in this part of the experiment were six Merino ewes, aged about $1\frac{1}{2}$ years, which originated from Naivasha Animal Husbandry Research Station, Kenya. They had been withdrawn from a flock rotation programme in which for all practical purposes they had been reared 'worm-free'. Three of the sheep possessed HbA and three of the sheep had HbB. Although male sheep are normally easier to sample for this type of experiment, the lack of sufficient males with HbA made it necessary to select all females.

Experimental design

Six worm-free Merino ewes, confined to standard sheep metabolism cages, were each experimentally infected with 150 *H. contortus* (L_3) larvae per kilogram of body weight.

Faecal egg counts and PCV were monitored twice weekly for 2 weeks, then daily. The sheep were weighed weekly during the experimental period.

On day 38 of the infection with H. contortus each animal was injected with an autologous ^{51}Cr labelled red cell suspension. Heparinised blood samples were collected daily for radioactivity determination and packed cell volume measurements. The total faecal output for each 24 hour period was weighed. To avoid any contamination of faeces by urine in these female sheep faecal samples for daily faecal egg counts and for radioactivity determination were taken directly from the rectum of each sheep.

The methods used for these techniques are described in General Materials and Methods II.

Part 2

Experimental animals

Two more Merino ewes, one with HbA and the other with HbB, aged $1\frac{1}{2}$ years, were brought from Naivasha Research Station where they had been reared on H. contortus endemic pasture. The same six animals from the previous part of the experiment were re-used. Although it would have been more satisfactory to use completely new groups of animals either 'worm-free' or having been reared under similar conditions the scarcity of HbA sheep necessitated this approach.

Experimental design

Eight Merino ewes, four with HbA and four with HbB were given anthelmintic treatment at 3 and 1 months before

the start of the experiment. They were confined to standard sheep metabolism cages and were experimentally infected with 50 H. contortus (L_3) larvae per kilogram body weight.

Faecal egg counts and PCV values were monitored daily and the sheep were weighed each week during the experimental period of 58 days.

Part 3

Experimental animals

The same sheep were used as in the last experiment. The sheep were subjected to anthelmintic treatment twice, i.e. 2 months and 1 month prior to the start of the experiment. The sheep were experimentally infected with 25 H. contortus (L_3) larvae per kg body weight and the same haematological and parasitological measurements were performed as in Part 2.

RESULTS

Part 1 (150L/kg)

Faecal egg counts

The daily mean faecal egg counts are illustrated in Figure 20 and the data recorded in Table P in the Appendix. The HbB sheep showed a slightly earlier rise in egg count than the sheep with HbA. After day 19 or 20 the mean faecal egg counts increased sharply and apart from normal fluctuations maintained a level of about 5000 epg in the HbB sheep whereas this reached only about 2000 epg in the HbA sheep. These

differences between the mean epg of the two groups were significant (see Table P in the Appendix) for most of the period of infection but at 40 days post-infection these differences became less marked. During the subsequent 2 weeks the mean egg counts became once again significantly different but on day 56 post-infection the HbA sheep unexpectedly 'self-cured'.

Haematology

Sheep with HbA had daily mean PCV values which were about 4% higher than those of the HbB sheep throughout the first part of the experiment. On some occasions these differences were statistically significant at the 5% level. (Figure 23 and Table Q in the Appendix.)

Pathogenesis

There was no significant difference between the mean blood volumes, the mean red cell or plasma volumes in sheep of the two haemoglobin types (Table 10). However, the amount of whole blood lost into the faeces of the HbB sheep was significantly (<0.02) higher than in the HbA sheep, i.e. 20.6 ml and 10.2 ml respectively (Table 11). Although the average amount of red blood cells lost into the faeces was 4.9 ml, in HbB sheep and only 2.8 ml in HbA sheep this difference between the loss was not statistically significant. The daily red cell clearance results indicated that HbB sheep were losing about 5 ml/day whereas HbA sheep were losing about 2 ml/day (Figure 24). The rate of disappearance of the labelled cells from the circulation expressed as 'half-life' values indicated that the red cells of HbB sheep had a significantly ($<.01$) shorter life span than the HbA sheep (Table 11).

Part 2 (50L/kg)

Faecal egg counts

The results of the mean daily faecal egg counts are illustrated in Figure 21 and the details recorded in Table P in the Appendix. HbB sheep again showed on average a shorter patency period than sheep with HbA, and HbB sheep generally maintained a higher mean daily egg count than the HbA sheep and on many occasions this difference was statistically significant. From 30 days post-infection the mean egg counts in the HbB sheep were maintained at about 2500 epg (maximum 4600 epg) whereas the HbA sheep had levels of only about 1500 epg (maximum 2800 epg).

Haematology

The mean PCV values are illustrated in Figure 23 and the individual results are recorded in Table Q in the Appendix. From the beginning of this part of the experiment HbA sheep had higher PCV values than the HbB sheep and in all cases the difference was significant. Both groups of sheep maintained more or less the same PCV throughout this part of the experiment, i.e. around 32% in HbA sheep and 25% in the HbB sheep.

Part 3 (10L/kg)

Faecal egg counts

The faecal egg counts began to increase after day 18 but they rose only gradually reaching about 500 epg after a further 8 days, i.e. day 26, in both groups. Between days 26 and 36 the HbB sheep sometimes had significantly higher egg counts but

thereafter the egg counts of the two groups began to converge i.e. NS . By day 35 the HbB sheep had stabilised at a mean egg count of about 2300 epg, whereas the HbA sheep reached only about 1500 epg. (Figure 22 and Table P in the Appendix).

Haematology

The mean PCV values are illustrated in Figure 23 and the individual values are recorded in Table Q in the Appendix. At the beginning of this part of the experiment the HbA sheep had mean PCV values which were about 3% higher than the HbB sheep and this disparity was statistically significant after day 27 The HbA sheep maintained these higher values and towards the end of this part of the experiment the PCV increased slightly to reach 31.5%. The HbB sheep maintained much lower PCV values, however, of around 27% throughout this part of the experiment. This difference between the two groups was slightly less than in the Part 2.

Body Weights of Sheep

The mean total body weights were similar in both HbA and HbB sheep and were generally maintained throughout each part of the experiment. There was no significant difference between the groups. (Table R in the Appendix.)

Figure 20

The mean faecal egg counts of HbA and HbB Merino sheep following experimental infection with H. contortus ($150L_3$/kg)

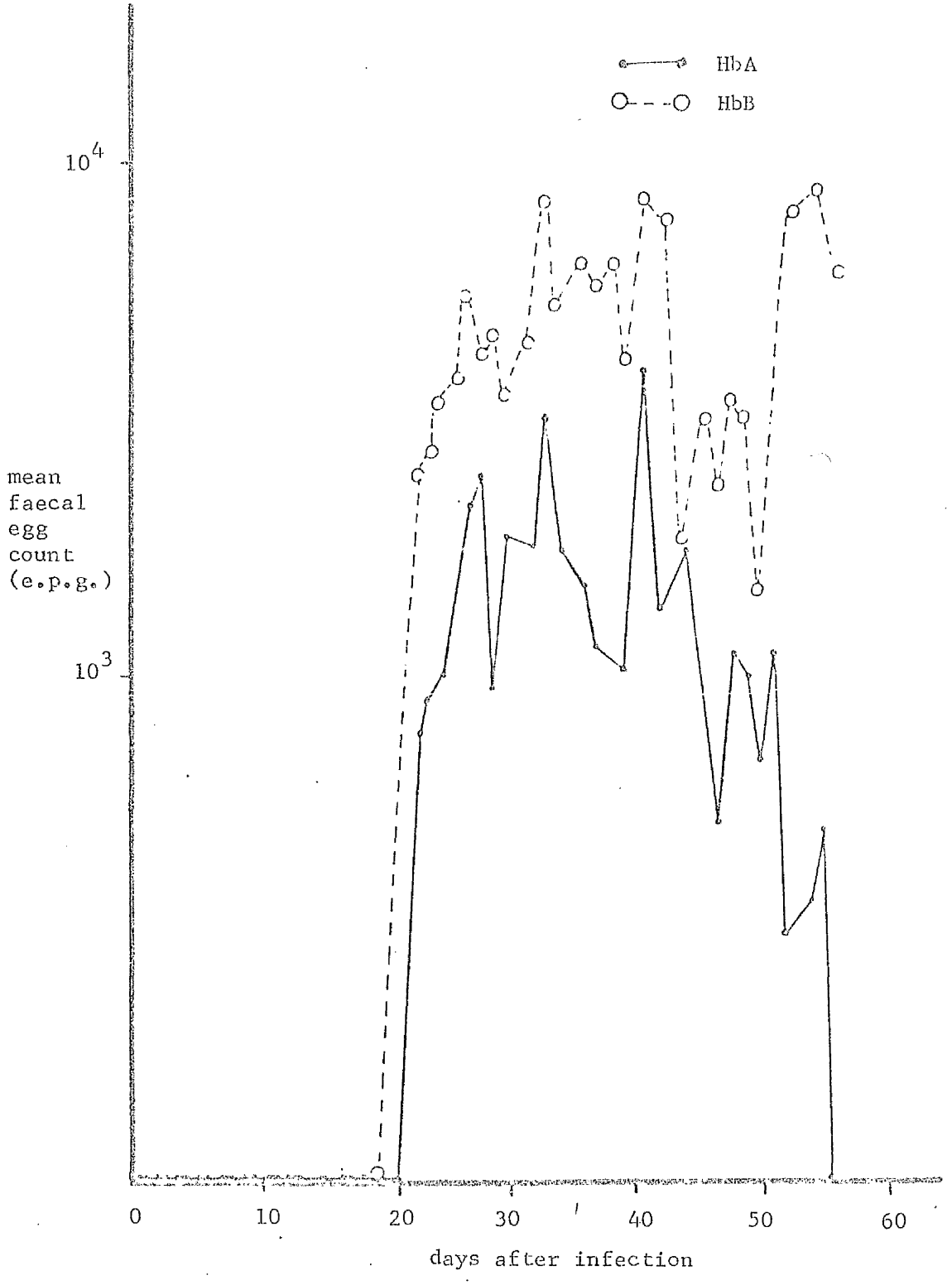


Figure 21

The mean faecal egg counts of HbA and HbB Merino sheep following experimental infection with H. contortus ($50L_3$/kg)

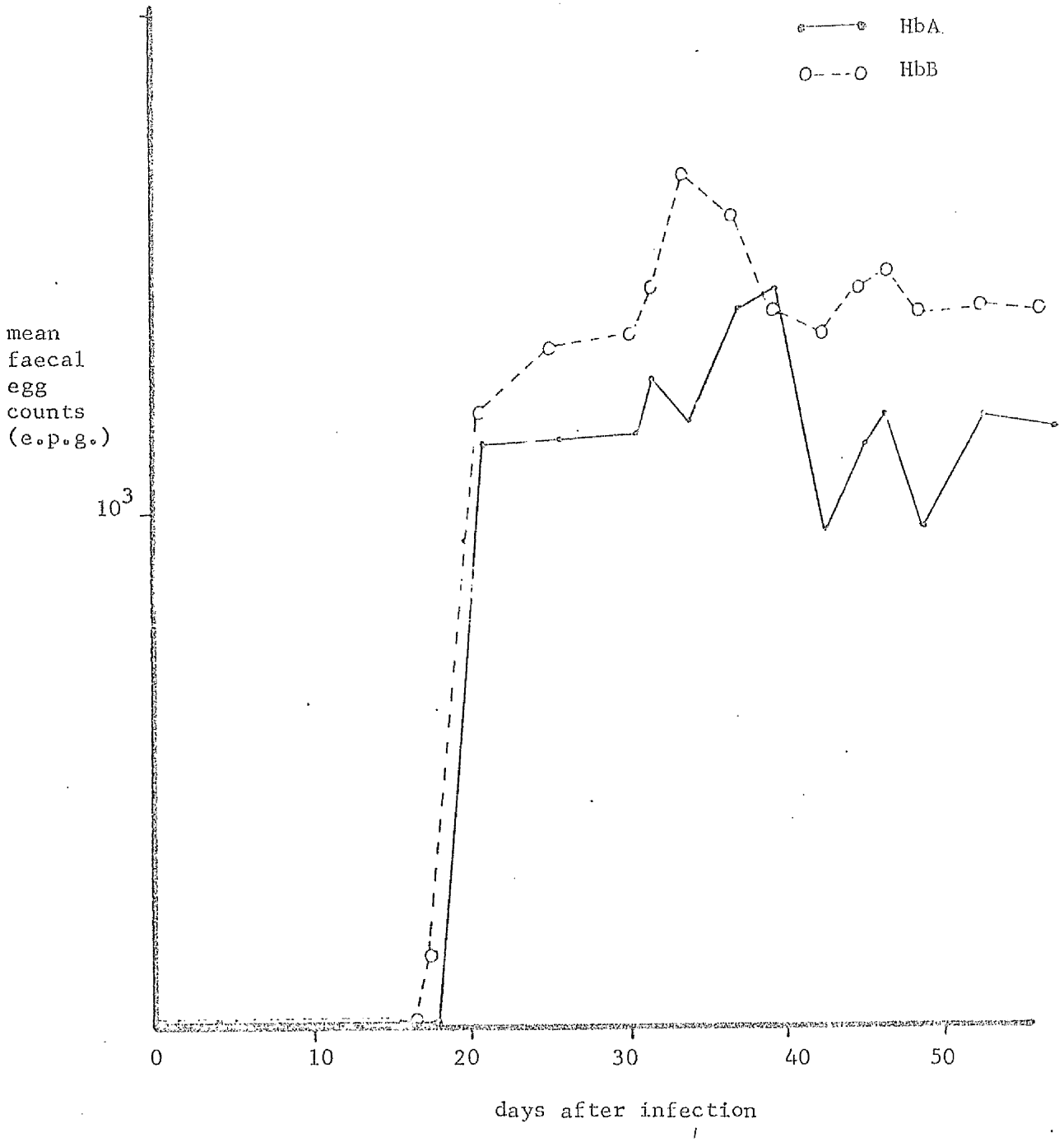
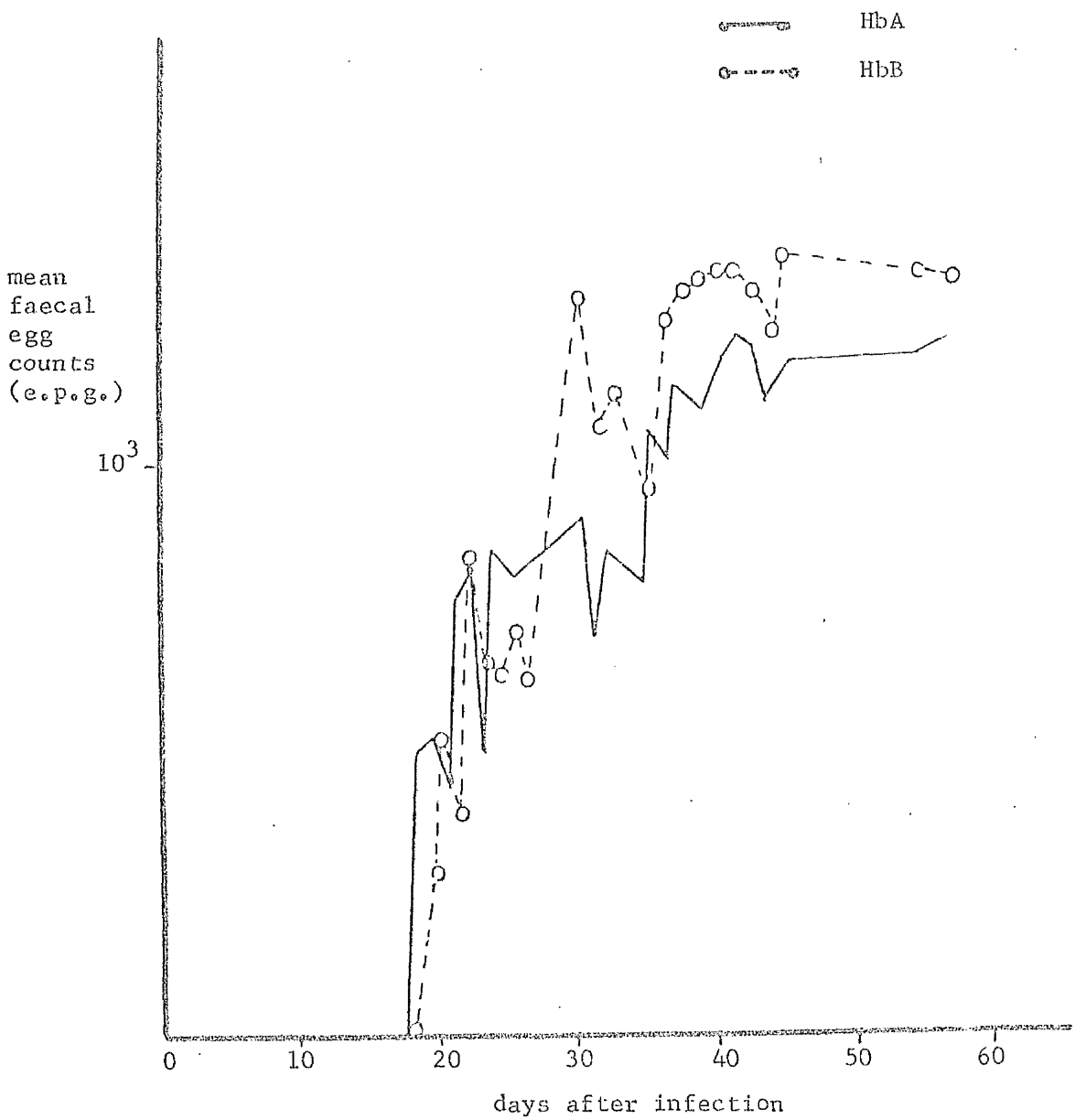


Figure 22

The mean faecal egg counts of HbA and HbB Merino sheep following experimental infection with H. contortus ($10L_3$/kg)



The mean PCV values of HbA and HbB Merino sheep following experimental infection with H. contortus at dosage rates of 10, 50 and 150 L_3 larvae per kg.

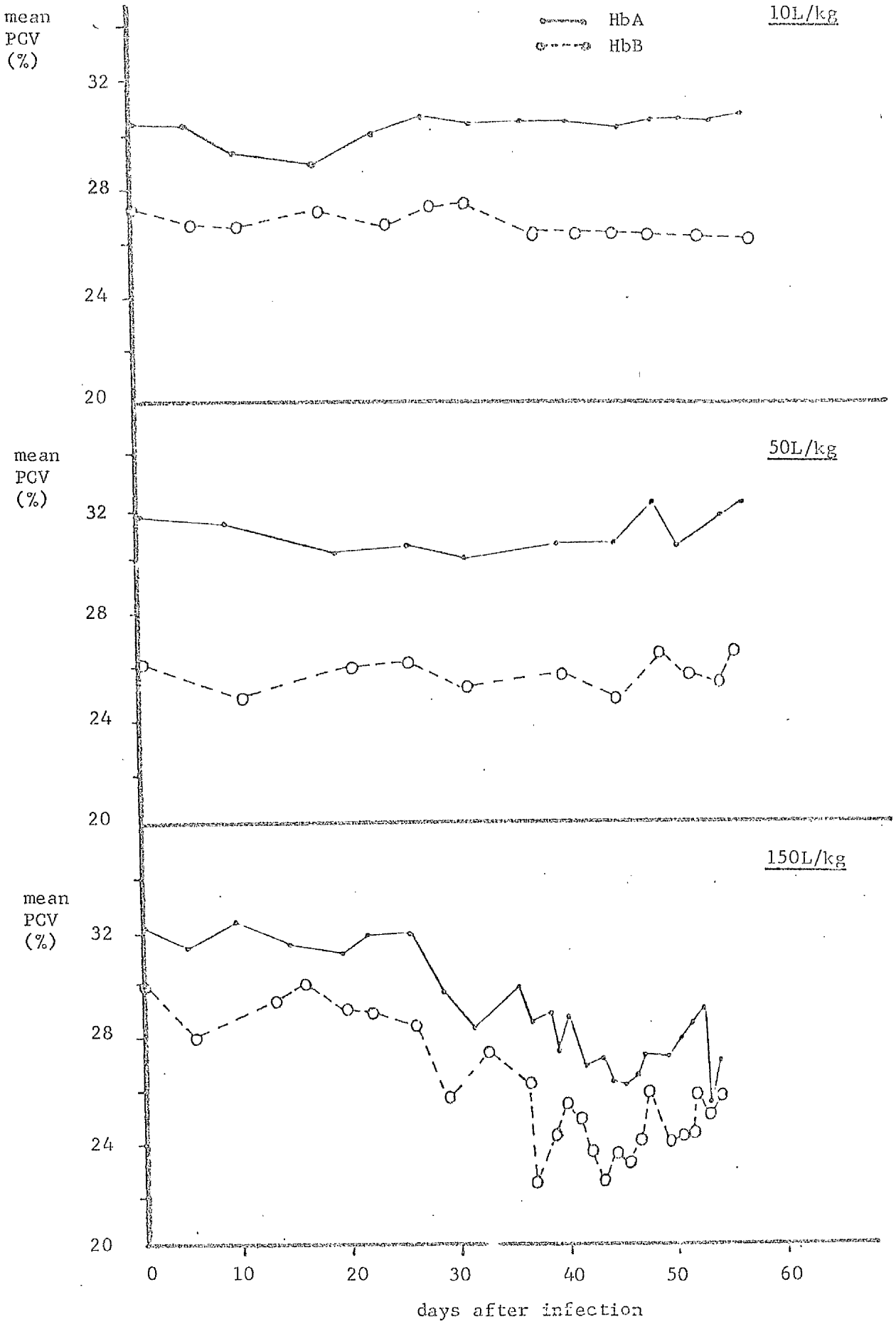


Figure 24

The daily mean faecal clearance of labelled red cells in HbA and HbB Merino sheep following experimental infection with *H. contortus* ($150L_3$/kg)

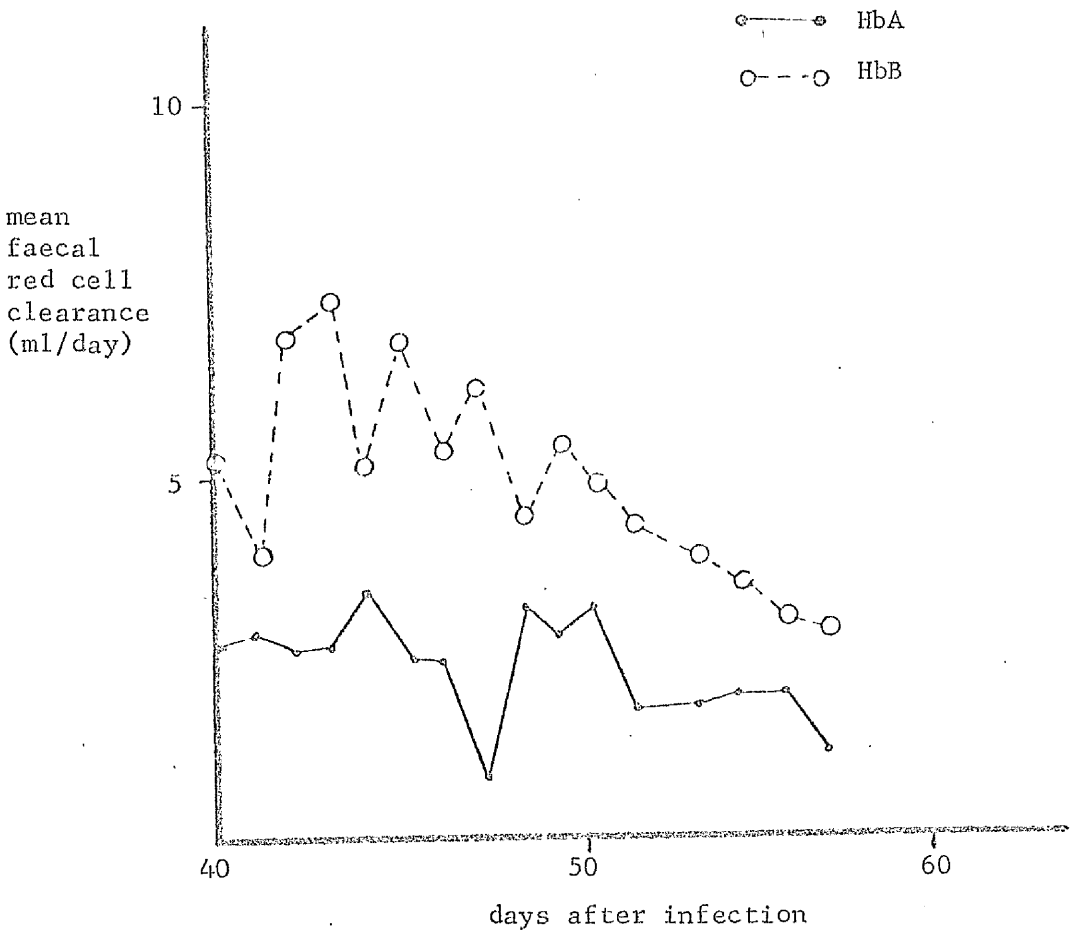


TABLE 10

Blood, red cell and plasma volumes and PCV of Merino sheep on day 38 of an infection with *H. contortus* (150L/kg)

Hb type	Sheep No.	BV (ml/kg)	Vc (ml/kg)	Vp (ml/kg)	PCV
AA	1	56.2	15.2	41.2	27.0
AA	2	65.3	17.3	48.0	26.5
AA	3	60.1	22.2	37.9	34.0
Mean		60.6 ± 2.6	18.3 ± 2.1	42.37 ± 2.9	29.16 ± 2.4
BB	4	62.9	13.8	49.1	22.0
BB	5	95.8	21.5	74.2	22.5
BB	6	70.8	16.9	53.8	24.0
Mean		76.5 ± 9.9	17.5 ± 2.2	59.0 ± 7.7	22.8 ± 0.6
	P	NS	NS	NS	< 0.05

TABLE 11

Faecal clearance of whole blood and red blood cells and half-life of red blood cells in Merino sheep of HbA and B from days 41 to 56 of a primary infection with H. contortus (150L/kg)

Hb type	Sheep no.	Faecal Clearance		$T_{\frac{1}{2}}$ (hrs)	
		whole blood	rbc	whole	rbc
AA	1	15.7	4.0	222.9	239.2
AA	2	7.7	2.2	258.0	241.6
AA	3	6.9	2.2	259.9	258.9
	Mean	10.2 ± 2.8	2.8 ± 0.6	246.9 ± 12.03	246.6 ± 6.2
BB	4	23.2	4.8	226.5	193.7
BB	5	12.4	3.3	230.8	197.4
BB	6	26.3	6.6	191.1	168.4
	Mean	20.6 ± 4.21	4.9 ± 0.95	216.2 ± 12.6	186.5 ± 9.1
	P	<0.02	NS	NS	<0.01

DISCUSSION

As in the previous experiment, the numbers of animals involved is quite small. There are several reasons for this; one is the availability of the facilities required for experiments of this nature where the sheep have to be kept in metabolism cages; also the volume of work involved in experiments of this kind where daily collection of blood, faeces and urine are necessary. In this series of experiments a number of sheep were re-used. It would have been preferable to have had different sheep for each experiment, e.g. worm-free. However the availability of animals, particularly of HbA type, made it necessary to indulge in this economy. Both HbA and HbB groups of sheep received exactly the same treatment so there was no reason why the results should not be completely valid as a comparative exercise.

It was apparent from this series of experiments that when relatively low experimental infections of H. contortus i.e. 150, 50 and 10 L/kg were administered to two groups of Merino sheep with HbA and B, a difference in the relative resistance of HbA and HbB sheep was evident. This resistance when expressed in the form of reduced blood loss, showed correspondingly lower erythropoiesis, low egg counts and higher haematocrit values in HbA sheep than in HbB sheep.

The combined results of the last two experiments indicate that the phenomenon of strain resistance of different Merino haemoglobin types to H. contortus infection was not absolute

but was apparently dependent on the size of the infection. For example, in higher levels of experimental infection (i.e. 300 L/kg), the strain difference in susceptibility became much less apparent.

Generally, the disease followed a similar course to that observed in the last experiment and as observed by Dargie and Allonby (1975) and Altaif and Dargie (1976). Eggs were observed in the sheep faeces from between 18 and 20 days after infection in all three experiments. From this point to around 25 days post-infection the faecal egg counts increased rapidly and, although fluctuations occurred, this was followed by a plateau-phase in which the faecal egg output was maintained at between 1000 and 8000 epg. In all three experiments HbB sheep maintained higher faecal egg counts compared with HbA sheep.

A slight decrease in haematocrit values occurred in both groups after day 25 of an infection with 150 L/kg, but this decrease did not occur when infections of 50 or 10 L/kg were used. In both these latter cases the haematocrit values maintained a steady level, probably due to both groups of sheep being able to compensate sufficiently for any blood taken by the relatively few numbers of parasites. For most of the period the HbA sheep had significantly higher haematocrits than the HbB sheep. The greatest difference in haematocrits between the haemoglobin groups was observed when the infective dose was 50 L/kg.

The HbB sheep lost significantly more whole blood into the faeces than HbA sheep and since red cell clearance figures were not significantly different this suggests that a higher proportion of plasma was lost in the HbB sheep than red blood cells.

For instance the daily red cell clearance results indicated that the HbB sheep were losing about 5ml/day of red blood cells, whereas HbA sheep were losing only about 2 ml/day and there was a corresponding increase in erythropoiesis in the HbB sheep when they were infected with 150 L/kg of H. contortus. Towards the end of the experiment the red cell loss decreased in both groups following the occurrence of 'self-cure' and probably expulsion of most of the worms.

The reason for the occurrence of 'self-cure' after day 55 remains obscure. However at all times during the experiment the sheep were confined to metabolism cages indoors and fed the same batch of hay which was also kept dry and indoors. Thus this rainfall could not have been responsible for a challenge of larvae and subsequent hypersensitivity reaction described by Gordon (1948), and Stewart (1953) and Soulsby and Stewart (1960) or a factor in the new grass described by Allonby and Urquhart (1973) each leading to 'self-cure'

Although Allonby and Urquhart (1973) were able to record that 'self-cure' occurred more often and effectively in HbA sheep they were unable to observe overall differences in the faecal egg counts between the haemoglobin types of Merino sheep naturally infected with H. contortus in Naivasha Kenya. However differences in faecal egg counts between

the haemoglobin groups were apparent in the Machakos field experiment except at certain times of the year related to periods of heavy challenge. These differences in observation may be related to the difference in size of the natural infections. Naivasha pasture may have had a higher incidence of H. contortus than Machakos. The size of the infection may also affect the extent of resistance in Florida Native sheep, as Jilek and Bradley (1969) and also Radhakrishnan et al. (1952) were unable to observe that HbA were more resistant to haemonchosis than HbB sheep by monitoring faecal egg counts although HbA sheep 'self-cured' more often.

These results lead to the conclusion that differences between haemoglobin types in resistance to H. contortus only became apparent under certain circumstances, and that the size of the challenge is most important.

SUMMARY

In Merino sheep there is a difference in susceptibility between sheep of haemoglobin type A and those of haemoglobin type B. It has been demonstrated that this strain difference in susceptibility is not absolute and that it is dependent on the size of the challenge infection, and as in the case of Scottish Blackface, at certain levels of infection the strain difference in susceptibility becomes obscure.

DISCUSSIONS AND CONCLUSIONS

DISCUSSIONS AND CONCLUSIONS

The results of the experiments in Section II of this thesis demonstrate that certain differences exist in the susceptibility between Merino sheep of the three haemoglobin types to H. contortus infection, i.e. HbA sheep were generally more resistant to H. contortus than HbB sheep, whilst sheep with HbAB occupied an intermediate position. This difference has been demonstrated both in parasitological terms, i.e. by monitoring faecal egg counts and worm burdens, and also by assessing the pathogenic effect of H. contortus infection on the host from various pathophysiological parameters. Apart from certain periods possibly related to periods of high challenge the investigation of naturally acquired infections showed that HbA sheep had lower egg counts and 'self-cured' more often and effectively than HbB sheep. From these findings it was concluded that this strain difference in susceptibility was not absolute since it appeared to be dependent on the size of the experimental infection, i.e. at high levels of infection the strain difference in susceptibility became obscure. It has also been shown that the HbB gene was predominant in most sheep in Kenya and it is thought that this is probably due to the apparent overriding selection for resistance to drought. However since the incidence of the A gene tended to increase in the wetter areas, it is possible that this gradient in haemoglobin type is related to the higher incidence of H. contortus in these regions rather than towards a selection towards the incidence of drought.

Subsequent studies on the response of each strain to experimental H. contortus infection indicated that the difference in resistance to haemonchosis within the haemoglobin groups was due to the differences in the establishment of adult H. contortus rather than a suppression of egg laying capacity or variations in the amount of blood taken by each worm. From the results of these studies it is clear that the conditions in the abomasum of the HbA sheep must have been more unfavourable in some way to the parasites than that in the HbB sheep. Recent work has indicated that IgA antibodies may be involved in the protective immunity to H. contortus infections in sheep. Using radioimmunoassay techniques Smith et al. (1977) found that seven month old sheep rendered immune to H. contortus by vaccination had high levels of specific mucous IgA as well as an increased number of IgA producing plasma cells in the abomasal mucosa, whereas two month old lambs, which could not be protected by vaccination, had significantly depressed IgA levels in the mucosa. Furthermore, Duncan et al. (1977) have shown in Kenya that the indigenous Masai sheep had higher levels of IgA than the exotic Merino sheep. However, one week after anthelmintic treatment the IgA levels in both breeds were significantly reduced. This might have been due to either there being no worms, or possibly due to some immunosuppressive activity in the drug. It would be interesting to assess the IgA levels in sheep of the three haemoglobin types to determine whether any differences also occur between the three strains.

Recent work performed concurrently to that described here has indicated that Masai sheep were by far the most resistant

to infection with H. contortus when compared to Merino, Blackhead Persian, Corriedale and Dorper breeds, using both parasitological and pathophysiological parameters (Allonby and Preston, 1977; Preston and Allonby, 1977). This breed difference also appeared to be associated with lower worm establishment since a marked expulsion of worms was found to occur during the prepatent period in the Masai sheep. However this breed difference was shown not to be dose dependent (Allonby, Preston and Aucutt, 1977; Aucutt et al., 1977) or related to different planes of nutrition (Allonby and Preston, 1977). Clearly there must be a profound immunological difference between strain differences and breed differences in susceptibility which is possibly related to the physical structure of the abomasum as well as physiological differences.

When reared worm free, the weight gains in Masai sheep were equal to those of Merino sheep (Allonby and Preston,1977). However, under traditional management Masai sheep are characterised by their stunted appearances, thought to be largely related to an early and chronic infection with H. contortus (Allonby, 1974). This apparent anomoly may be related in some way to the interesting phenomenon which arose from the pathophysiology experiments described in this section. After a second infection with H. contortus haematocrit values increased dramatically, although there was also a substantial increase in red blood cell loss in both haemoglobin types (see Figs. 11 and 12). This may be due to a homeostatic response of fluid shift, but it also could be that there was an increased loss of plasma into the abomasum. This might be due to a hypersensitivity reaction

occurring against the worms at the mucosal wall. A corresponding deleterious effect of the nitrogen balance may occur, effecting weight gains of the sheep if food and water was not present in plentiful supply to compensate. This could possibly explain how even a partially immune animal may still appear stunted as is the case of Masai sheep reared on endemic pasture under traditional management.

The information presented in Section II could have a number of practical applications. One such application would be the selection and development of haemoncho-tolerant strains of sheep in the traditional low investment/low risk management systems of Africa in which the high incidence of 'self-cure', often the only mechanism in which the sheep periodically lose their worm burden, would be advantageous. However, it could equally well be implemented in more sophisticated animal breeding programmes. There has been an increase in public opinion against the use of 'drugs' on consumable items such as fruit and vegetables; and a reduction in the use of pesticides has already seen the highly successful development of many disease-resistant plant strains. Chemical contaminants in butter, milk and meat are also becoming increasingly unacceptable and hence public opinion against the use of acaricides and anthelmintics plus the ever increasing incidence of resistance to these chemicals and their intrinsic high costs, could make the breeding of haemoncho-tolerant strains of sheep a commercially viable proposition, particularly if combined with a cross-breeding programme. Interest in this resistance of certain strains is also growing in connection with other diseases. Bangham and Blumberg (1958) observed an association between haemoglobin type and resistance to

trypanosomiasis in cattle; and more recently Anderson et al. (1972) indicated that the high resistance of young cattle to anaplasmosis may be associated with the presence of foetal haemoglobin.

Another practical application of the information on strain would be in relation to the choice of animals in research work. As early as 1931 Fourrie noticed a big spectrum in response to H. contortus and in many field and indoor experiments documented so far there are always individual animals which react differently from the main group. The choice of sheep of similar strains would obviously help to minimise these variations in the future selection of experimental animals.

One of the disadvantages of working with large animals in an "indoor" experiment is that the number of animals involved are necessarily low because of the facilities for maintaining them. The advantage of the N. brasiliensis-rat system studied in the experiments described in Section I of this thesis is that large numbers of rats can easily be used. This system has proved to be a useful laboratory model of a host-parasite relationship and its use in combination with the H. contortus-sheep system could continue to be advantageous due to the similarities between the two systems, i.e. in both systems a strain difference of the host has been found to influence the balance of the host-parasite relationship 'self-cure' which is itself influenced by the hormonal effects of lactation. It would be of interest to perform further studies on the N. brasiliensis system to determine whether strain differences are also dose dependent. Another possible useful approach would be to investigate the development of immune

competency in lambs of different haemoglobin types and to use both helminth systems as models to investigate in more detail the way in which immunological and other factors regulate the host-parasite relationship. This might then lead the way to the rational development of immunisation procedures against helminths which would be of inestimable value to the welfare of both man and animals throughout the world.

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APPENDIXES

TABLE A

^{32}P-uptake in both sexes of London and Aberdeen strains of Hooded Lister rats infected with N. brasiliensis

Days after infection	London strain		Aberdeen strain	
	Male rats	Female rats *	Male rats	Female rats
4	-	-	18.3 ± 1.5	-
5	36.0 ± 10.9	-	41.4 ± 6.6	73.2 ± 32.6
6	39.1 ± 9.2	104.8 ± 7.5	44.3 ± 5.4	159.7 ± 31.1
7	42.3 ± 9.5	110.2 ± 23.2	20.4 ± 5.4	85.3 ± 36.5
8	31.9 ± 9.3	35.4 ± 6.1	15.9 ± 3.4	81.4 ± 52.3
9	36.2 ± 15.3	10.6	5.8 ± 2.9	88.7 ± 34.9
10	18.3 ± 4.6	4.3 ± 1.3	32. ± 0.8	20.5 ± 4.6
11	11.1 ± 5.2	8.4 ± 1.0	0.3	

* The results of Henney et al., 1971 are incorporated in this table

TABLE B

Worm wet weights (mg) and ^{32}P-uptake during the course of an infection with N. brasiliensis in 4-5 week old male Hooded Lister rats of Aberdeen strain

Days after infection	Worm wet weights (mg)		
	Young rats		Adult rats
6	15.0 ± 5.3	NS	25.6 ± 2.72
7	22.9 ± 8.5	NS	26.7 ± 3.4
8	15.2 ± 5.9	NS	23.6 ± 5.5
9	14.2 ± 4.7	NS	12.4 ± 3.9
10	13.9 ± 5.7	NS	16.4 ± 3.9
11	13.4 ± 3.3	<0.2	3.5 ± 3.5
13	6.9 ± 6.9	<0.5	0

Days after infection	^{32}P-uptake		
	Young rats		Adult rats
6	51.2 ± 5.9	NS	44.3 ± 5.4
7	27.5 ± 9.3	NS	20.4 ± 6.9
8	20.3 ± 8.2	NS	15.9 ± 3.4
9	17.2 ± 3.7	NS	5.8 ± 3.0
10	9.1 ± 3.1	NS	3.2 ± 0.8
11	6.2 ± 2.7	NS	0.3 ± 0.3
13	1.8 ± 1.8		-

TABLE C

Worm wet weights (mg) and ^{32}P-uptake during the course of infection with N. brasiliensis in female Hooded Lister rats of Aberdeen strain experiencing proestrus, oestrus or dioestrus

Days after infection	Worm wet weights (mg)				
	Rats in proestrus		Rats in oestrus		Rats in dioestrus
5	19.3 ± 16.7	NS	60.6 ± 10.9	NS	26.4 ± 14.5
6	58.9 ± 14.0	NS	52.8 ± 8.6	NS	23.1 ± 20.9
7	36.8 ± 11.2	<0.05	15.9 ± 6.5	NS	30.2 ± 11.9
8	36.4 ± 7.4	<0.01	3.4 ± 1.5	NS	23.2 ± 11.0
9	26.2 ± 7.0	<0.05	6.2 ± 2.1	NS	11.5 ± 3.1
10	17.2 ± 7.2	NS	9.9 ± 6.9	NS	1.9 ± 1.9

Days after infection	^{32}P-uptake				
	Rats in proestrus		Rats in oestrus		Rats in dioestrus
5	49.3 ± 4.3	<0.05	35.3 ± 2.9	NS	36.2 ± 7.4
6	76.9 ± 11.6	NS	66.1 ± 8.9	NS	79.6 ± 19.6
7	40.0 ± 11.7	NS	15.7 ± 0.7	NS	32.3 ± 9.8
8	23.6 ± 7.9	NS	17.5 ± 3.5	NS	23.1 ± 3.0
9	15.3 ± 6.2	NS	13.3 ± 3.2	NS	17.9 ± 2.2
10	7.2 ± 0.6	NS	10.3 ± 1.7	NS	12.7 ± 1.0

TABLE D

^{32}P-uptake in male Hooded Lister rats of Aberdeen strain infected with N. brasiliensis larvae. Experimental group injected with 0.02 ml of Depoprovera on day 7 of infection

Days after infection	^{32}P-uptake Control group		Experimental group
4	18.3 ± 1.5		
5	41.5 ± 6.6		
6	44.3 ± 5.4		
7	20.4 ± 5.4		
8	15.9 ± 3.4	NS	12.5 ± 2.0
9	5.8 ± 2.9	NS	7.7 ± 0.9
10	3.2 ± 0.8	NS	3.6 ± 0.4
11	0.3 ± 0.3	NS	1.2 ± 0.4

TABLE E

Mean faecal egg counts of the three groups of sheep with different haemoglobin types reared on H. contortus-endemic pasture at a farm near Machakos

Week of sampling	Mean faecal egg counts		
	HbA	HbAB	HbB
0	2 525	1 980	2 600
3	200	400	260
6	525	850	1 150
9	325	80	770
12	850	1 000	660
15	175	850	700
18	330	210	425
21	100	350	506
24	400	1 100	909
27	350	1 700	1 784
30	67	250	348
33	0	190	480
36	166	418	638
39	100	209	696
42	25	92	414
45	50	182	396
48	400	691	1 324
51	1 100	2 191	2 340
54	1 300	1 008	1 653
57	1 033	2 527	1 540
60	975	1 066	1 105
63	400	425	965
66	1 125	1 612	1 815
69	333	900	538
72	0	227	638
75	400	290	417
78	225	138	272
84	900	660	965
87	667	1 060	3 236
90	1 025	1 010	3 692
93	0	11	314
96	25	63	91
98	50	178	600

.../

Week of sampling	Mean faecal egg counts		
	HbA	HbAB	HbB
100	900	114	051
102	1 100	1 356	2 054
104	400	925	1 504
106	400	1 120	2 350
108	850	1 873	3 146
110	1 733	3 023	5 167

TABLE F

Phase I

Whole blood, red cell and plasma volumes and PCV of Merino sheep prior to experimental infection with H. contortus (300L/kg)

Hb type	Sheep no.	BV ml/kg)	Vc (ml/kg)	VP (ml/kg)
AA	1	38.0	12.6	25.5
AA	2	42.6	14.1	28.6
AA	3	46.9	14.1	32.8
	Mean	42.5 ± 2.6	13.6 ± 0.5	28.9 ± 2.1
AB	7	52.2	15.1	36.6
AB	8	30.4	9.0	21.3
AB	9	75.8	15.0	60.7
	Mean	52.8 ± 13.1	13.2 ± 2.1	39.5 ± 11.5
BB	4	31.3	7.8	23.3
BB	5	35.9	12.1	23.4
BB	6	57.9	15.6	45.4
	Mean	41.7 ± 8.2	11.8 ± 2.3	30.7 ± 7.4
		NS	NS	NS

TABLE G

Phase 1

Whole blood, red cell and plasma volumes and PCV of Merino sheep 55 days after a primary experimental infection with H. contortus (300L/kg)

Hb type	Sheep no.	BV ml/kg)	Vc (ml/kg)	VP (ml/kg)
AA	1	57.3	9.2	48.4
AA	2	50.2	9.0	41.2
AA	3	51.2	6.2	45.1
	Mean	52.9 ± 2.2	8.1 ± 0.9	44.9 ± 3.1
AB	7	55.1	12.2	42.9
AB	8	43.2	9.5	33.7
AB	9	45.5	5.5	40.0
	Mean	47.2 ± 3.6	9.1 ± 1.9	38.9 ± 2.7
BB ,	4	54.2	6.5	47.7
BB	5	56.2	12.3	43.8
BB	6	42.6	5.1	37.5
	Mean	50.9 ± 4.2	8.0 ± 2.2	43.0 ± 2.9
		NS	NS	NS

TABLE H

Phase 1

Faecal clearance of whole blood and red cells in Merino sheep during the control, primary and secondary periods of experimental infection

Hb type	Sheep no.	Faecal clearance					
		control period		during primary infection		during secondary infection	
		whole blood	rbc	whole blood	rbc	whole blood	rbc
AA	1	35.5	9.9	22.7	4.5	56.5	12.6
AA	2	40.2	11.2	43.8	7.7	90.4	15.6
AA	3	24.0	6.7	53.4	6.4	58.6	9.9
	Mean	33.3 ± 4.8	9.2 ± 1.3	40.0 ± 9.1	6.2 ± 0.9	68.5 ± 10.9	12.7 ± 1.6
AB	7	45.8	13.7	42.8	6.2	69.4	14.3
AB	8	16.6	6.2	17.7	3.6	47.9	12.6
AB	9	31.4	7.7	49.2	5.8	82.0	14.0
	Mean	31.3 ± 8.4	9.2 ± 2.3	36.6 ± 9.6	5.2 ± 0.8	66.4 ± 9.9	13.6 ± 0.5
BB	4	52.2	13.2	45.4	5.7	59.2	9.1
BB	5	18.4	5.6	36.0	7.9	78.2	21.6
BB	6	24.4	7.0	29.6	3.8	129.2	27.5
	Mean	31.7 ± 10.4	8.6 ± 2.3	37.0 ± 4.6	5.8 ± 1.2	88.9 ± 20.9	19.4 ± 5.4
		NS	NS	NS	NS	$<.05$	$<.05$

TABLE I

Phase 2

Whole blood, red cell and plasma volumes of Merino sheep prior to an infection with *H. contortus* (300L/kg)

Hb type	Sheep no.	BV (ml/kg)	Vc (ml/kg)	VP (ml/kg)
AA	4	55.2	17.7	37.5
AA	5	49.0	12.3	36.8
AA	6	55.1	16.0	39.1
	Mean	53.1 ± 2.1	15.3 ± 1.6	37.8 ± 0.7
AB	3	45.1	13.5	31.6
AB	7	46.3	13.9	32.4
AB	9	56.8	16.5	40.3
	Mean	49.4 ± 3.7	14.6 ± 0.9	34.8 ± 2.8
BB	1	46.9	10.8	36.2
BB	2	41.9	10.5	31.5
BB	8	38.4	9.9	28.4
	Mean	42.4 ± 2.5	10.4 ± 0.3	32.0 ± 2.3
P AA/BB		<.05	<.05	NS

TABLE J

Phase 2

Whole blood, red cell and plasma volumes of Merino sheep 49 days after a primary infection with H. contortus (300L/kg)

Hb type	Sheep no.	BV (ml/kg)	Vc (ml/kg)	VP (ml/kg)
AA	4	57.4	16.1	41.4
AA	5	54.8	12.1	42.7
AA	6	61.2	14.7	46.5
	Mean	57.8 ± 1.9	14.3 ± 1.2	43.5 ± 1.5
AB	3	46.4	12.5	33.9
AB	7	50.3	10.1	40.2
AB	9	58.8	10.6	48.3
	Mean	51.8 ± 3.7	11.1 ± 0.7	40.9 ± 4.2
BB	1	52.9	10.1	42.9
BB	2	44.5	9.4	35.2
BB	8	54.7	10.9	43.8
	Mean	50.7 ± 3.1	10.1 ± 0.4	40.6 ± 2.7
P AA/BB		NS	<.05	NS

TABLE K

Phase 2

Faecal clearance of whole blood and red cells in Merino sheep during the control period and period of primary experimental infection

Hb type	Sheep no.	Faecal clearance			
		control period		during primary infection	
		whole blood	rbc	whole blood	rbc
AA	4	6.2	2.3	11.8	2.5
AA	5	20.9	5.9	7.8	1.8
AA	6	2.8	1.7	8.3	2.2
	Mean	9.9 ± 5.5	3.3 ± 1.3	9.3 ± 1.2	2.1 ± 0.2
AB	3	1.3	0.4	6.3	2.0
AB	7	4.0	0.9	17.2	6.6
AB	9	7.2	1.9	9.8	2.6
	Mean	4.1 ± 1.7	1.0 ± 0.4	11.1 ± 3.2	3.7 ± 1.4
BB	1	8.8	2.3	11.0	2.5
BB	2	1.9	0.48	14.9	3.5
BB	8	11.5	2.9	8.0	1.9
	Mean	7.4 ± 12.8	1.8 ± 0.7	11.3 ± 2.0	2.6 ± 0.4
		NS	NS	NS	NS

TABLE L

Phase 2

Faecal clearance of whole blood and red cells of Merino sheep prior to and during a secondary infection with H. contortus

(300L/kg)

Hb type	Sheep no.	Faecal clearance			
		control period		during primary infection	
		whole blood	rbc	whole blood	rbc
AA	4	1.6	1.2	0.73	0.5
AA	5	34.0	7.7	23.2	6.9
AA	6	16.4	4.8	12.9	3.6
	Mean	17.3 ± 9.4	4.5 ± 1.9	12.3 ± 6.5	3.67 ± 1.8
AB	3	10.8	3.0	10.6	2.6
AB	7	19.0	5.1	6.5	2.0
AB	9	2.7	0.9	2.7	0.9
	Mean	10.6 ± 4.7	3.0 ± 1.2	6.6 ± 2.3	1.8 ± 0.5
BB	1	21.9	4.5	19.4	4.3
BB	2	14.0	2.9	1.7	0.6
BB	8	50.8	7.0	38.3	9.7
	Mean	28.9 ± 11.2	4.8 ± 1.2	19.8 ± 10.5	4.8 ± 2.6
		NS	NS	NS	NS

TABLE M

Mean faecal egg counts of three groups of Merino sheep infected with 300L/kg H. contortus larvae

Phase 1	Hb type			
days after infection	HbA	HbAB	HbB	P A:B
18	0	0	0	
20	200 ± 100	67 ± 33	167 ± 86	NS
27	8000 ± 2816	5333 ± 2444	9433 ± 754	NS
28	8500 ± 1852	9233 ± 3329	7000 ± 917	NS
30	7367 ± 176	11567 ± 2258	9267 ± 953	NS
32	16200 ± 1474	11967 ± 2378	14333 ± 2677	NS
33	18000 ± 3163	16300 ± 2884	17667 ± 2171	NS
34	15767 ± 2992	22733 ± 2267	17300 ± 3493	NS
35	16467 ± 2829	20500 ± 755	13900 ± 3817	NS
36	15667 ± 3797	13133 ± 3120	17300 ± 1457	NS
38	11700 ± 4650	22100 ± 4513	20733 ± 2115	NS
41	7367 ± 1789	16633 ± 2326	16133 ± 1934	<0.05
42	6933 ± 2264	5267 ± 2397	6100 ± 1286	NS
43	5800 ± 2955	2970 ± 2525	5200 ± 2254	NS
44	9500 ± 2673	3267 ± 3117	4425 ± 1785	NS
46	9333 ± 1878	4633 ± 3709	11067 ± 933	NS
47	10167 ± 4579	7033 ± 4206	11900 ± 4539	NS
48	6667 ± 2572	3800 ± 2603	9200 ± 1255	NS
50	9767 ± 3996	6367 ± 3702	11989 ± 868	NS
51	5567 ± 2206	3033 ± 1449	6967 ± 2450	NS
52	7733 ± 1954	1133 ± 589	4133 ± 865	NS
54	8833 ± 3991	3567 ± 2378	14533 ± 3205	NS
58	11600 ± 5302	4600 ± 2318	8067 ± 2477	NS
61	11233 ± 1980	7400 ± 2759	11033 ± 2643	NS
63	9833 ± 4684	5333 ± 2049	7367 ± 3166	NS
65	10567 ± 2890	5400 ± 2079	7600 ± 3722	NS
67	7433 ± 2631	5833 ± 2404	6733 ± 3317	NS
69	5133 ± 2111	3733 ± 2322	2100 ± 3962	NS
72	5600 ± 1986	4633 ± 2684	5533 ± 2618	NS
73	5600 ± 1823	5000 ± 3591	6133 ± 3034	NS
74	5700 ± 2417	6767 ± 4355	6500 ± 4382	NS
75	3867 ± 1125	7500 ± 4680	5200 ± 2352	NS

Phase 2	Hb type			
days after infection	HbA	HbAB	HbB	P A:B
19	0	0	0	NS
20	0	0	0	NS
24	265 ± 88	1350 ± 233	1650 ± 1184	NS
28	617 ± 216	2617 ± 1217	2433 ± 1909	NS
33	3733 ± 3508	28683 ± 25429	4367 ± 707	NS
35	3750 ± 3026	20017 ± 4273	10747 ± 4741	NS
40	7850 ± 2837	6217 ± 3089	9300 ± 3352	NS
42	6417 ± 3034	33117 ± 20628	10833 ± 2450	NS
45	6417 ± 2800	30100 ± 16450	9800 ± 3010	NS
49	6800 ± 3204	15000 ± 6050	9000 ± 2693	NS
52	6850 ± 2300	15250 ± 7280	10000 ± 3390	NS
56	7750 ± 2457	15000 ± 7070	9700 ± 2626	NS
61	7750 ± 2319	15250 ± 7456	9683 ± 1937	NS
62	10067 ± 4932	1900 ± 1450	6033 ± 3332	NS
63	4883 ± 2455	700 ± 264	11033 ± 6624	NS
64	4100 ± 3802	3600 ± 2298	5133 ± 2614	NS
65	3133 ± 2603	1633 ± 1390	2367 ± 1260	NS
67	11650 ± 5911	7417 ± 4621	7100 ± 2696	NS
68	12483 ± 5896	7400 ± 4629	5883 ± 1335	NS
69	5233 ± 2984	8083 ± 6320	4733 ± 3679	NS
70	9883 ± 4619	6150 ± 3655	3000 ± 2358	NS
71	7150 ± 3919	10950 ± 7765	8883 ± 7320	NS
73	6167 ± 2634	6883 ± 4819	3183 ± 1711	NS
75	567 ± 296	600 ± 503	767 ± 328	NS

Mean PCV values of three groups of Merino sheep infected with 300L/kg H. contortus larvae

Phase 1	Hb type			
days after infection	HbA	HbAB	HbB	P A:B
0	31.0 ± 2.0	27.5 ± 1.9	28.0 ± 2.6	NS
1	31.0 ± 1.6	27.0 ± 1.5	27.5 ± 1.4	NS
2	31.7 ± 1.2	28.7 ± 2.1	30.3 ± 2.9	NS
3	30.7 ± 1.2	27.0 ± 2.0	30.3 ± 2.2	NS
4	31.0 ± 1.3	29.0 ± 2.6	29.0 ± 2.1	NS
5	30.0 ± 0.9	29.0 ± 1.7	28.7 ± 1.9	NS
6	32.0 ± 0.6	27.7 ± 1.8	31.7 ± 1.5	NS
7	27.7 ± 2.3	30.7 ± 1.8	28.7 ± 1.3	NS
8	29.0 ± 0.0	30.0 ± 2.1	29.0 ± 2.0	NS
9	28.0 ± 0.6	30.0 ± 2.1	28.3 ± 1.2	NS
10	29.0 ± 0.6	30.0 ± 2.1	28.7 ± 1.8	NS
11	28.7 ± 1.3	28.0 ± 1.5	27.5 ± 1.0	NS
12	29.0 ± 1.1	30.0 ± 1.6	30.3 ± 1.4	NS
13	27.7 ± 1.2	29.7 ± 1.4	28.7 ± 0.9	NS
14	28.0 ± 1.0	29.0 ± 1.5	28.5 ± 2.0	NS
15	26.7 ± 1.8	29.0 ± 1.9	29.3 ± 2.0	NS
16	28.0 ± 0.6	29.0 ± 2.6	30.2 ± 2.8	NS
17	26.5 ± 1.3	25.8 ± 1.6	26.7 ± 1.8	NS
18	27.7 ± 1.3	27.2 ± 1.8	29.0 ± 2.0	NS
19	25.0 ± 1.2	25.7 ± 1.2	25.8 ± 1.6	NS
20	24.7 ± 0.9	26.5 ± 1.3	25.8 ± 2.1	NS
21	23.7 ± 0.7	24.0 ± 1.9	24.5 ± 0.9	NS
55	15.3 ± 1.8	18.7 ± 3.3	15.3 ± 3.3	NS
56	16.7 ± 2.4	19.7 ± 3.3	15.3 ± 3.0	NS
57	15.5 ± 2.5	16.2 ± 2.8	14.0 ± 3.5	NS
58	16.7 ± 2.9	16.8 ± 3.0	16.8 ± 4.1	NS
59	17.0 ± 2.1	18.0 ± 3.1	17.3 ± 3.6	NS
60	15.7 ± 2.2	16.7 ± 2.6	14.7 ± 1.2	NS.
61	16.3 ± 1.9	17.2 ± 2.7	17.5 ± 2.8	NS
62	17.7 ± 1.2	18.0 ± 2.1	16.3 ± 2.4	NS
63	16.8 ± 2.1	19.3 ± 3.3	17.3 ± 2.8	NS

days after infection	Hb type HbA	HbAB	HbB	P A:B
64	17.3 ± 1.9	18.3 ± 2.5	19.3 ± 5.4	NS
65	17.7 ± 1.9	18.0 ± 2.6	17.3 ± 3.1	NS
66	16.7 ± 2.3	20.8 ± 2.2	18.0 ± 2.5	NS
67	18.0 ± 3.2	21.2 ± 2.0	18.8 ± 2.7	NS
68	18.2 ± 3.2	22.2 ± 1.8	19.7 ± 2.9	NS
69	18.5 ± 4.0	20.0 ± 2.1	19.7 ± 3.2	NS
70	19.5 ± 3.3	21.0 ± 2.0	21.5 ± 2.8	NS
71	20.2 ± 3.2	22.3 ± 2.8	22.8 ± 3.2	NS
72	20.8 ± 2.6	20.8 ± 2.7	20.8 ± 2.8	NS
73	21.3 ± 3.2	22.7 ± 2.9	21.0 ± 2.1	NS
74	23.3 ± 3.3	23.8 ± 3.8	24.7 ± 3.6	NS
75	23.2 ± 3.5	23.2 ± 3.1	23.7 ± 3.4	NS
76	22.7 ± 2.8	23.8 ± 3.2	22.3 ± 2.5	NS
Phase 2				
0	28.7 ± 2.0	29.7 ± 0.3	24.7 ± 0.9	NS
1	28.3 ± 1.2	29.0 ± 1.5	25.7 ± 1.7	NS
2	28.0 ± 1.0	30.3 ± 1.5	27.7 ± 1.5	NS
3	27.3 ± 0.7	29.3 ± 1.5	26.3 ± 0.9	NS
4	27.7 ± 0.9	29.7 ± 1.7	26.3 ± 1.8	NS
5	27.0 ± 1.5	29.0 ± 0.0	25.7 ± 1.5	NS
6	27.3 ± 1.7	29.3 ± 0.7	25.0 ± 1.2	NS
7	27.3 ± 0.3	29.0 ± 1.0	24.3 ± 1.2	NS
8	28.0 ± 1.5	30.7 ± 0.7	25.0 ± 1.2	NS
9	27.3 ± 0.3	30.3 ± 1.2	24.7 ± 1.3	NS
10	26.7 ± 0.3	29.0 ± 1.0	25.0 ± 1.0	NS
11	28.0 ± 1.2	29.3 ± 0.9	25.3 ± 1.7	NS
12	26.7 ± 0.9	30.0 ± 0.6	25.0 ± 1.5	NS
13	26.3 ± 0.3	29.7 ± 1.2	24.3 ± 0.9	NS
14	25.7 ± 0.9	30.3 ± 1.7	25.7 ± 1.2	NS
15	27.0 ± 0.0	30.7 ± 1.2	26.0 ± 1.0	NS
16	27.0 ± 1.7	29.7 ± 1.8	26.3 ± 0.9	NS
17	27.0 ± 1.0	30.3 ± 1.5	26.0 ± 1.2	NS
18	27.7 ± 2.0	31.0 ± 1.5	27.7 ± 0.9	NS
19	28.7 ± 1.5	32.0 ± 1.5	27.7 ± 1.2	NS
20	28.7 ± 1.3	31.3 ± 1.9	26.0 ± 0.6	NS

Phase 2	Hb type			
days after infection	HbA	HbAB	HbB	P A:B
21	27.0 ± 1.5	28.7 ± 0.7	26.3 ± 0.3	NS
22	26.3 ± 2.0	28.0 ± 1.0	24.0 ± 0.9	NS
23	27.3 ± 1.9	28.7 ± 0.9	24.0 ± 1.2	NS
24	25.3 ± 1.7	28.3 ± 1.5	25.3 ± 1.2	NS
25	26.3 ± 1.5	27.0 ± 0.0	24.0 ± 1.3	NS
26	26.0 ± 0.6	27.3 ± 0.2	24.0 ± 1.5	NS
27	25.0 ± 2.1	28.7 ± 1.7	24.0 ± 1.2	NS
28	27.3 ± 0.3	29.3 ± 0.7	24.7 ± 1.5	NS
29	27.3 ± 0.7	27.3 ± 0.9	24.3 ± 0.9	NS
49	25.3 ± 1.5	21.7 ± 2.7	20.0 ± 0.6	<0.05
50	25.7 ± 1.2	26.3 ± 2.0	17.7 ± 1.5	<0.01
51	29.3 ± 2.9	29.0 ± 1.5	18.7 ± 1.5	<0.05
52	28.0 ± 3.0	29.7 ± 0.3	18.7 ± 1.5	<0.02
53	27.3 ± 2.7	28.3 ± 1.2	18.0 ± 2.0	<0.02
54	28.0 ± 1.5	29.3 ± 1.2	18.3 ± 2.2	<0.01
55	27.3 ± 2.3	28.3 ± 1.3	17.7 ± 2.8	<0.05
56	26.0 ± 2.6	27.3 ± 0.9	18.3 ± 3.2	<0.05
57	26.7 ± 1.5	28.3 ± 1.2	21.0 ± 3.0	<0.05
58	28.0 ± 1.7	29.3 ± 1.9	20.7 ± 2.4	<0.02
59	27.3 ± 2.4	29.3 ± 2.0	20.0 ± 1.5	<0.02
60	27.0 ± 1.2	28.3 ± 2.9	21.7 ± 1.5	<0.05
61	28.3 ± 2.4	28.0 ± 2.9	22.7 ± 0.9	<0.05
62	29.0 ± 2.3	29.7 ± 1.5	22.0 ± 0.6	<0.02
63	28.0 ± 2.5	28.7 ± 1.9	22.0 ± 1.0	<0.05
64	28.0 ± 1.5	28.0 ± 1.7	23.7 ± 1.7	<0.05
65	28.7 ± 2.6	28.3 ± 1.7	23.3 ± 1.5	<0.05
66	29.0 ± 1.2	27.3 ± 1.9	23.3 ± 1.2	<0.05
67	29.3 ± 1.5	28.0 ± 2.0	24.7 ± 0.7	<0.05
68	29.3 ± 1.2	29.7 ± 1.9	25.7 ± 0.7	<0.02
69	30.3 ± 0.6	30.0 ± 2.1	25.0 ± 1.2	<0.05
70	30.3 ± 0.7	29.7 ± 1.9	24.7 ± 1.7	<0.02
71	29.0 ± 0.6	29.7 ± 1.9	24.3 ± 1.3	<0.05

TABLE O

Total body weights of Merino sheep of different haemoglobin types during the two experiment periods

Hb type	Sheep no.	Days after infection Phase 1			Phase 2				% gain/loss
		0	55	77	0	49	61	71	
AA	4	24.0	25.0	25.0	26.0	27.0	28.0	28.5	18.8
AA	5	22.5	22.5	23.0	24.0	24.5	26.0	25.0	11.1
AA	6	24.0	25.0	26.0	27.0	27.5	29.0	28.0	16.7
									15.5 ± 2.3
AB	3	23.0	26.0	26.0	26.5	27.0	27.5	27.5	19.6
AB	7	24.0	25.5	25.0	26.0	26.0	27.0	27.5	14.6
AB	9	23.0	25.0	25.0	25.0	25.0	25.0	26.0	13.0
									15.7 ± 1.9
BB	1	23.5	24.0	25.0	25.0	26.0	27.0	26.7	12.8
BB	2	23.0	25.0	25.0	26.0	26.0	27.0	26.0	13.0
BB	8	25.0	26.0	26.0	26.0	27.0	27.5	27.0	8.0
									11.3 ± 1.6
									NS

TABLE P

Mean faecal egg counts of two groups of Merino sheep infected with 10L/kg, 50L/kg and 150L/kg H. contortus larvae

10L/kg				50L/kg				150L/kg			
days after infection	HbA	HbB	P	days after infection	HbA	HbB	P	days after infection	HbA	HbB	P
0	0	0		0	0	0		0	0	0	
15	0	0		13	0	0		15	0	0	
16	0	0		14	0	0		19	0	0	
17	0	0		15	0	0		20	0	33 ± 33	NS
18	325 ± 149	100 ± 71	NS	16	0	0		21	67 ± 33	500 ± 208	<.05
19	350 ± 287	200 ± 135	NS	17	0	0		22	300 ± 203	1267 ± 353	<.05
20	275 ± 118	350 ± 166	NS	18	100 ± 71	375 ± 179	NS	23	753 ± 348	2367 ± 328	02
21	300 ± 196	250 ± 144	NS	21	1367 ± 696	1600 ± 612	NS	24	900 ± 462	2667 ± 441	<.02
22	675 ± 333	775 ± 342	NS	25	1367 ± 731	2025 ± 702	NS	25	1000 ± 519	3367 ± 706	<.05
23	300 ± 122	475 ± 144	NS	31	1433 ± 722	2300 ± 612	NS	26	1400 ± 666	3733 ± 674	<.05
24	725 ± 562	450 ± 144	NS	32	1867 ± 906	2800 ± 864	NS	27	2167 ± 1167	5400 ± 2364	<.05
25	650 ± 429	525 ± 189	NS	34	1500 ± 737	4675 ± 1972	<.05	28	2500 ± 700	4066 ± 1882	<.05
26	650 ± 456	425 ± 155	NS	37	2500 ± 1322	3925 ± 246	NS	29	867 ± 176	4533 ± 1567	<.02
30	825 ± 217	2075 ± 617	<.05	40	2833 ± 1433	2550 ± 228	NS	30	1800 ± 874	3367 ± 742	NS
31	525 ± 175	1225 ± 335	<.05	43	900 ± 458	2350 ± 443	<.02	32	1767 ± 809	4600 ± 1677	<.05
32	725 ± 189	1450 ± 601	NS	45	1367 ± 784	2775 ± 705	NS	33	3200 ± 1496	8500 ± 346	<.01
34	625 ± 118	950 ± 126	NS	47	1600 ± 755	2950 ± 656	NS	34	2700 ± 1405	5033 ± 1703	<.05
35	1275 ± 478	1675 ± 511	NS	49	933 ± 418	2625 ± 322	<.01	36	2500 ± 1021	6367 ± 521	<.05
36	1075 ± 397	1900 ± 279	<.05	53	1600 ± 656	2675 ± 709	NS	37	2133 ± 1338	5567 ± 968	<.05
37	1425 ± 439	2175 ± 322	NS	57	1533 ± 689	2675 ± 357	<.05	39	2000 ± 1179	6367 ± 961	<.02
38	1350 ± 405	2225 ± 256	<.05					40	4233 ± 3099	4033 ± 1934	NS
39	1550 ± 333	2300 ± 474	<.05					41	1300 ± 1054	8500 ± 2478	<.05
41	1750 ± 497	2225 ± 487	NS					43	1766 ± 1551	7700 ± 2312	<.05
42	1725 ± 427	2100 ± 442	NS					44	1233 ± 1135	1733 ± 318	NS
43	1300 ± 286	1825 ± 455	NS					46	500 ± 404	3167 ± 617	NS
44	1550 ± 232	2525 ± 403	<.05					47	1166 ± 1167	2100 ± 666	NS
54	1675 ± 243	2325 ± 673	NS					48	1000 ± 611	2467 ± 1039	NS
56	1700 ± 376	2250 ± 444	NS					49	666 ± 666	3267 ± 825	<.05
								50	1100 ± 700	2333 ± 186	NS
								51	300 ± 185	2333 ± 578	<.02
								53	366 ± 185	7600 ± 3539	<.05
								54	500 ± 264	8367 ± 367	<.05
								55	0	8367 ± 3374	<.05
								56	33 ± 33	6067 ± 2515	<.05

TABLE Q

Mean PCV values of two groups of Merino sheep infected with 10L/kg, 50L/kg and 150L/kg H.contortus larvae

10L/kg				50L/kg				150L/kg			
days after infection	HbA	HbB	P	days after infection	HbA	HbB	P	days after infection	HbA	HbB	P
0	31.0 ± 1.7	27.0 ± 2.1	NS	0	31.0 ± 0.5	25.0 ± 0.9	<001	0			
5	30.3 ± 0.1	26.5 ± 2.1	NS	10	31.0 ± 0.6	25.5 ± 0.3	<001	10			
10	29.5 ± 0.6	26.5 ± 2.9	NS	25	31.0 ± 0.6	26.0 ± 0.5	<001	26	30.3 ± 2.1	30.2 ± 2.0	NS
17	29.5 ± 0.6	27.3 ± 3.0	NS	30	30.7 ± 1.0	25.5 ± 0.9	<01	29	30.5 ± 1.5	27.2 ± 2.0	NS
23	30.3 ± 0.6	27.0 ± 2.1	NS	40	31.3 ± 1.5	25.8 ± 0.3	<01	32	27.8 ± 1.5	27.7 ± 2.1	NS
27	31.0 ± 0.7	27.6 ± 0.9	<05	45	31.5 ± 1.8	25.4 ± 1.5	<05	36	30.3 ± 1.6	27.3 ± 1.2	NS
30	31.0 ± 0.7	27.6 ± 0.9	<05	48	32.7 ± 1.4	26.8 ± 1.7	<05	37	29.2 ± 2.4	22.8 ± 0.6	<05
37	31.0 ± 0.5	26.5 ± 0.9	<01	51	31.0 ± 1.5	25.9 ± 0.3	<02	39	29.7 ± 2.4	26.0 ± 0.9	NS
41	31.3 ± 0.9	26.4 ± 0.9	<02	54	32.3 ± 1.3	25.6 ± 0.9	<01	40	28.0 ± 1.5	25.0 ± 1.0	NS
43	31.0 ± 0.5	26.3 ± 1.3	<01	57	32.3 ± 1.8	26.4 ± 1.0	<05	41	30.3 ± 0.8	24.7 ± 1.5	<05
52	31.0 ± 0.6	26.3 ± 1.1	<02					42	27.3 ± 1.5	23.3 ± 1.2	NS
57	31.5 ± 0.9	26.3 ± 2.1	<05					43	27.7 ± 1.4	23.8 ± 1.0	<05
								44	28.3 ± 1.5	23.0 ± 0.6	<05
								45	27.3 ± 2.1	23.7 ± 0.6	NS
								46	27.3 ± 1.1	25.8 ± 0.5	NS
								47	26.8 ± 1.0	23.8 ± 0.9	<05
								48	29.0 ± 1.5	23.7 ± 0.6	<05
								49	28.0 ± 1.6	24.0 ± 0.5	<02
								50	28.0 ± 1.6	24.0 ± 0.5	<05
								51	29.0 ± 1.5	26.2 ± 0.9	NS
								52	29.9 ± 1.5	25.2 ± 0.8	<05
								53	30.7 ± 2.0	26.3 ± 0.5	NS
								54	27.7 ± 1.0	25.6 ± 1.2	NS
								55	26.0 ± 1.5	26.3 ± 1.0	NS
								56	28.0 ± 1.0	25.3 ± 1.3	NS

TABLE R

Total body weights of Merino sheep of different haemoglobin types during two experimental infections with H. contortus i.e. 10L/kg and 50L/kg

10L/kg		Days after infection			
Hb type	Sheep No.	0	27	56	% gain/loss
AA	1	35.0	24.0	35.0	0.0
AA	2	40.0	44.0	43.0	+7.5
AA	3	42.0	44.5	43.0	+2.4
AA	4	44.0	36.5	40.0	-10.0
					-0.3
BB	5	39.5	34	35	-13.0
BB	6	41.0	40	40	-2.5
BB	7	41.0	45	43	+5.0
BB	8	42.0	41	42	0.0
					-2.6

50L/kg		Days after infection			
Hb type	Sheep No.	0	30	45	% gain/loss
AA	1	36.0	36.0	37.0	+3.0
AA	2	40.0	42.0	42.0	+5.0
AA	3	40.0	44.0	42.0	+5.0
AA	4	40.0	41.0	45.0	+13.0
					+6.5
BB	5	40.0	39.5	44.0	+10.0
BB	6	49.0	49.0	46.0	-7.0
BB	7	42.0	45.0	46.0	+10.0
BB	8	37.0	37.0	39.5	+7.0
					+5.0