SOME ASPECTS OF LOCAL IMMUNITY AND PATHOGENESIS IN RODENTS INFECTED WITH NIPPOSTRONGYLUS BRASILIENSIS OR TRICHOSTRONGYLUS COLUBRIFORMIS

by

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DECLARATION

I hereby declare that the work presented in this thesis is original and was conducted solely by the author with the exception of some of the immunological tests in Section I which were carried out in collaboration with Dr. H. Wedrychowicz and the immunological tests in Section II, Chapter 3, which were carried out in collaboration with Mr. M. Abebe and Dr. H. Wedrychowicz.

I also certify that no part of this thesis has been submitted previously by the author for the award of a degree at any university.

CONTENTS

Summary

In Section I of this study rats infected with Nippostrongylus brasiliensis were used as a model for the study of local intestinal antibody responses to gastrointestinal parasitic infections. In Chapter 1 local immune responses to N. brasiliensis were compared in rats which had been immunosuppressed by gamma radiation and in non-irradiated rats. It was demonstrated that in the irradiated rats there was a depletion of Peyer's patches and IgA containing cells in the intestinal lamina propria. There was also a virtual absence of parasite-specific IgA and haemagglutinating antibodies in mucosal extracts in the irradiated hosts. In the non-irradiated infected rats there was a proliferation and hypertrophy of Peyer's patches, a hyperplasia of IgA secreting cells in the intestinal lamina propria and elevated anti-parasite haemagglutinating antibody levels in mucosal extracts and in serum. These changes correlated with the intestinal phase of the infection. The results showed that Peyer's patches responded to the presence of N. brasiliensis in the intestine.

In Chapter 2 changes in immunoglobulin concentrations and in anti-parasite antibodies were measured in rats following primary infection with N. brasiliensis. Coproantibody measurements were compared with concentrations of immunoglobulins and anti-parasite antibodies in corresponding samples of small intestine mucosa and serum. Coproantibodies were also measured in a group of rats following second and third infections with N. brasiliensis. The results showed that following N. brasiliensis infection, immunoglobulin levels increased

in intestinal mucosa, in faecal extracts and in serum. The close correlation between changes in coproantibody levels following infection with N. brasiliensis and other immunological parameters led to the suggestion that coproantibody measurements could provide a useful index of local intestinal immunity. It was also shown that although not the most abundant immunoglobulin in faeces of infected rats, secretory IgA proved to have the highest immunological activity.

In Chapter 3 coproantibody and anti-parasite antibody levels in lung extracts were measured in rats vaccinated with irradiated N. brasiliensis larvae. The results supported the previous findings that coproantibody measurements closely reflect local intestinal anti-parasite antibody responses and also showed that low levels of antigenic stimulation in the intestine can elicit a high degree of protective immunity. It was also demonstrated that anti-parasite antibodies could be detected in the lungs of vaccinated and challenged rats.

In Chapter 4 local and systemic humoral responses were measured in N. brasiliensis infections in normal rats and in rats immuno-suppressed by infection with Trypanosoma brucei. The results indicated that both local and systemic antibody responses to N. brasiliensis were modified as a result of T. brucei infection. In addition the results showed that T. brucei immunosuppression led to a depression of both systemic antibody responses and local IgA and IgG responses in the lungs of N. brasiliensis infected rats. There was evidence that these depressed responses may enhance parasite establishment in the intestine.

Local intestinal antibody responses were measured in groups of rats following vaccination with living intestinal stages of N. brasiliensis and with adult nematode somatic and metabolic antigens and following challenge with L3 larvae (Chapter 5). The results obtained failed to show any correlation between local intestinal responses to the antigens used in the tests and the degree of protection to challenge in the vaccinated rats. However there was a clear positive correlation between anti-ESP haemagglutinating antibody titres in faecal extracts, both before and after challenge, and the degree of protection recorded.

In Chapter 6 the relationship between bronchial antigenic stimulation and local antibody responses to N. brasiliensis in the rat and the possible role of lung and faecal anti-parasite IgA responses to N. brasiliensis antigens was measured using different sites and different levels of antigenic stimulation. The results showed that strong local antibody responses, particularly IgA responses, occurred in the lungs of rats vaccinated with irradiated larvae. However comparisons of lung anti-larval IgA antibodies in rats resistant to or susceptible to challenge failed to demonstrate a direct role for lung IgA antibodies in specific host immunity. Levels of faecal haemagglutinating antibodies against adult nematode excretory-secretory antigens showed a significant positive correlation with the numbers of worms recovered from the intestines following vaccination and also with the degree of resistance to reinfection. Preincubation of adult N. brasiliensis in media containing faecal IgA from primary infected rats had no adverse effect on the ability of these worms to establish and survive in naive rats.

The results obtained in Chapters 2, 3 and 4 indicated that coproantibody levels correlated closely with levels of antibody in both small intestine contents and mucosal extracts. These findings showed that local antibody responses to gastrointestinal parasites can be monitored by coproantibody measurement.

In Section II the suitability of the Mongolian gerbil (Meriones unguiculatus) as a laboratory host for Trichostrongylus colubriformis was investigated. The results of Chapter 1 showed that the pathogenic effects of T. colubriformis in the gerbil were essentially similar to those observed in sheep and thereby suggested that the gerbil is a suitable laboratory host for the sheep nematode T. colubriformis. Furthermore, they showed that, because of the high level of establishment and the extended duration of infection, the gerbil is superior to other small animal hosts previously described.

The pathogenisis of a drug-susceptible and a drug-resistant strain of T. colubriformis was compared in Chapter 2. The results showed that the drug-susceptible strain of T. colubriformis used in the infections was more pathogenic than the drug-resistant strain. These findings were similar to observations previously reported by other workers investigating drug resistance in mixed T. colubriformis and H. contortus infections in sheep.

The results of Chapter 2 also showed that the histopathology of T. colubriformis infections in the gerbil was similar, in many aspects, to T. colubriformis infections in sheep. These findings supported those of Chapter 1 and also indicated that T. colubriformis infections in the gerbil could provide a suitable model for the study of anthelminthic resistance.

In Chapter 3, local and systemic antibodies were detected in gerbils following vaccination with irradiated or non-irradiated T. colubriformis larvae and after challenge with non-irradiated larvae. Coproantibodies were shown to reflect antibody levels in intestinal contents and mucosal extracts. These results further supported one of the main findings of this thesis, i.e. that copro-antibody measurement provides a sensitive index of immunity to intestinal parasites at mucosal surfaces.

GENERAL INTRODUCTION

Local Immunity

Parasitic diseases of domestic animals, particularly those caused by helminth parasites which live on or near intestinal bronchial or hepatic mucosal surfaces result in very substantial economic losses to the livestock industry throughout the world. These losses can be quantified in terms of increased mortality, reduced weight gains, depressed milk and wool production and impaired reproductive performance.

Among the more important intestinal helminth parasites infecting domestic animals are Haemonchus contortus and Ostertagia spp. infections in sheep and cattle, Trichostrongylus colubriformis in sheep and lambs and Trichostrongylus axei in lambs. Dictyocaulus viviparus which causes bovine bronchitis and Fasciola hepatica infections in the bile ducts of ruminants and especially sheep, also result in major economic losses.

It has been observed that many animals acquire a natural immunity to helminth infections and the quest for an understanding of the immune mechanisms in such infections has stimulated many studies (see review by Urquhart, Jarrett and Mulligan, 1962). Much of the early research on helminth diseases was concerned with documenting the various effects on the host resulting from infection, rather than investigating the mechanisms of immunity. However a great deal of effort has also been expended on the search for effective vaccines against helminth diseases. The ultimate aim was to identify antigens capable of stimulating protective immunity at mucosal surfaces and to find the optimum dosage and best route of presentation of such antigens.

Unfortunately to date the search for effective vaccines against helminth infections has been largely unsuccessful. However one notable exception was the developement of a successful X-irradiated vaccine against D. viviparus infections in young cattle (see review bt Urquhart, et al, 1962). The absence of effective vaccines against other helminth diseases has led to increasing reliance on anthelminthic drugs as a method of disease control.

In studies of immunity, in general, serological responses to infections were measured and serum antibody titres were assumed to reflect the immune status of the host. However in some cases it was observed that there was a poor correlation between immunity and circulating antibodies. Burrows, Elliot and Havens (1947) and Burrows and Havens (1948) in investigating the effect of cholera vaccine in pigs made such an observation and also noted that immunity was more closely related to levels of faecal antibody (coproantibody).

In one of the first reports of the detection of local antibody responses to intestinal infections, Davis (1922) showed that specific antibodies to Bacillus dysenteriae groups of organisms could be detected in the stools of patients suffering from dysentry and in similar experimental infections in animals. Except for the work of Burrows, et al, (1947) and Burrows and Havens (1948) who detected coproantibodies in the faeces of animals experimentally infected with cholera, little interest was shown for a number of years in local immune responses at mucosal surfaces.

However during the past two decades there has been a growing interest in local immune responses to intestinal antigens and the subject has now become an important new area of immunological research.

Most of the reported investigations have concerned studies of local immune responses to intestinal and bronchial bacterial and viral infections (see reviews by Bienenstock, 1974; Bazin, 1976; Parrott, 1976; Bienenstock and Befus, 1980; Tomasi, Larson, Challacombe and McNabb,1980).

In some of the early investigations of local immunity to intraluminal antigens, it was observed that a consequence of antigenic stimulation of the intestinal mucosa was a proliferation of immunoglobulin secreting plasma cells in the intestinal lamina propria. It was noted that, in particular, there was a proliferation of immunoglobulin A (IgA) secreting cells. Immunoglobulin A was first isolated and characterised as β 2A - globulin by Heremans, Heremans and Schultze (1959) and shortly afterwards Hanson (1961) found that IgA was the predominant immunoglobulin in milk. The belief that IgA was the most abundant immunoglobulin secreted at mucosal surfaces influenced the direction of much of the subsequent research into local immune responses.

Several workers have investigated local IgA responses following antigenic stimulation of the small intestine. It was shown for example by Crabbe, Nash, Eyssen and Heremans (1968) that the intestinal mucosa of normal mice was rich in IgA secreting cells but in contrast less than one tenth of these numbers of IgA cells were seen in germ-free mice. A corresponding reduction in IgA concentration of serum, intestinal secretions and mucosal tissue was noted in the germ-free mice compared with normal animals. These results led the above workers to suggest a causal relationship between micro-organisms in the lumen of the intestine and the

development of secretory IgA cells in the intestine. Histochemical examination of the small intestines of mice 30 days after oral immunisation with horse ferritin showed that the local secretory immune response was almost totally due to a proliferation of IgA secreting plasma cells (Crabbe, Nash, Bazin, Eyssen and Heremans, 1969). Similar findings were reported by Dolezel and Beinenstock (1971). These latter authors used autoradiographic and immuno-fluorescent techniques to analyse the response in hamsters to orally administered bovine albumin solution. They found that most of the immunoglobulin cells secreting specific anti-bovine antibodies were of the IgA class and that the response was maximal 25 days after the commencement of immunisation. Bazin, Levi and Doria (1970) subjected germ-free mice to exposure and challenge with orally administered sheep red cells. After initial exposure and after challenge the response was expressed mainly in the proliferation of IgA containing cells in the spleen and mesenteric lymph nodes although 25 days after the first immunisation and after restimulation there was also evidence of some increase in immunoglobulin G (IgG) and immunoglobulin M (IgM) cells in these tissues. Other workers have reported similar responses to orally administered sheep red cells. Robertson and Cooper (1972) found that in rats, the major response to intraduodenal injections of sheep red cells was the appearance of antibody producing cells in the mesenteric lymph nodes and in the small intestine lamina propria and submucosa.

It was shown by Crabbe, Nash, Bazin, Eyssen and Heremans (1970) that plasma cells are not present in the intestines of mice at birth, but that they appear at about 10 days of age presumably as a response to antigenic stimulation.

The question of primary stimulation and migration of plasma cell precursors became the subject of much speculation. Some workers put forward the view that lymphocytes already in situ in the intestinal lamina propria were sensitised by contact with antigen in the intestinal lumen and consequently began to secrete immunoglobulins. However the work of Ferguson and Parrott (1972) did not support this view. These workers implanted germ-free isografts of mouse foetal small intestine under the kidney capsules of adult mice and showed that despite the lack of intraluminal antigenic stimulation, these grafts were populated with intraepithelial lymphocytes and plasma cells. Moreover it was subsequently demonstrated that plasma cells in similar germ-free isografts secreted and stored IgA in the graft lumen (Ferguson, 1974). These results indicated that immunoglobulin secreting effector cells must migrate to their secreting site via the blood and must be sensitised at some other location.

The possibility that Peyer's patches may provide a vehicle for the antigenic stimulation of lymphocytes has been investigated by several workers. Peyer's patches are small nodular aggregates of lymphoid cells found along the sub-mucosa of the small intestine. Although Peyer's patches do not contain antibody forming cells (Bienenstock and Dolezel, 1971; Muller-Schoop and Good, 1975) they do appear to play a significant role in the local immunological system.

The nodular areas of Peyer's patches contain germinal centres and are composed mostly of B-lymphocytes and the internodular areas contain T-cells. T-lymphocytes are also present in the dome areas and near the mucosal layer. The proportion of T and B cells in these tissues varies with age.

Pollard and Sharon (1970) observed that Peyer's patches in germ-free mice were relatively inactive but responded to direct antigenic stimuli with Salmonella paratyphi and meningitis virus. In contrast, when the antigens were administered parenterally there was no Peyer's patch response. Craig and Cerba (1971) transferred Peyer's patch, peripheral blood and popliteal lymph node cells into allogenic rabbits which had been lethally irradiated to produce immunosuppression. The proliferative and differentiative potential of the transferred donor cells were assessed by quantifying the IgA producing cells found in the gut of the recipients. It was shown that Peyer's patches were a highly enriched source of lymphocytes which could proliferate and differentiate into IgA secreting cells. Peyer's patch cells were significantly more efficient at 'seeding' the gut of irradiated recipient rabbits with IgA cells than lymphocytes from peripheral blood or lymph nodes. This work gave further support for the hypothesis that Peyer's patches were an important site of lymphocyte sensitisation. Evidence that Peyer's patches might act as 'samplers' of antigenic material in the lumen of the intestine was presented by Bockman and Cooper (1973). These workers demonstrated the absorption, by pinocytosis, of particles of ferritin from the surface of Peyer's patch epithelium.

It was shown that there is no afferent drainage into Peyer's patches but there is an efferent drainage system into the mesenteric lympn nodes from Peyer's patches (see reviews by Bienenstock, 1974; Parrott, 1976 and Tomasi, et al, 1980). The dome area of Peyer's

patches is covered by cuboidal epithelial cells but goblet cells have not been identified. Specialised epithelial cells which occur in areas of epithelium overlying lymphoid follicles in human Peyer's patches were described by Owen and Jones (1974). These specialised cells are characterised by luminal surface microfolds rather than microvilli and were called M cells. The M cell processes form a latticework which allows lymphoid cells to approach within 3 μm of the intestinal lumen. These specialised epithelial or M cells contain multiple cytoplasmic vacuoles which suggest that the cells could have a transport function, i.e. the transfer of antigenic material from the lumen across the Peyer's patch epithelium. A cell similar to Peyer's patch M cells was found in the epithelium of bronchus associated lymphoid tissue (Bienenstock and Johnston, 1976). It was subsequently shown (Owen, 1977) that Peyer's patch M cells transport potentially antigenic material to lymphocytes below the epithelium.

The importance of Peyer's patch cells in local immunity was further investigated by Befus, O'Neill and Bienenstock (1978). Lymphocytes from Peyer's patches, lamina propria and popliteal lymph nodes were given intravenously to irradiated allogenic rabbits. A group of control rabbits were irradiated but not given cells. Between 4 and 6 days after transfer of lymphocytes from Peyer's patches or lamina propria there was a proliferation of IgA containing cells in the spleen, mesenteric lymph nodes and intestinal lamina propria of the irradiated recipients. Transferred Peyer's patch cells produced a greater response than cells transferred from the lamina

propria. The control rabbits, irradiated only, showed no signs of repopulation with IgA cells six days after irradiation, nor did the recipients which had been given popliteal lymph node lymphocytes. This latter fact showed that transfer of allogenic popliteal lymph node cells alone did not lead to IgA cell repopulation. These results indicated that Peyer's patches, and to a lesser extent intestinal lamina propria, are rich sources of precursors of immunoglobulin secreting plasma cells in the gut.

The findings of the work reviewed above strongly suggest that the following sequence of events leads to the appearance of IgA secreting cells in the small intestine. Intraluminal antigens enter Peyer's patches via the dome M cells and stimulate both B and T lymphocytes. Peyer's patches are on the route of recirculating lymphocytes (Gowans and Knight, 1963) and sensitised lymphocytes leave the patches by the efferent lymphatic system, migrate via the mesenteric lymph nodes and the thoracic duct to the bloodstream. By some mechanism which has not been elucidated the sensitised B lymphocytes, the precursors of the IgA secreting plasma cells, then migrate to the lamina propria of the small intestine and begin to secrete IgA.

Immunoglobulin A secreted at mucosal surfaces is known as secretory IgA or S-IgA and this has been shown to be different in several respects from circulating IgA. Circulating immunoglobulin A is monomeric and has a molecular weight of 160,000. S-IgA is dimeric and is synthesised locally by plasma cells. It is dimerised intracellularly with a cysteine-rich polypeptide J-chain.

The dimeric IgA binds to a substance called the secretory component (SC), present on the surface of the cell in which the dimer was produced. S-IgA has a molecular weight of about 390,000.

In addition to IgA secreting cells, plasma cells in the intestinal lamina propria and in other mucosal sites secrete immunoglobulins G, M and E. Immunoglobulin G found in mucosal secretions appears in the monomeric state and has a molecular weight of 150,000. This immunoglobulin is known to be the most abundant of the immunoglobulins present in internal secretions. Immunoglobulin M is found as a polymer of five peptide subunits and has a molecular weight of 900,000. Immunoglobulin M also contains a J chain and is thought to combine with SC in a manner similar to that in the formation of S-IgA. Immunoglobulin E, the major reaginic antibody, is also known to be secreted at mucosal surfaces (see review by Bienenstock and Befus, 1980). This immunoglobulin does not share the same transport system as IgG and IgM and almost nothing is known about the migration of the precursor cells of IgE secreting plasma cells. The study of IgE responses to parasitic infections is outwith the scope of this thesis.

The translocation of immunoglobulins across the intestinal epithelium in humans has been studied by many workers. It is now accepted that IgA and IgM are synthesised in submucosal plasma cells and then secreted across the epithelium, while IgG is produced by transudation from serum. IgA is synthesised and dimerised

intracellularly with a polypeptide J-chain. The IgA dimer is then transported across columnar epithelial cells containing the secretory component, a process which involves the formation of cytoplasmic vesicles (Brown, Isobe and Nakare, 1976). The dimeric IgA combines with the SC during the transcellular transport and is secreted as S-IgA into the lumen of the intestine. These workers showed that the polymer IgM was produced and transported across columnar epithelial cells in combination with SC in the same way as that observed for IgA. The absence of IgG from SC-containing epithelial cells suggested that other mechanisms must operate in the transport of IgG across mucosal surfaces. These findings confirmed previous reports (Brandtzaeg, 1975) which suggested that, in contrast to IgG, IgA and IgM were true secretory immunoglobulins. The function of the SC has been the subject of some speculation. Some workers have claimed that it is a 'transport piece' although there is no conclusive evidence for this. However the findings of Brown et al, 1976, tend to suggest a transport function for the SC. Combination with SC is thought to stablise S-IgA against proteolysis and this may be an important function (Johnson, 1970; Shuster, 1971).

The concept of a common mucosal immunological system, i.e. a system of lymphoid tissue common to intestinal, bronchial and mammary lamina propria has been postulated (see review by Bienenstock, 1974). This system is now generally referred to as the mucosal associated lymphoid tissue (MALT). Several workers have subsequently

shown that antigenic stimulation of intestinal mucosal surfaces can lead to the appearance of immunoglobulin containing cells and/or the production of secretory antibody not only at intestinal mucosal surfaces but at mucosal surfaces in the lung and in the mammary gland. The appearance of secretory IgA in the milk of humans infected with Salmonella typhimurium was demonstrated by Allardyce, Shearman, McLelland, Marwick, Simpson and Laidlaw (1974). Similarly IgA producing cells were detected in the milk of women orally immunised with Escherichia coli (Goldblaum,Ahlstedt, Carlsson, Hansen, Jodal, Lindin-Janson and Sohl-Akerlund, 1975). Other workers demonstrated that the ingestion of Streptococus mutans vaccine led to the production of specific IgA antibodies in the saliva of rats (Michalek, McGhee, Mestecky, Arnold and Bozzo, 1976) and of humans (Mestecky, McGhee, Arnold, Michalek, Prince and Babb, 1978). It was also shown that bronchial or gastric immunisation of rabbits with Pneumococcus antigen produced IgA responses in milk, saliva, bronchial fluid and intestinal fluid (Montgomery, Connelly, Cohn and Skandera, 1978).

Further evidence of a common MALT system was given by Rudzik, Clancy, Perey, Day and Bienenstock (1975). These authors subjected rabbits to 1krad X-irradiation to denude the gut and bronchial lamina propria of plasma cells. Lymphocytes from allogenic rabbits, collected from either bronchus-associated lymphoid tissue or Peyer's patches were then given intravenously to the irradiated rabbits. Six days later both intestinal and bronchial lamina propria were found to be repopulated with IgA containing cells. In contrast, rabbits which had been subjected to the same radiation

dose and had not received cells showed no evidence of plasma cell repopulation at mucosal surfaces. The above results suggested that the repopulation of both intestinal and lung lamina propria was via an organised common lymphoid tissue system.

McDermott and Bienenstock (1979) injected 3H thymidine labelled mesenteric lymph node cells into syngenic female mice. Twenty four hours later 3H labelled cells were observed in the gut, reproductive tract, mammary gland and mesenteric lymph nodes (MLN) of the recipient mice. The predominent immunoglobulin content of these cells was IgA. In peripheral lymph nodes most of the transferred MLN cells were expressing IgG and only 8% were of the IgA isotype. These workers also labelled peripheral lymph node cells and bronchial lymph node cells with 3H thymidine and injected the labelled cells into recipient mice. Peripheral lymph node cells tended to return to their site of origin and to be equally divided between IgA and IgG containing cells. The labelled bronchial lymph node cells tended to localise in the lungs. These findings provided further support for the concept of a common mucosal immunological system.

As a result of the many studies carried out on local immune responses at mucosal surfaces to both viral and bacterial infections a greater understanding of immune responses to these organisms has been achieved. However, although a number of workers have examined local immune responses to gastrointestinal parasites, the subject has not been widely studied.

One of the first reported studies on local immune responses to gastrointestinal helminths was that of Douvres (1962), who

investigated local immunity in Oesophagostomum radiatum infections in cattle. It was shown that antibodies were present in intestinal mucosal samples taken from infected cattle. Furthermore it was shown in an in vitro test that developing larvae were coated with antibodies. Studies on Oesophagostomum columbianum in sheep showed a proliferation of lymphoid tissue in the gut of infected animals and also demonstrated lower serum antibody titres than antibody titres within the gut mucosa at the same time of infection (Dobson, 1967). This phenomenon was explained by the possible leakage of antibody away from the gut, although the conclusion was drawn that the responses of sheep to this parasite were largely local and centred on the alimentary tract where there was cellular proliferation and local production of antibody.

Serum and small intestine contents of mice infected with Heligmosomoides polygyrus were examined for levels of IgA, IgG and IgM and also for antibody levels after initial and challenge infections by Crandall, Crandall and Franco (1974). The main immunoglobulin response detected in serum and in intestinal contents after first and subsequent infections was an increase in IgG levels. This corresponded with a proliferation of IgG containing cells in the submucosa of infected animals. The main antibody response in serum was in IgG after both initial and challenge infections although antibody responses were detected in other immunoglobulin classes in serum. In immunised mice IgG levels were high in intestinal contents and only IgG antibody was detected. However in later studies of Heligmosomoides polygyrus infections in mice,

Cypess, Ebersole and Molinari (1977) found high levels of IgA and IgG containing cells in the lamina propria of intestinal mucosa of infected animals. Radial immuno-diffusion of intestinal perfusates showed that there was a marked increase of anti-parasite IgA and IgG after infection. The former workers, Crandall et al (1974) speculated that their failure to detect a significant stimulation of secretory IgA response to infection could possibly be due to the procedures used.

An important contribution to our knowledge of local immune responses to helminth infections was made by Musoke, Williams, Leid and Williams (1975). These workers were the first to show a protective role for IgA in helminth infections. The numbers of metacestodes developing in the liver of rats was significantly reduced by concurrent inoculation of freshly hatched Taenia taeniaeformis oncospheres and colostral IgA antibody into isolated gut loops. Further evidence of a protective role for secretory IgA in T. taeniaeformis infections was reported by Lloyd and Soulsby (1978). These workers showed that recipient mice could be protected against Taenia taeniaeformis if they were fed IgA isolated from colostrum or IgG isolated from serum of donor mice which had previously been infected orally with T. taeniaeformis. In addition they demonstrated a role for IgA locally secreted in the intestine. Thus passive immunity was transferred to 4 week old mice by the intraduodenal injection of intestinal IgA from donor mice. In contrast intestinal IgG from the same immune donors had no protective effect. The ability of the intestinal IgA to confer protection against infection was largely eliminated by absorption with hatched, activated oncospheres of T. taeniaeformis.

The first report of Haemonchus contortus specific mucosal IgA was recorded by Smith (1977) who demonstrated the presence of antilarval IgA and IgG in the abomasal mucus of infected sheep. Examination of the ratio of mucus IgG : serum IgG led to the suggestion that while the IgG was serum derived, the IgA was locally produced. In 1978 Smith and Christie showed that if sheep which had been vaccinated with irradiated H. contortus larvae were challenged, there were greatly elevated mucosal IgA and IgG responses compared with unvaccinated controls. These workers suggested that intestinal immunoglobulin secretions played a part in conferring resistance to H. contortus. Serum and abomasal mucosa IgA and IgG were also measured in young lambs and in sheep which had been vaccinated with irradiated Haemonchus contortus larvae prior to challenge with normal larvae (Duncan,Smith and Dargie, 1978). In the young lambs vaccination did not give protection and there was no elevation of serum or mucosal antibody levels. In the adult animals where vaccination did induce protection there was a rise in serum and mucosal IgA and IgG concentrations. The increase in mucosal IgA was more marked than the corresponding rise in serum IgA. These results added further support to the view that local immune response may be important in host responses to gastrointestinal parasites.

Other evidence of local immune responses to intestinal parasites was produced by Cripps and Rothwell (1978) who found that when sheep resistant to Trichostrongylus colubriformis were challenged there was a significant increase in intestinal IgA.

Elevated IgA levels in the caecal contents of birds harbouring Eimeria tenella infections were observed by Davis, Parry and Porter (1978). In these infections the lack of any correlation between protection and circulating antibody led to the suggestion that intestinal secretory IgA might be associated with protection. The presence of an active local secretory immune response to the stomach worm Hyostrongylus rubidus in pigs was demonstrated by Smith, Herbert and Davis (1979). Immunofluorescent studies showed that following a single dose of 1×10^5 larvae there was an increase in both IgA and IgM immunocytes.

Evidence of a local intestinal immunoglobulin response to Nippostrongylus brasiliensis infection was first reported by Poulain, Luffau and Pery (1976a). These workers observed a marked increase in IgA levels in intestinal fluid of infected animals coinciding with the presence of adult worms in the gut. Further evidence of a strong local antibody response to N. brasiliensis in the rat was demonstrated by Sinski and Holmes (1977) who used an adaptation of a technique described by Movsesijan and Lalić (1971) to measure local and circulating IgA and IgG responses following infection. The results showed that infection provoked a more pronounced local IgA response than that observed in serum. Serum IgG values were found to be higher than IgG levels detected in small intestine mucosal extracts. Further evidence that local specific IgA antibodies are closely associated with immunity to N. brasiliensis was produced by Sinski and Holmes (1978) who demonstrated an almost 10 fold increase in mucosal IgA in the 12 days following infection. Serum IgG levels rose more slowly and were not so closely associated with worm

expulsion. Haemagglutinin titres in both mucosal extracts and in serum reflected the elevated levels of IgA and IgG respectively and a close temporal relationship was shown to exist between the elevated local anti-worm antibodies and the presence of parasites in the intestine.

None of the above workers produced any conclusive evidence to show how locally produced immunoglobulins might be implicated in immunity to parasites and it appears that this is an area of helminth immunity which requires further investigation.

So far, estimations of local antibody responses to helminth infections have been limited to post-mortem preparations of sections of gut, of mucosal extracts or of bile. Such techniques have obvious disadvantages, especially in studies using domestic animals where their high cost frequently limits the numbers of animals available for experiments. An additional serious disadvantage of measuring local antibody responses in post-mortem preparations is that sequential monitoring of immune responses in the same individual animal is not possible. In many instances it is desirable to examine changes in immune responses following challenge infection or drug treatment. Results obtained from investigations into the immune response to bacterial and viral infections of the gastrointestinal tract suggest that an estimation of local immune response may be made during the life of infected animals by examination of faecal extracts.

Biologically active antibodies have been detected in faeces of humans and animals suffering from a number of viral and bacterial infections. As early as 1922, Davis showed the presence of anti-<u>Shigella</u> agglutinins in stools from patients suffering from bacillary

dysentery. These antibodies were detected before the appearance of circulating antibodies in the serum. Burrows and Havens (1948) demonstrated high titres of specific anti-cholera antibodies in the faeces of guinea pigs experimentally infected with Vibrio cholerae and speculated that this faecal or coproantibody was formed locally. These workers also noted that the faecal antibody titres reached a high level prior to the development of any appreciable serum titre. The presence of antibodies to Vibrio cholerae in the faeces of infected guinea pigs was also demonstrated by Burrows and Ware (1953). High levels of coproantibody associated with developing immunity to cholera in humans were observed by Freter, Mondal, Shrivastava and Sunderman (1965). The latter workers also observed that there was little elevation in serum antibody at the same time of infection and they concluded that serum antibodies played little or no part in the developing immunity but concluded that coproantibodies were involved in immunity. In studies on V. cholerae infections in mice Fubara and Freter (1972) found high coproantibody titres in vaccinated mice and concluded that these antibodies were S-IgA. The hypothesis was offered that these antibodies could be the result of local synthesis or could be serum derived.

A number of workers have demonstrated coproantibodies and intestinal antibodies to poliovirus (Lipton and Steigman, 1963; Kawakami, Tatsumi, Tatsumi and Kono, 1966; Kono, Ikawa, Yaoi, Hamada, Ashihara and Kawakami, 1966; Berger, Ainbender, Hodes, Zepp and Hevizy, 1967; Keller and Dwyer, 1968; Orga and Karzon, 1969). The predominant immunoglobulin identified in these studies was S-IgA.

Several additional reports of coproantibody detection have been documented. Hepatitis-associated antigen-antibody complexes were detected in faeces of patients suffering from hepatitis (Orga, 1973). Antibodies to Newcastle disease virus were found in faeces of infected chickens by Kono, Akao, Sasagawa and Nomura (1969). The latter authors made the suggestion that the immunity was probably B cell dependent and that the antibody was IgA produced locally in the lamina propria.

Haneberg and Tonder (1973) found IgA present, in variable quantities, in faeces of a number of healthy children. Although IgG and IgM were detected in some samples, IgA was the predominant faecal immunoglobulin. These workers suggested that IgG and IgM were degraded by enzymes in the gut whereas IgA was resistant to such action.

Further evidence that faecal samples can be used to monitor local immune responses by measuring the immunoglobulins present in the faeces was reported by Haneberg and Aarsog (1975). Infants recovering from infectious enteritis showed elevated faecal IgA and agglutinating antibody levels.

In studies on coproantibody production in swine dysentery, Jenkins and Roberts (1980) detected IgA, IgG and IgM and as in studies of other infections IgA predominated. Again in common with several unrelated studies peak coproantibody levels were detected prior to the production of serum antibody. These workers suggested that coproantibody measurements might provide a useful diagnostic tool.

However it appears that there are no reports of coproantibody measurements in man or animals infected with gastrointestinal helminths.

A major part of this study is devoted to coproantibody measurements in animals harbouring such parasites.

Section I of this study describes a series of experiments which examine local immune responses in rats infected with Nippostrongylus brasiliensis.

In Section II, the suitability of the Mongolian gerbil, Meriones ungiculatus, as a laboratory small animal host for the study of Trichostrongylus colubriformis infection is assessed. Following this investigation, local immune responses to T. colubriformis in the gerbil are examined.

GENERAL MATERIALS AND METHODS

A. Experimental Animals

(i) Rats

Female Hooded Lister rats (Olac 1976 Ltd., Bicester, England) about 8 weeks old and weighing approximately 150g were used in the experiments described in section I.

The rats were housed in polypropylene cages with wire mesh floors suspended above sawdust trays and were fed a commercial pelleted rodent diet (No. 4 Angus Milling Co., Perth, Scotland). Water was available ad libitum.

(ii) Gerbils

Gerbils (Meriones unguiculatus) approximately 8 weeks old and of both sexes obtained from Intersimian Ltd., Abingdon, England, were used in the experiments described in section II.

The animals were housed in single sex groups in polypropylene cages with wire mesh floors and fed the commercial ration already described.

B. Parasitological Techniques

Nippostrongylus brasiliensis in the Rat

The parasite used in these experiments was maintained for 20 years in the Veterinary Physiology Department, University of Glasgow. Female Hooded Lister rats were used for repeated passage of the parasite.

(i) Preparation of Infective Larvae

The culture of N. brasiliensis larvae was as described by Jennings, Mulligan and Urquhart (1963).

Faeces were collected from rats with a patent infection, usually between 7 and 9 days after larval inoculation. The faecal pellets were ground to a stiff paste with a little warm water using a pestle and mortar and a portion of the paste was spread on a circle of filter paper 7 cm in diameter (Whatman No. 1, B.D.H., Ltd., England). The faecal smear was approximately 3 cm in diameter and care was taken to leave the periphery of the paper clear. The paper was then dipped in water and placed on a pad of synthetic sponge, which had previously been soaked in water, in a plastic petri dish (Figure 1). After replacing the lids, the dishes were stored in a humid incubator at 27°C.

After 5 days the larvae could be seen collecting in a fringe on the periphery of the filter paper. The larvae were always harvested after an incubation period of between 5 and 10 days and were used to infect rats on the same day as harvesting. Harvesting was accomplished by flooding the petri dish with water at 37°C, thus allowing the larvae to swim off into the water. The warm water containing the larvae was collected and filtered under suction in a large Buchner funnel using Green's No. 904 filter paper (J. Green, Maidstone,England), 18.5 cm in diameter. This paper was then inverted and placed on an Endecott sieve (mesh 400) in a Baermann apparatus filled with water at 37°C. The larvae swam down the sieve while the faecal debris was retained. The larvae collected at the bottom of the funnel and were run off into a suitable container.

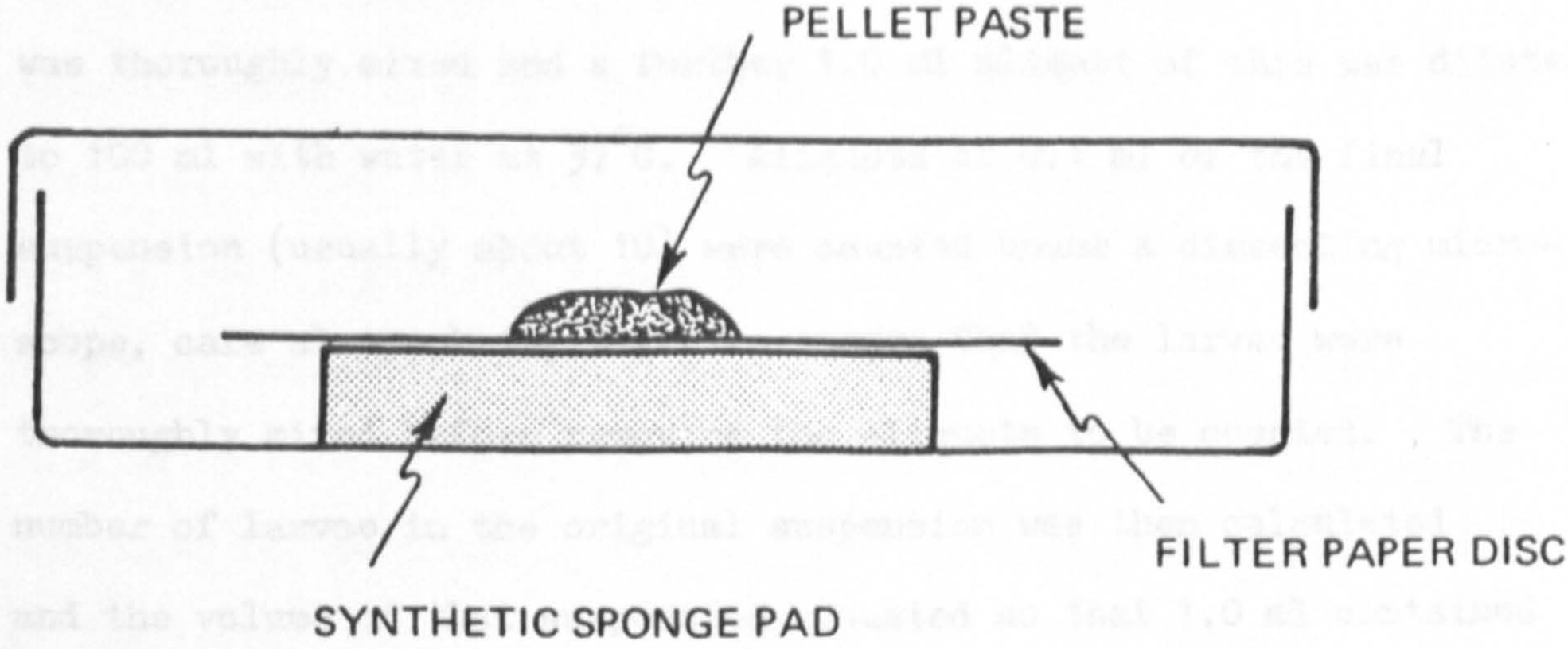

Fig. 1.

(ii) Counting of Larvae

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

(iii) Infection of Rats

The rats, while lightly sedated with trichloroethylene (Trilene, I.C.I., Ltd., England) were injected subcutaneously in the groin using a 1.0 ml syringe. The level of infection in most of the experiments was approximately 5,000 N. brasiliensis larvae per rat.

(iv) Faecal Egg Counts

Faeces was collected on a sheet of absorbent paper which covered the area under the grid floor of the cages. A 1.0 g random sample of faeces was gathered from a 24 hour collection of faeces. This sample was homogenised in 10.0 ml of water, then passed through a nylon tea strainer. After centrifuging at 350 g the residue was suspended in 15 ml of saturated NaCl solution. Both chambers of a McMaster slide were filled with the suspension and the eggs counted. Each chamber has a volume of 0.15 ml. The mean number of eggs in each chamber x 10 was equivalent to the number of eggs per gramme of faeces.

(v) Recovery of Adult N. brasiliensis

All rats were killed by Trilene anaesthesia followed by cervical dislocation. The skin and abdominal wall were slit along the mid-ventral line and the entire small intestine removed. The first two thirds was slit longitudinally with blunt scissors, cut into 4 inch lengths and placed in a nylon tea strainer suspended in a 250 ml beaker filled with saline at 37°C. This was then placed in a water bath at 37°C for 1 hour, by which time more than 98% of the worms had swum out of the strainer and collected at the bottom of the beaker.

(vi) Counting of Adult N. brasiliensis

Worms collected from each rat, as described above, were transferred to a petri dish and the total number or appropriate aliquot counted under a dissecting microscope. In general freshly collected worms were counted but if this was not possible they were preserved in formol saline till required.

Trichostrongylus colubriformis in Gerbils

(i) Infective Larvae

The strains of T. colubriformis used in the present study were maintained by regular passage through parasite-free lambs and infective larvae were kindly supplied by Dr. Lewis of the Pfizer Central Research Laboratories, Kent, or by Dr. Coop of the Moredun Institute, Edinburgh and stored in the laboratory at 4°C till required.

(ii) Counting of Larvae

Counts were carried out as described in B (ii) above. The larvae were examined microscopically to ensure that they were normal and active before being used to infect gerbils.

(iii) Infection of Gerbils

The gerbils were lightly sedated with trichloroethylene and dosed *per os.* with the required number of infective *T. colubriformis* larvae by means of a curved 8 cm long 14 gauge ball-ended dosing needle attached to a syringe. The larval suspension was well mixed before each administration.

(iv) *T. colubriformis* Faecal Egg Counts

These were carried out as in B(v) above.

(v) Recovery of Adult *T. colubriformis*

The gerbils were killed by deep Trilene anaesthesia followed by cervical dislocation. Immediately after death the small intestine was removed and opened longitudinally, the contents were placed in a nylon tea strainer which was suspended for 4 hours in 250 ml of physiological saline at 37°C. The worms disengaged from the intestinal mucosa and settled at the bottom of the beaker. The worms were washed three times in ice cold saline prior to being counted under a dissecting microscope.

(vi) Counting of Adult *T. colubriformis*

Adult parasites collected as described above were transferred to a petri-dish and the total number (or appropriate aliquots) counted under a dissecting microscope or preserved in formol saline until counted.

C. Sampling Methods and Extract Preparation

(i) Phosphate Buffered Saline Solution (PBS)

A stock solution was prepared by dissolving 67.40 g anhydrous Na_2HPO_4, 3.90 g NaH_2PO_4 and 21.25 g NaCl to give 5 l of solution in distilled water. This was diluted 6:4 with distilled water to give isotonic phosphate buffered saline (PBS) pH 7.4.

(ii) Preparation of Small Intestine Mucosal Extracts

Small intestine mucosal samples were collected post-mortem by placing the open small intestine on a clean glass plate and scraping the mucosa with a scalpel blade. The mucosal sample was then weighed and homogenised in an ice bath using an Ultra Turax (r)-type T P /8/10 disrupter with 0.2 M acetate buffer pH 5.5 (6.8 ml 0.2 M acetic acid + 43.2 ml 0.2M sodium acetate containing 0.01% soy bean trypsin inhibitor and 0.01% sodium azide). Five ml of buffer was added for each 1 g of mucosal scrapings.

After centrifuging for 15 minutes at 4,000 g the supernatant was removed and clarified by ultra centrifugation at 30,000 g for 1 hour. The clear supernatant was then dialysed for 24 hours against PBS and the protein content was measured. The samples were used immediately in immunological tests or stored at $-20^{o}C$.

Where worm burdens and intestinal mucosal extracts were required at necropsy, the small intestine was halved longitudinally. One half was used to prepare mucosal extract and the worm numbers in the other half were ascertained as previously described. Worm burdens were obtained by doubling the number in half of the intestine and adding any worms recovered from contents solid material (see iii below).

(iii) Preparation of Globulins from Rat and Gerbil Small Intestine Contents

At necropsy the small intestine was removed and the contents were flushed out with 20 ml cold PBS pH 6.0 containing 0.01% soybean trypsin inhibitor. After mixing and centrifuging (3,000 g) for 15 minutes at 4°C the supernatant was removed and inactivated at 56°C for 30 minutes.

The solid material remaining was placed in a nylon tea strainer suspended in physiological saline at 37°C. Adult parasites trapped in this solid material swam out of the strainer and collected at the bottom of the beaker. These worms were counted and added to the numbers recovered from segments of small intestine (vide supra).

The pH was then adjusted to 7.0 with 0.1 N NaOH and the globulin fractions were precipitated by adding saturated ammonium sulphate solution in the ratio of 2 ml saturated $(NH_4)_2SO_4$ to 3 ml supernatant. After mixing and allowing to stand for 30 minutes at room temperature, the precipitated globulins were removed by centrifugation for 30 minutes at 4,000 g. The supernatant was discarded and the globulins were dissolved in approximately 2 ml of PBS and dialysed against PBS for 24 hours at 4°C. Total globulin concentration was estimated and immunological analysis carried out immediately or after storage at -20°C.

(iv) Preparation of Rat and Gerbil Large Intestine Globulins

The contents of the large intestine were washed out with 20 ml cold PBS in a similar manner to that described above and processed in the same way as the small intestine globulins.

(v) Preparation of Rat and Gerbil Faecal Globulins

Faeces excreted during each collection period (16 hour or 24 hour periods) from groups of infected or non-infected animals were collected on absorbent paper placed beneath the grid floor. From each collection a 10 g aliquot was taken and cleaned of any food dust contamination by briefly washing on a sieve under running cold water. The faeces were then placed in PBS pH 7.4 containing 0.02% sodium azide in the ratio of 1 g faeces to 3 ml of PBS, homogenised and extracted for 24 hours at 4°C. Insoluble residues were removed by repeated centrifugation (30 minutes at 6,000 g). The globulin fraction was separated by precipitation with saturated ammonium sulphate pH 7 at room temperature. Protein precipitates were isolated by centrifugation, redissolved in 0.1 M PBS and dialysed overnight against 0.1 M PBS pH 7.4 which contained 0.02% sodium azide. After dialysis, the protein concentration was estimated and immunological analysis was carried out immediately or after preservation at -20°C.

(vi) Preparation of Lung Extracts in Normal and Infected Rats

Lungs removed at necropsy were rinsed in cold PBS to remove superficial blood, blotted dry with filter paper and weighed. They were then homogenised and extracted with cold PBS in the ratio of 3 ml PBS to 1 g tissue. After ultracentrifugation (30,000 g) for 1 hour at 4°C the globulin fractions were precipitated from the supernatant with saturated ammonium sulphate solution as previously described in the preparation of small intestine globulins. The globulins were redissolved in 0.1 M PBS and after dialysing for 24

hours against 0.1 M PBS the protein concentrations were measured. Immunological analysis was carried out either immediately or after storage at -20°C.

(vii) Serum Collection

Blood for serum preparation was collected from infected and normal animals under deep anaesthesia prior to death by cardiac puncture with a 10 ml syringe using a 20 gauge needle. The blood was transferred to a centrifuge tube and allowed to clot before the serum was removed by centrifugation.

D. Preparation of Parasite Antigens

Antigens of both N. brasiliensis from infections in rats and T. colubriformis from infections in sheep and gerbils were prepared as described below. T. colubriformis larvae harvested from sheep faeces were used to prepare larval somatic extracts (L3SE) and adult worms recovered from infected gerbils were used to prepare adult somatic extracts (ASE).

(i) Infective Larval Somatic Extracts (L3SE)

Infective (L3) N. brasiliensis or T. colubriformis larvae were collected and carefully washed in 0.01 M PBS containing 0.02% sodium azide to inhibit bacterial growth. The larvae were given 10 washes using the ratio of 50 ml PBS to 1 ml of sedimented larval suspension. The larvae were then homogenised in a glass tissue grinder surrounded by ice. The homogenate was cleared by ultra centrifugation for 1 hour at 30,000 g, filtered through a bacterial filter (Millex - HA 0.22μm, Millipore Corporation). Finally the protein concentration was ascertained.

(ii) Adult Worm Somatic Extract (ASE)

These extracts were prepared from adult N. brasiliensis of T. colubriformis parasites essentially as described above for L3SE antigens. For N. brasiliensis ASE, worms were collected 9 days after a first infection.

(iii) Adult Worm Excretions and Secretions (ES Products)

Freshly isolated adult parasites (for N. brasiliensis antigen worms from day 10 - 12 after first infections were used) were carefully washed 10 times with 0.1 M PBS containing 0.02% sodium azide. After washing the worms were counted and placed in sterile petri dishes in the proportion of 5,000 worms to 15 ml 0.1 M PBS (pH 7.4 containing 300 I.U./ml penicillin and 100 μg/ml streptomycin) and incubated at 37°C for 24 hours.

If at least 95% of the worms were still alive they were removed by centrifugation for 5 minutes at 1,000 g and the supernatant filtered through a bacterial filter (Millex - HA 0.22 μm). Finally the protein concentration was ascertained. If worm viability was less than 95% after incubation the incubate was discarded.

(iv) Excretory-Secretory Products (ESP)

Antigen preparations were stored at -20°C until required.

E. Biochemical Analyses

(i) Protein Concentration of Extracts and Globulin Preparations

These estimations were carried out by the Folin Method (Lowry, Rosebrough, Farr and Randall, 1951) as modified by Ayre and Goldberg (1966).

(ii) Serum Total Protoin Estimations

These are estimated by continuous flow analysis (Standard Technicon Auto-Analyser II), based on the Modified Biuret Method (Weichselbaum, 1946).

F. Immunological Techniques

(i) Immunoglobulin Estimations

Immunological quantification of IgA, IgG and IgM in serum, intestinal and faecal samples was carried out by the single radial immunodiffusion technique described by Mancini, Carbonara and Heremans (1965). In this method antisera to the immunoglobulins to be assayed were incorporated into agar gels and the samples containing the immunoglobulins were allowed to diffuse into the agar, producing precipitin arcs of immunoglobulins and anti-immunoglobulin anti-serum. The diameters of the arcs are proportional to the quantities of immunoglobulins in the sample.

The required concentration of anti-serum in the gel is dependent on the concentration of immunoglobulin in the sample. It was found that serum had higher immunoglobulin concentrations than intestinal, faecal or lung samples and consequently higher concentrations of anti-sera were required to measure IgA, IgG and IgM levels in serum than in the other samples.

Sheep anti-rat IgA, IgG and IgM sera, alpha, beta and mi chain specific (Eivai Bios Laboratories Ltd., England) were used for incorporation into the agar gels and mouse immunoglobulin reference standards were obtained from Meloy, Springfield, U.S.A. Rat immunoglobulin reference standards were not available commercially.

Table 1

Measurement of Serum Immunoglobulins

Immunoglobulin Measured	Anti-Serum Concentration in Agar %	Immunoglobulin Standard Dilutions mg/ml	Volume of Sample or Immunoglobulin Standard Applied to Sample Well
IgA	6.0	26.0 13.0 6.5 3.25	4 µl
IgG	4.0	4.0 2.0 1.0 0.5	4 µl
IgM	7.0	2.5 1.25 0.62 0.31	4 µl

Three grammes of agar and 4 g polyethylene glycol (Mol. Wt. 4,000) were dissolves in 100 ml of distilled water to produce the agar gel. The agar gel plates were prepared as described by Mancini et al (1965).

(a) Measurement of serum immunoglobulins

Serum samples were tested undiluted. See Table 1 for experimental details.

(b) Measurement of Intestinal Faecal and Lung Immunoglobulins

The globulin fractions of intestinal contents were concentrated by dialysis using carbowax (polyethylene glycol Mol. Wt. 20,000) in which water was removed from the samples by osmosis. The final volumes were adjusted to give the required concentrations.

Lung and intestinal contents were concentrated to 0.5 ml per 1.0 g tissue and faecal globulins precipitated from 20 g faeces were diluted to give a volume of 4 ml. Because lower concentrations of immunoglobulins were present in these samples compared with serum, lower concentrations of anti-sera and immunoglobulins were used (see Table 2).

After incubation of the plates in a humid atmosphere at 20°C for 48 hours the plates were washed in saline and then 4% tannic acid was layered over the gel surface for 10 minutes. The latter treatment increased the visibility of the precipitin rings. After intensification of the immunoprecipitates, the plates were washed for 10 minutes in distilled water and the diameters of the precipitin rings were measured. This method allowed the rings to be measured without staining. Immunoglobulins were expressed as percentages of total globulins in the sample.

Table 2

Measurement of Intestinal, Faecal and Lung Immunoglobulins

Immunoglobulin Measured	Anti-serum Concentration in Agar %	Immunoglobulin Standard Dilutions mg/ml	Volume of sample or Immunoglobulin Standard Applied to Sample Well
IgA	1.7	2.0 1.0 0.5 0.25	8 µl
IgG	1.4	6.0 3.0 1.5 0.75	8 µl
IgM	2.1	2.5 1.25 0.625 0.32	8 µl

(ii) Double Diffusion Tests

Gel double immunodiffusion tests were carried out in agar gels essentially as described by Ouchterlony (1953) on plates or slides. The required volume of 1% agar was poured into either disposable plastic petri dishes or on slides pre-coated with agar, all plates and slides being on a level surface. The required well pattern was cut using commercially available punches and the agar plugs were removed by suction. The diameters of the central antigen well and the peripheral antibody wells were 8 mm and 6 mm respectively in the agar plate method.

Test sera and extracts from small and large intestine contents and faecal, mucosal and lung extracts were tested against the prepared L38E, ASE and ESP antigens as appropriate. The plates or slides were incubated for 48 hours at room temperature and then washed with physiological saline for 24 hours followed by a final wash in distilled water for approximately 16 hours. After drying at 37°C the plates and/or slides were stained with saturated Amido Black solution and examined for precipitin lines.

(iii) Passive Haemagglutination Tests

Titration of antibodies was carried out using 1% sheep tanned red blood cells by the Taketsy micromethod (Herbert, 1975). Ten ml of blood from a sheep, frequently used for this test, was collected into a 50 ml flask containing glass beads. The blood was defibrinated by gentle whirling and the plasma removed by centrifugation. The sedimented red cells were washed three times with physiological saline by centrifugation at 700 g for 15 minutes after gentle mixing with saline solution. The supernatant was removed by a Pasteur pipette connected to a water suction pump. This was then followed

by three washes using 0.1 M PBS pH 8.0. The required numbers of 0.7 ml aliquots of the packed washed erythrocytes were dispensed into universal 20 ml bottles and washed three times with PBS by centrifugation and sedimentation. Finally the washed cells in each bottle were suspended in 10 ml PBS.

Ten ml of tannic acid solution (50 - 80 mg in 500 ml of PBS) was added to each bottle and the cell suspensions were gently mixed, after which they were incubated for 15 minutes at 37°C. Red cells later to be coated with somatic or ESP antigens were incubated for 10 minutes. The cells were then washed two times with PBS as described above.

After the second wash, the cells to be coated with antigens were resuspended in 10 ml PBS and 10 ml of antigen solution (PBS containing 72.5 µg, 78 µg and 84 µg per ml of L3SE, ESP and ASE respectively) was added. The cells were then incubated for 1 hour at 37°C. The cell preparation was gently mixed every 15 minutes. This was then followed by an overnight incubation at 4°C. They were later centrifuged and the supernatant discarded. The coated cells along with tanned, but uncoated, erythrocytes (the latter being used as controls and for absorption of heterophile agglutinins) were washed three times with 0.1% bovine albumin in PBS before the total volume was adjusted to 50 ml to give a 1% RBC suspension.

Fresh cells were prepared for every batch of samples tested in this study.

Table 3

Sample	Concentration
Faecal Extracts	1 g per 1 ml
Small and Large Intestinal Contents	1 ml per intestine
Mucosal and Lung Extracts or Precipitated Globulin Fraction from Above	1 g tissue per 3 ml 1 g tissue per 1 ml
Serum	Undiluted

Absorption of Heterophile Agglutinins

Non-specific agglutinins were removed from large and small intestinal contents, serum, small intestine mucosal extracts, lung and faecal extracts by absorbing the samples with uncoated tanned red cells before they were used in the test. Samples were mixed in the ratio 0.4 ml of control cells to 0.1 ml sample. After incubation at room temperature for 30 minutes, the mixture was centrifuged for 15 minutes at 700 g and the clear supernatant was collected in vials for immediate use or storage at -20°C till required.

Titration of Antibodies in Faecal Extracts, Small and Large Intestinal Contents, Mucosal and Lung Extracts and Serum

Before titration the volumes of the above samples were adjusted to give the concentrations shown in Table 3.

U-shaped multi-well plastic microtitre plates were used for the titrations. Twenty five µl of the test samples were serially diluted with 0.15% bovine serum albumin in PBS as diluent.

Twenty five µl of coated tanned erythrocytes were added to each well and the titres read after 2 hours. The end-point was taken as the lowest dilution to agglutinate completely the red cells in the well. In each test, control samples from non-infected animals were used.

In most cases the results were transformed to log 10.

G. Statistical Methods

The standard 't' test was used to evaluate the results. P values less than 0.05 were considered significant.

SECTION I

Local Immune Responses to *Nippostrongylus brasiliensis* Infections in the Rat

INTRODUCTION

Nippostrongylus brasiliensis

The nematode Nippostrongylus brasiliensis occurs naturally in the intestine of the rat, Rattus norvegicus and Rattus rattus and in the mouse, Mus muscularis (Haley, 1961). The life cycle was first described by Yokagawa (1922) and the infections can be easily established in laboratory rats and mice.

Details of the parasitic and preparasitic stages of N. brasiliensis and a review of the relevant literature was given by Haley (1962). Eggs which appear in the faeces of infected animals by day 6 after infection hatch out in about 24 hours. By day four after hatching two larval moults have taken place to give third stage (L3) infective larvae. L3 larvae penetrate the skin of the host (experimental infections are conveniently produced by subcutaneous injection) and migrate, probably via blood and lymph, to the lungs by 12 - 15 hours post infection. However there is still some speculation about the exact route of migration to the lungs (see review by Ogilvie and Jones, 1971).

The larvae grow in the lungs (18 - 32 hours), moult to give fourth stage larvae and sex differentiation takes place by 32 to 40 hours after infection. The L4 larvae migrate via the bronchi, trachea, oesophagus and the stomach to the small intestine 50 to 60 hours after infection, where rapid growth takes place and the final moult occurs at about 100 hours after infection. The adult nematode lives in the anterior portion of the small intestine. Eggs appear at day six of the infection and egg production rises

quickly to reach a plateau at about 8 to 10 days after infection (DAI), after which a rapid fall occurs to give very low counts by day 12 and negative counts a few days later. In parallel with this the worm population is rapidly expelled after day 11 to reach a very low level by day 13 after infection.

Immune Responses to Nippostrongylus brasiliensis

The sudden expulsion of the worm population at about day 12 of infection is a feature of N. brasiliensis infections and the phenomenon is known as 'self-cure', a term first used by Stoll (1929) to describe sudden dramatic falls in faecal egg counts in lambs infected with Haemonchus contortus. This fall was assumed to be due to the expulsion of the worm burden. In N. brasiliensis infections the self-cure was first described by Africa (1931). Taliaferro and Sarles (1937) showed that the loss of parasite burden was not the result of ageing of the worms but of a host immune response to the parasite. Immune expulsion is a phenomenon which occurs in many host parasite systems but is seen at its most dramatic in rats infected with N. brasiliensis. Following the immune expulsion of a first infection the host exhibits a strong resistance to subsequent infections.

In many ways this is a convenient parasite system for laboratory study and N. brasiliensis infections in rats have been extensively used in the study of immunity to gastrointestinal parasites. The experimental techniques are relatively simple and the prepatent period is short, thus minimising the problems involved in producing adequate supplies of animals and infective material.

To a lesser extent mice and hamsters have been used as laboratory hosts in the study of N. brasiliensis infections.

Although all the factors involved and the exact sequence of events leading to the termination of a N. brasiliensis infection have still to be elucidated, a great deal of information has been amassed on the circulating, reaginic and cellular antibody responses associated with the kinetics of expulsion and on the changes observed in the intestinal mucosa of the host as the infection progresses (see reviews by Urquhart, Jarrett and Mulligan, 1962; Soulsby, 1968; Jarrett and Urquhart, 1971; Ogilvie and Jones, 1971; Murray, 1972; Ogilvie and Jones, 1973; Ogilvie and Love, 1974; Ogilvie and MacKenzie, 1977; Ogilvie and Parrott, 1977 and Askenase, 1980).

The role of humoral antibody in the sequence of events has been investigated. Passive immunity was demonstrated by the transfer of immune serum followed by larval challenge (Sarles and Taliaferro, 1936; Chandler, 1938 and Neilson, 1965). It was further shown that where adult parasites were transferred to rats which had been passively immunised with hyperimmune serum expulsion was accelerated (Mulligan, Urquhart, Jennings and Neilson, 1965 and Ogilvie and Jones, 1968).

However the latter workers demonstrated that relatively large quantities of hyperimmune serum were required to produce evidence of immunity and the efficacy of different batches of immune and hyperimmune serum was variable. It was also shown (Mulligan et al, 1965) that immune serum had little or no effect

on worms *in vitro*. The accumulated evidence suggested that the presence of circulating antibody alone was insufficient to cause worm expulsion and an attempt was made to identify some other factor or combination of factors which could produce immune expulsion of *N. brasiliensis*.

In a primary infection the sudden termination of the worm population (Africa, 1931 and Mulligan *et al*, 1965) gave rise to the theory that worm expulsion might involve an anaphylactic reaction in the gut. However when an anaphylactic reaction was produced in the gut of infected sensitised animals using ovalbumin antigen the worms were not expelled (Barth *et al*, 1966 and Ogilvie, 1967). The former workers also showed that if immune serum was administered prior to the ovalbumin shock worm expulsion occurred, whilst the administration of the same immune serum on its own had little effect.

These findings gave rise to the theory that expulsion involved a two stage process (Mulligan *et al*, 1965 and Urquhart *et al*, 1965). This hypothesis suggested that a local anaphylactic reaction caused an increased capillary permeability which then allowed significant quantities of anti-worm antibodies to leak into the gut and exert a direct action on the parasites. It was shown that in a primary infection of *N. brasiliensis* the macromolecular leak into the intestine was relatively massive and reached a peak at about 10 to 12 days after infection, i.e. at the time of maximum worm expulsion (Neilson, 1965 and Mulligan, 1968).

However the 'leak lesion' theory lost credence following the reports by Ogilvie and Hockley (1968) and Lee (1969) of structural and other changes occurring in the worms during the course of an infection. These workers showed that the worms had suffered irreversible damage by the tenth day after infection, i.e. the parasites were damaged presumably as a result of developing immunity before the maximum macromolecular leak occurred. The suggestion was made that the immunological damage might precede the macromolecular leak (see review by Ogilvie and Jones, 1971) and that the termination of the infection might involve the expulsion of worms which had already been irreversibly damaged by immunological action.

Further evidence that adult N. brasiliensis were damaged by host immunity as early as day 8 after infection was produced by Henney, Maclean and Mulligan, (1971, 1973) and Maclean (1977). These workers intravenously injected radiolabelled substances, ^{32}P sodium phosphate, ^{75}Se selenomethionine and ^{14}C glucose into rats harbouring N. brasiliensis populations. It was shown that the ability of the parasites to take up the radioisotopic label from the host's tissue fluids was impaired from day 8 after infection. A rapid drop in worm metabolic activity was recorded between 8 and 11 DAI. This process was assumed to be the result of developing immunity since it was shown that where worm expulsion was delayed by immunosuppression or accelerated as in second infections, there was a corresponding delay or acceleration in the impairment of worm metabolism. The conclusion was drawn

from these studies that damage to the worms preceded maximum macromolecular leak by several days. These findings therefore supported the conclusions of Ogilvie and Hockley (1968) and Lee (1969) that structural damage preceded maximum macromolecular leak.

However it is still generally agreed that there are two steps in the termination of a worm population, worm damage as a result of developing host immunity, and later, expulsion of the damaged worms by a further process.

It was shown that the immune response in rats was closely associated with the appearance, in serum, of antibodies resembling human reagins (Ogilvie, 1964, 1967). These antibodies were identified as IgE immunoglobulins (see Ogilvie and Jones, 1971). It was thought that IgE antibodies played a significant part in the expulsion of a Nippostrongylus brasiliensis population. However there were situations where IgE antibodies occurred and yet worm expulsion did not take place. For example IgE levels in lactating or in neonatally N. brasiliensis infected rats are much the same as in N. brasiliensis infections in normal mature rats yet worm expulsion does not take place in the former groups (Jarrett, Jarrett and Urquhart, 1966; Connan, 1973). Conversely it was shown that rats could be passively immunised against N. brasiliensis by antiserum free of detectable IgE (Jones, Edwards and Ogilvie, 1970). Later it became evident that the action of antibodies alone was not suffient for worm expulsion, although in some circumstances antibody action could cause a gradual loss in worm burdens, e.g. in neonates or in heavily infected rats. It appeared that the combined actions of antibodies, lymphocytes and another cell,

thought to be the mast cell were essential for the expulsion of adult N. brasiliensis (see review by Ogilvie and Love, 1974).

It was suggested by Edwards Burt and Ogilvie (1971) that worms were attacked by circulating antibodies which led to both the structural changes reported by Ogilvie and Hockley (1968) and Lee (1969) and to a marked increase in acetylcholinesterase activity in the adult worms between days 7 and 14 of infection. The suggestion has been made that acetylcholinesterase may have a biological holdfast action which allows the worms to maintain their position in the villi of the mucosa and that anti-acetylcholinesterase antibodies act by interfering with the 'holdfast' action and force the worms into the lumen of the gut where the environment is less favourable (see review by Ogilvie and Jones, 1971). However this hypothesis is speculative and even if it was valid it would not, in itself, explain worm expulsion.

The role of intestinal mast cells in the immune response to N. brasiliensis has been the subject of much controversy (see reviews by Murray, 1972; Askenase, 1980 and Ferguson and Miller, 1979).

Mast cells occur widely in the body in extravascular species. These cells contain granules which are storage sites for vasoactive amines. The cells are believed to migrate to the tissues and are found near epithelial and epidermal surfaces. A series of studies has produced evidence strongly suggesting that the response of mast cells to nematode infections is T cell dependent (see review by Askenase, 1980). Results produced by Ruitenberg and Elgersma (1976) and Olsen and Levy (1976) confirm this view and Nawa and Miller (1979) suggested that mast cells may be derived from lymph-borne precursors.

It is believed that mast cells have receptors for the Fc portion of the IgE molecule and that the action of antigen on the mast cell - IgE complex leads to degranulation of the mast cells with the release of vasoactive amines.

It was observed that soon after the arrival of N. brasiliensis in the intestine mast cells in the area of worm concentration degranulated and disappeared from the area but reappeared by about day 10 and continued to increase exponentially even after expulsion had taken place (Miller and Jarrett, 1971). Moreover the rise in mast cell numbers continued although the worms were removed by anthelmintic treatment. It was also shown (Murray, Miller, Sandford and Jarrett, 1971) that the mast cells did not degranulate and discharge in the absence of the parasites, i.e. degranulation is dependent on a factor associated with the parasite.

Jones and Ogilvie (1971) suggested that the second stage in worm expulsion might involve the action of released amines from mast cells on worms previously damaged by antibody. This view is in conflict with the leak lesion theory (Mulligan et al, 1965, Urquhart, et al, 1965) which postulated that local mucosal anaphylaxis leads to increased gut permeability which allows circulating antibody to attack the parasites which were then expelled by a further but undefined process.

Doubts on the essential role of the mast cell response in immune expulsion of N. brasiliensis were cast by Ogilvie, Love, Jarra and Brown, (1977) and Befus and Bienenstock (1979) who showed that antibody damaged worms could be expelled from recipient rats whose

mast cell population had been eliminated by sub-lethal irradiation. While these findings might be controversial (see review by Askenase, 1980) further evidence that the mast cell response was not an essential component of the immune expulsion of nematodes was produced by Uber, Roth and Levy (1980) who showed that expulsion occurred in a strain of mice lacking mast cells. However uncertainty has continued as to the role of mast cells in host immunity to N. brasiliensis.

An interesting hypothesis is that the function of the mast cell response to a first infection may be to restore the intestinal structure after expulsion (Woodbury, Gruzenski and Lagunoff, 1978) and that this process may play some part in protection against further infections.

It is generally known that there is proliferation of mucosal goblet cells at the time of N. brasiliensis expulsion. Miller and Nawa (1979) collected thoracic duct lymphocytes (TDL) from rats 10 days after infection and from hyperimmune rats (1 week after a third infection). These workers showed that when TDL were transferred intravenously to infected rats they stimulated goblet cell differentiation. Thoracic duct lymphocytes lacking surface immunoglobulin were as effective as unfractionated TDL or hyperimmune TDL. Although expulsion of a parasite population is associated with an increase in goblet cells, no direct evidence of their involvement in expulsion has been offered. Mucus produced by goblet cells may protect the mucosa, in some way, perhaps preventing 'damaged' worms from adhering to the mucosal surface and so facilitating expulsion.

Evidence was later produced which suggested that the following sequence of events was involved in worm expulsion. First worms are

damaged by antibody but not expelled and then the action of cells must follow in order to achieve expulsion, the cells responsible being lymphocytes (see review by Ogilvie and Love, 1974). It had been suggested that bone marrow derived cells were required to effect the final phase of worm expulsion (Kelly, Dineen and Love, 1973). However it was subsequently shown by Ogilvie et al (1977) that sensitised T cells alone can bring about the expulsion of a population of antibody damaged worms and immun o globulins appeared to play no part in the final phase of worm expulsion. They also suggested that it is the co-operation of eosinophils, macrophages and T cell effectors which bring about the final expulsion of a N. brasiliensis population.

It is generally accepted that N. brasiliensis is a complex antigenic organism and the immune response is commonly considered to be a multifactoral process involving antibodies, T-cells and perhaps local inflammatory responses. Although the literature contains a great deal of information on changes in the small intestine of the host as a result of infection and on changes in worm infectivity, reproduction, metabolic status and morphology as a result of developing host immunity, many of the hypothoses put forward remain controversial.

The literature accumulated over many years on gastrointestinal parasitic infections contains many references to systemic immune responses, particularly in Nippostrongylus brasiliensis infections in the rat. In contrast, until recently there have been few reports of the study and evaluation of local intestinal immunity to parasitic infections. Investigations into local immune responses to N. brasilinesis in the rat have been limited to reports by Poulain et al (1976a). Sinski and

Holmes (1977) and Sinski and Holmes (1978). These reports were reviewed in the General Introduction.

Section 1 of this thesis describes the results of a number of experiments designed to identify and measure local immunity to N. brasiliensis in the rat by a number of immunological tests. The initial experiment examines these responses in terms of the stimulation of Peyer's Patches and the appearance of IgA containing plasma cells in the intestinal lamina propria. The experiments described in the remainder of the section investigate in greater detail local antibody responses in N. brasiliensis infected rats by the detection and measurement of immunoglobulins and specific anti-parasite antibodies in faecal extracts. These results are compared with similar measurements in serum, small and large intestinal fluids, in mucosal extracts and in lung extracts.

CHAPTER 1

The Kinetics of Intestinal Mucous Associated Lymphoid Tissue (MALT) Responses Following Infection with *Nippostrongylus brasiliensis* in Normal and Immunosuppressed Rats

Introduction

A review of the relevant literature in the General Introduction has shown that a consequence of the introduction of antigenic material into the intestine is usually a proliferation of immunoglobulin secreting cells in the intestinal lamina propria. Investigation of the sequence of events leading to the appearance of these immunoglobulin containing cells in the intestinal mucosa has led several workers to conclude that Peyer's patches play an important role in the sensitisation and transport of lymphoblasts, the precursors of the immunoglobulin secreting plasma cells. In particular the specialised M cells in the Peyer's patch mucosa are thought to allow antigenic material to cross from the intestinal lumen, thus facilitating sensitisation of the lymphoblasts prior to their transport to their mucosal secreting sites.

This experiment was designed to examine, in particular, changes in the size and number of Peyer's patches resulting from the presence of adult N. brasiliensis worms in the rat intestine. Changes in parasite-specific mucosal IgA and in mucosal and serum haemagglutinating antibody levels were studied. In addition the numbers of IgA containing cells in the intestinal lamina propria were also measured. These results were compared with similar measurements in other rats in which the ability to mount local immune responses had been impaired by exposure to sub-lethal gamma irradiation 24 hours prior to N. brasiliensis infection. Systemic antibody responses were also compared in both groups of rats.

Many workers have shown that gamma radiation has immunosuppressive effects on systemic responses to infections. Ogilvie and Jones (1971) showed that in N. brasiliensis infections in rats, gamma radiation destroyed a factor which was essential for the immune expulsion of the worms. Although most attention has been devoted to the effects of irradiation on systemic responses some workers have shown that local responses are also suppressed by gamma radiation. Rudzik et al (1975) showed that 1000 rad X-irradiation of rabbits destroyed the IgA cell population in the gut and bronchial lamina propria and there was little evidence of repopulation six days after irradiation. Befus et al (1978) reported similar findings.

The radiation dose given to an animal to produce sub-lethal immunosuppression must be carefully chosen. Radiation doses of 300 - 400 rad (Taliaferro and Taliaferro, 1951) and 400 - 500 rad (Targett, 1973) have been used to produce sub-lethal immunosuppression of trypanosome infections in rats. The chief limiting factor in the choice of irradiation dose is the susceptibility of the intestinal epithelium and Targett (1973) recommended a dose of 400 - 500 rad to produce sub-lethal immunosuppression in rats. Since the habitat of N. brasiliensis is the small intestine a radiation dose at the lower end of the range, i.e. 400 rad, was used in this experiment.

It is well known that the suppressive effects of sub-lethal irradiation are transitory. The effects of sub-lethal radiation on the host is maximal between 24 and 48 hours after irradiation

Table 1.1

Experimental Plan

	No. of Rats	Larval Infection	Irradiation (rads)	Necropsy on days after infection							
				3	6	9	13	16	20	24	27
Infected (non-Irradiated) Group I	37	3,000	-	5	5	5	5	5	5	4	3
Infected/ Irradiated Group II	37	3,000	400	5	5	5	5	5	5	4	3
Irradiated (Non-infected) Group III	35	-	400	5	5	5	5	5	5	-	-
Control Rats Group IV	15	-	-	-	5	-	5	-	-	5	-

although the lymphoid tissue remains inactive for at least a week after irradiation (Targett, 1973). In the current experiment the host rats were irradiated 24 hours prior to N. brasiliensis infection.

Materials and Methods

Experimental Animals

One hundred and twenty four rats were used in this experiment. They were divided into four groups (I, II, III and IV). Groups II and III were subjected to gamma radiation in a Cobalt (^{60}Co) MK IVB irradiation chamber (Nuclear Engineering Ltd., Reading, England), so that each rat received a whole body dose of 400 rad. Twenty four hours after irradiation Group II along with a group of non-irradiated rats (Group I) were infected subcutaneously with 3,000 N. brasiliensis third stage (L3) larvae. A fourth group of animals acted as non-irradiated worm-free controls.

Experimental Plan

Five rats from each group were killed and dissected every 3 or 4 days after infection (DAI), (see Table 1.1). N. brasiliensis egg output was measured daily throughout the experiment. Following dissection the numbers of worms in the rat intestines were counted. Changes in the numbers of Peyer's patches and in the numbers of IgA-containing cells in the small intestine lamina propria were estimated. Haemagglutinating antibody levels against L3SE antigens were measured in serum and in intestinal mucosal extracts.

Parasite-specific IgA levels in intestinal mucosal extracts were measured on 6, 9 and 13 DAI. Serum precipitating antibody levels were also measured against larval and adult somatic extract antigens.

Parasitological Techniques

These were carried out as described in the General Materials and Methods.

Peyer's Patch Counts

The number of Peyer's patches larger than 2 mm in diameter was counted in the length of small intestine 30 cm distal to the pylorus.

Histological Techniques

A portion of small intestine, one centimetre in length was taken for immunohistological investigation from a point 20 cm distal from the pylorus. This piece of intestine was immediately immersed in liquid nitrogen. A direct immunofluorescent test was performed on cryostat sections prepared by the method of Allen and Porter (1970). Serial sections 5 μm thick were fixed in absolute methanol for 20 mins at room temperature. The fixed sections were pre-treated with Evans blue 1:10,000 to reduce the non-specific uptake of fluorescent reagents by polymorphonuclear cells.

A double staining procedure was employed for identifying IgA-containing cells in the lamina propria of the small intestine. The sections were sequentially incubated with fluorescin-conjugated sheep anti-rat IgA, alpha chain specific, (Eivai Bios Laboratories Ltd., Horsham, Sussex, England) and then fluorescin-conjugated goat

anti-rabbit Ig antiserum (Nordic Laboratories, Maidenhead, England). The preparations were examined on a Zeiss standard universal microscope (Carl Zeiss, (U.K.) Ltd., London) with a 3RS incident light illuminator equipped with FITC and BG12 filters. IgA-containing cells in the lamina propria in each group of rats were counted for 20 villus crypt units (VCU) per section. Ten sections were examined from each specimen.

Parasite-Specific IgA Levels in Mucosal Extracts

Anti-parasite IgA levels in mucosal extracts were measured essentially as described by Sinski and Holmes (1977) in a radio-immunoassay technique using whole parasite antigen.

Mucosal extracts to be tested were inactivated at 56°C for 30 minutes prior to testing. Approximately 50 mg of fresh parasites which had been washed several times in PBS were placed in tubes with 0.2 ml PBS. These worms were then incubated for 2 hours at 37°C with 0.2 ml of the previously inactivated test material. The parasites were then washed five times with PBS and incubated for 1 hour with 0.1 ml of ^{125}I rabbit anti-rat IgA (chain-specific, Eivai Bios Laboratories Horsham, England) labelled by the method described by Hunter and Greenwood (1962).

After incubation the parasites were washed five times in PBS and resuspended in 0.2 ml PBS for radioactivity determination. The amount of anti-IgA bound gives a measure of the quantity of IgA bound to the parasites.

Specific IgA binding was measured by the ^{125}I radioactivity bound per minute per mg 'fresh weight' of worms.

Mucosal extracts from the non-infected control animals were tested and the test was also performed in a series of blank tubes (containing no parasites) to give background radioactivity for the test.

Preparation of Parasite Antigens

N. brasiliensis larval somatic antigen (L3SE) and adult somatic antigen (ASE) were prepared as described in the General Materials and Methods.

Passive Haemagglutinating Tests

The titration of mucosal extract and serum antibodies against L3SE antigens was carried out by the Taketsy micromethod (Herbert, 1975).

Serum Precipitating Antibody Tests

These tests were performed by the method described by Ouchterlony (1953) using mucosal samples from Groups I and II and L3SE and ASE *N. brasiliensis* antigen preparations.

Results

Adult Worm Burdens

The numbers of worms recovered between days 6 and 20 of infection in Groups I and II, are shown in Figure 1.1. These results show that in Group II, the irradiated infected rats, there was an increase in the number of worms recovered on days 6 and 9 of infection and that worm expulsion was delayed by about 7 days compared with the normal infection (Group I). In the latter stages of the infection in Group II rats, the worms were seen to be located

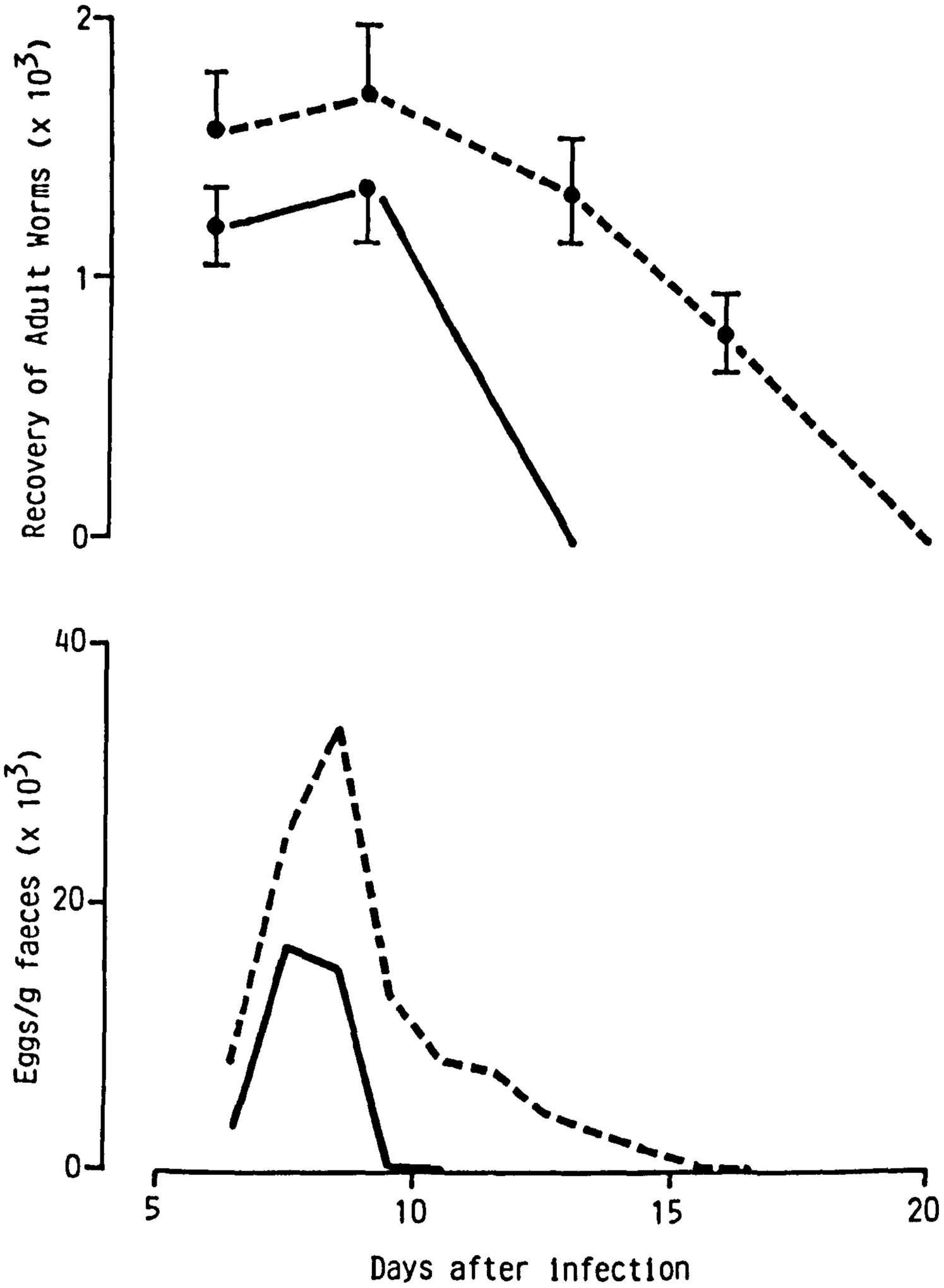

Fig. 1.1. Numbers (mean ± S.E.) of adult N. brasiliensis recovered and egg output (eggs/g faeces) in irradiated and non-irradiated rats infected with 3,000 N. brasiliensis.

——— Group I (infected, non-irradiated)

------- Group II (infected, irradiated)

in a lower part of the small intestine and, as late as day 27 of infection, a small number of worms were recovered from Group II rats on necropsy.

Faecal Egg Counts

Daily faecal egg production was monitored from 7 till 17 DAI (see Fig. 1.1). In Group II there was a marked elevation in egg production between 7 and 11 DAI and egg output continued at a reduced rate till 17 DAI, in contrast to Group I in which egg output terminated by day 11. Hence the immunosuppressive effects of the irradiation in Group II rats was manifested by both an enhancement in numbers and duration of egg production and a delay in worm expulsion.

Peyer's Patches

Changes in both the size and number of Peyer's Patches in each group of rats were compared at intervals throughout the experiment (see Fig. 1.2).

In Group I a significant increase in both size and number of patches was observed on 6 and 9 DAI, compared to the non-infected rats (Group IV). In Groups II and III the immunosuppressive effect of the radiation was shown by a significant reduction in Peyer's patch sizes and numbers between 3 and 13 DAI and thereafter a gradual increase towards normal levels. In Group I the number of Peyer's patches fell to control levels by 13 DAI. At no time during the experiment was there any significant difference between the numbers of Peyer's patches in Groups II and III (see Appendix A, Table 3).

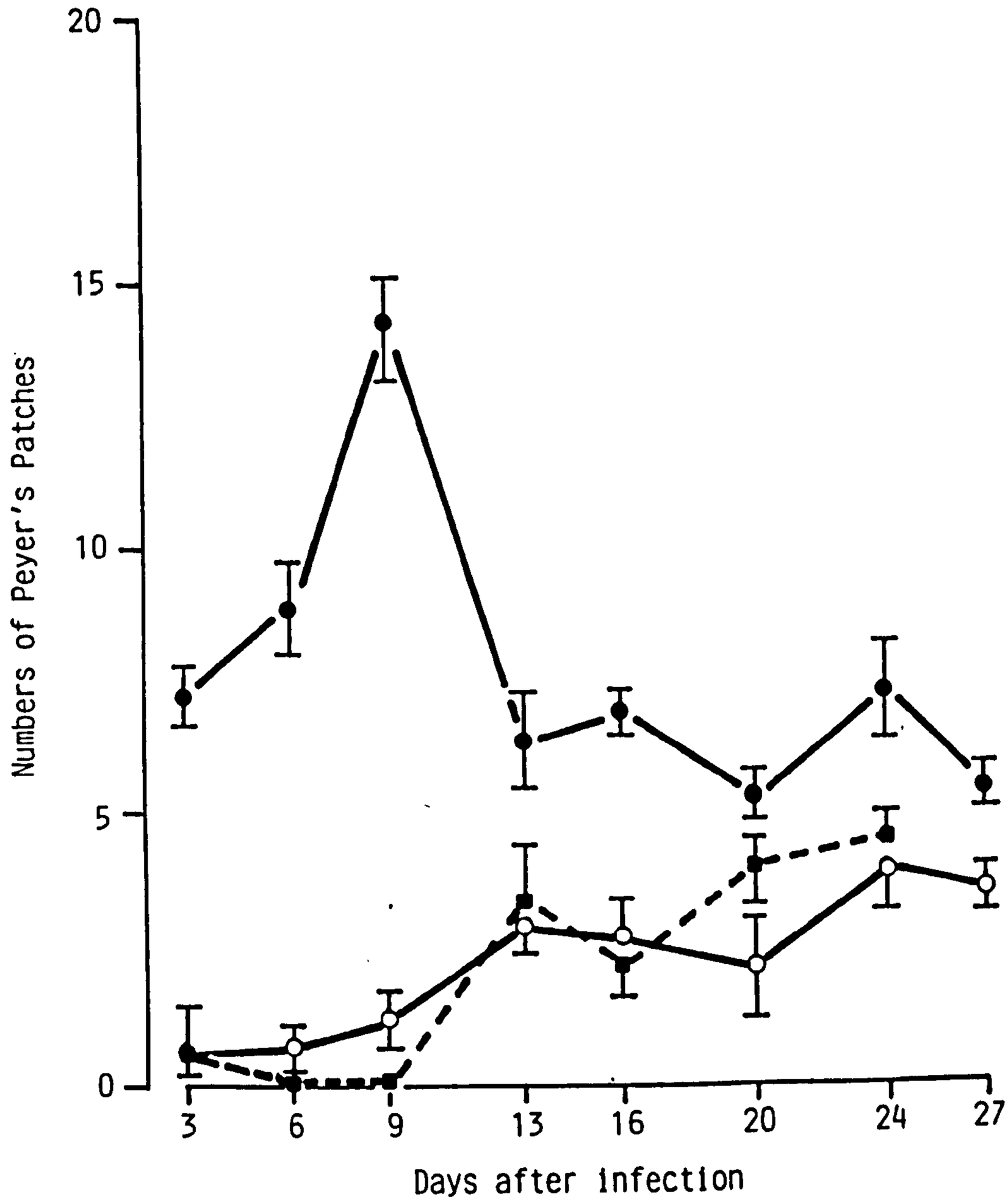

Fig. 1.2. Numbers (mean ± S.E.) of Peyer's patches in the first 30 cm of small intestine in irradiated and non-irradiated rats infected with 3,000 N. brasiliensis

Group I (Infected/non-irradiated) ——●——

Group II (Infected/irradiated) ——○——

Group III (Non-infected/irradiated) - - - - - ■ - - -

IgA Plasma Cells

The number of IgA-containing cells per villus crypt unit (mean of 200 VCUs) in the small intestine mucosa of one rat from each group taken at intervals throughout the experiment is shown in Fig. 1.3. Between days 6 and 16 of the infection a marked increase in the number of IgA-containing cells in Group I was observed compared with normal levels (Group IV). At the same time in both irradiated groups (Groups II and III), the number of such cells was greatly depleted. Seventeen days after irradiation there was evidence of a substantial repopulation of the intestinal mucosa of the irradiated rats, Groups II and III,with IgA containing cells.

Serum Precipitating Antibody Levels

The results of the gel double-diffusion tests are shown in Table 1.2. In the normal infection (Group I), high antibody concentrations were observed, especially against the adult worm antigen. There were no detectable antibodies present in the sera of the irradiated infected rats, Group II, throughout the period studied.

Parasite Specific IgA Levels in Mucosal Extracts

Table 1.3 shows a comparison of the parasite-specific IgA levels in mucosal samples prepared from the small intestines of rats necropsied on days 6, 9 and 13 after infection. On days 6 and 9 there was evidence of greatly elevated IgA levels in the mucosa of the small intestine in a normal infection in rats, Group I. However by day 13 after infection their mucosal IgA levels had

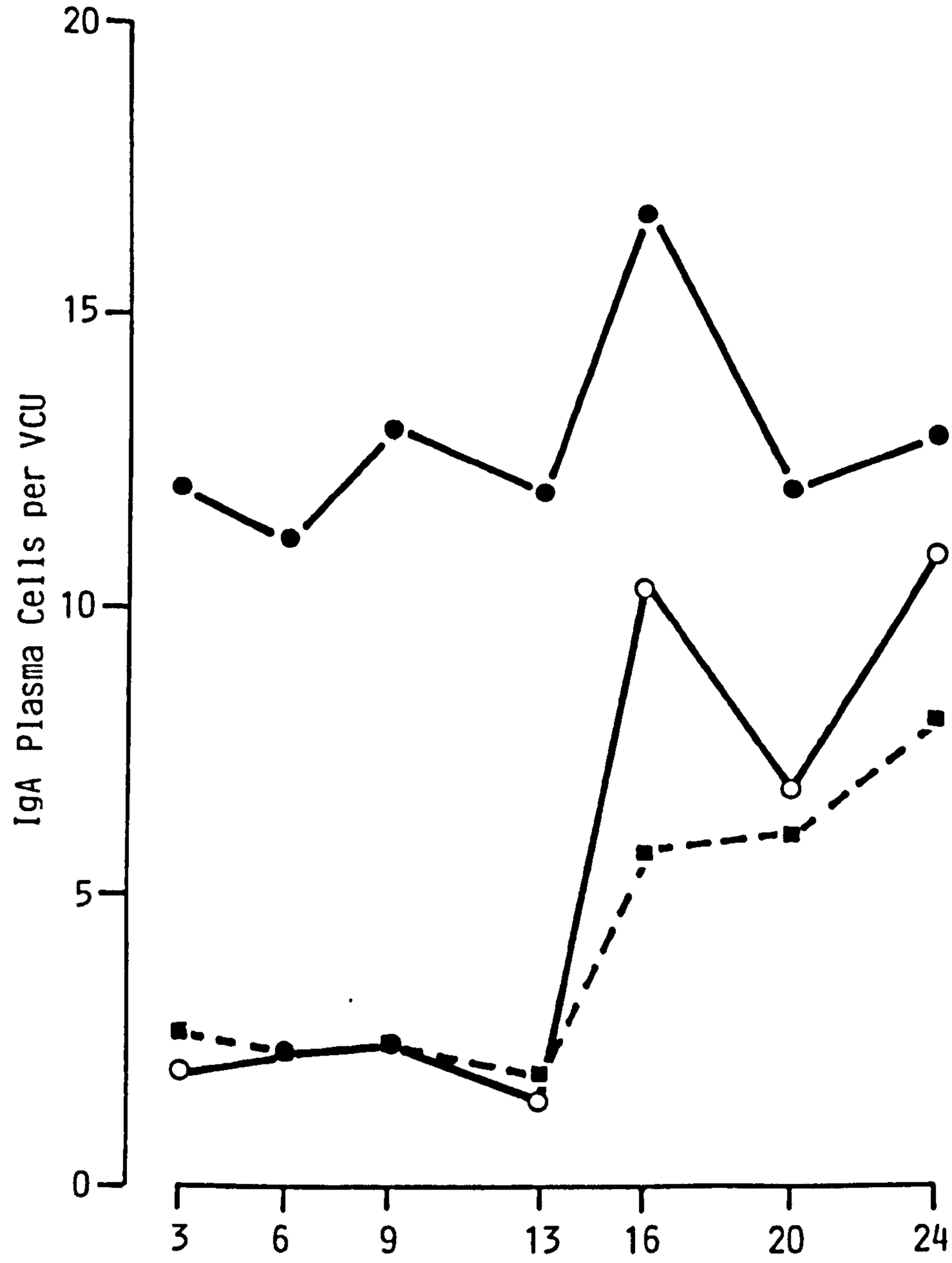

Fig. 1.3. Numbers of IgA-containing cells per villus crypt unit (mean of 200 VCU) in irradiated and non-irradiated rats infected with 3,000 N. brasiliensis.

Group I (Infected/non-irradiated) ——●——

Group II (Infected/irradiated) ——○——

Group III (Non-infected/irradiated) ------■------

Table 1.3

Parasite-specific IgA levels* in Small Intestine Mucosal Extracts from Irradiated and Non-irradiated Rats Infected with 3,000 *N. brasiliensis*

	Days after *N. brasiliensis* Infection		
	6	9	13
Group I (normal infection)	697 ± 260	1488 ± 446	403 ± 156
Group II (irradiated/infected)	31 ± 9	155 ± 57	137 ± 45
Group I/II ('t' test)	P < 0.05	P < 0.05	N.S.
Group IV (worm free/non irradiated)	275 ± 66	-	-

* Expressed as counts/min/mg 'fresh weight' worms ± S.E.

fallen to control values. In contrast, in mucosal extracts of infected irradiated rats there was little evidence of increased levels of parasite-specific IgA following infection.

Haemagglutinating Antibody Levels

Mucosal and serum haemagglutinating antibody levels against L3SE antigens are shown in Fig. 1.4. Antibody titres in mucosal extracts showed significant increases following the appearance of the adult N. brasiliensis in the intestine at 6 DAI. Mucosal antibody titres reached maximum levels at 13 DAI, the time of rapid worm expulsion. Serum antibody levels between 6 and 16 DAI closely reflected those in mucosal extracts. In the two irradiated groups, II and III, and in the worm free controls, Group IV, mucosal and serum antibodies were barely detectable throughout the period of study.

Discussion

The results clearly demonstrated that the sub-lethal radiation dose used in this experiment produced immunosuppression in rats (Group II). This was manifest both in terms of a greatly reduced rate of expulsion of the parasites and also elevated levels and increase in the duration of egg production.

In the non-irradiated infected rats there was a proliferation in both the numbers and size of Peyer's patches coinciding with the presence of the worms in the intestine between 6 and 13 DAI. The numbers of Peyer's patches reached a peak prior to worm expulsion and declined at about the same rate as the worm population to reach

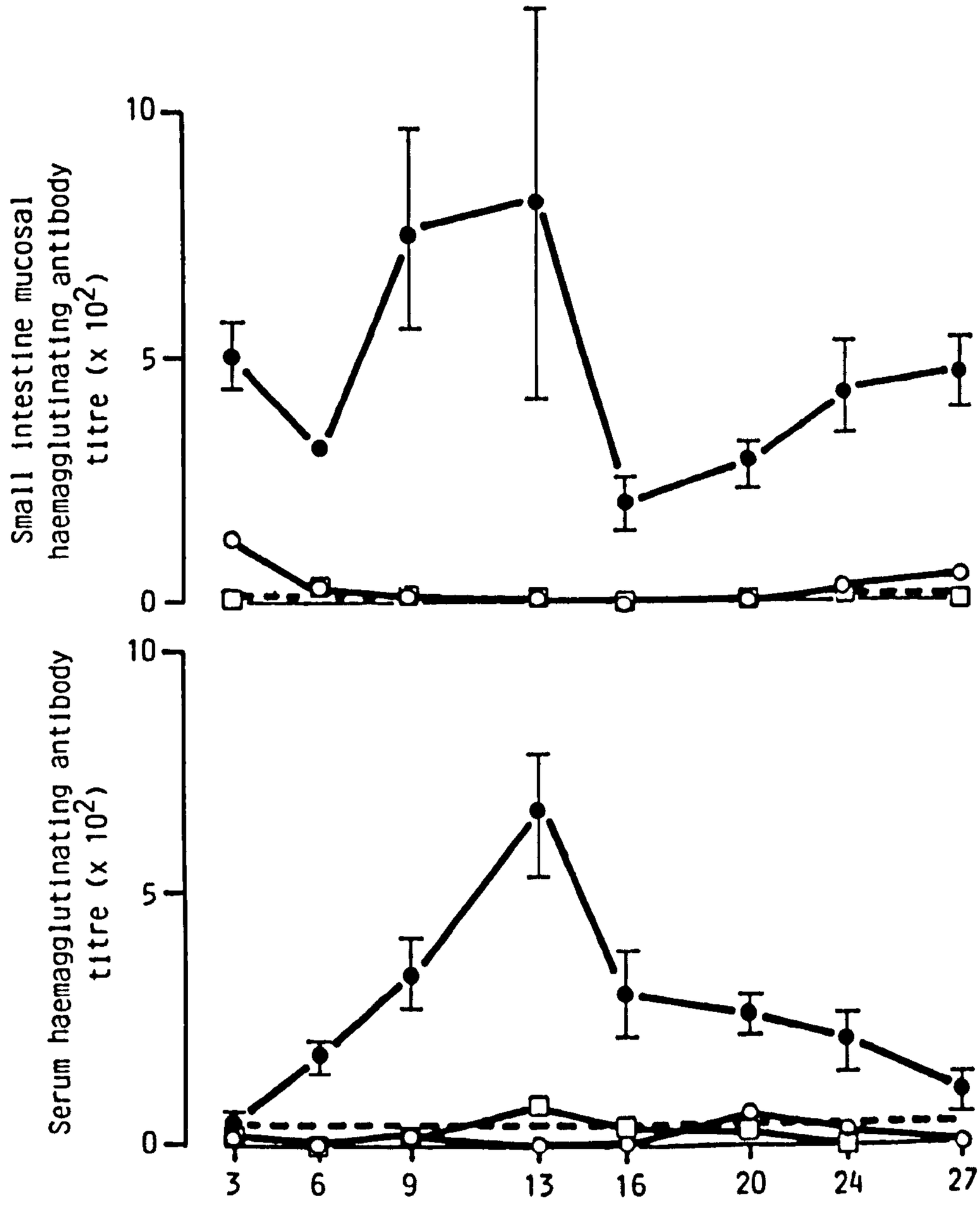

Fig. 1.4. Haemagglutinating antibody titres (mean ± S.E.) against L3SE antigen in small intestine mucosal extracts and serum in irradiated and non-irradiated rats infected with 3,000 N. brasiliensis and in worm-free controls.

Group I (Infected/non-irradiated) ——●——
Group II (Infected/Irradiated) ——○——
Group III (Non-infected/irradiated) ——□——
Group IV (Worm-free controls) ----------

normal levels at about day 13 when expulsion was complete. This sequence of events suggests that the Peyer's patches were stimulated by worm antigens and returned to normal levels once this stimulus had been removed. It has previously been reported by Pollard and Sharon (1970) and Muller-Schoop and Good (1975) that enlargement of Peyer's patches with the appearance of germinal centres can be stimulated by bacterial antigens.

In both groups of irradiated rats, Groups II and III only a few Peyer's patches were observed for the first 10 days following irradiation. The first signs of a return to normal numbers of Peyer's patches in these groups occurred 14 days after irradiation. Changes in Peyer's patch numbers in both infected Groups, I and II, correlated well with the onset of worm expulsion and the completion of the expulsion process. In non-irradiated infected rats (Group I), changes in mucosal haemagglutinating antibody titres and parasite-specific mucosal IgA levels correlated well with changes in worm numbers between the establishment of the nematodes in the intestine and their expulsion at about 13 DAI.

There is an apparent discrepancy in the timing of maximum IgA-containing cell numbers in the small intestine mucosa and the timing in changes in the other parameters measured. It appeared that the maximum IgA-containing cell population in the small intestine occurred several days after maximal mucosal and serum haemagglutinating antibody levels, after maximum parasite-specific IgA

mucosal levels and after worm expulsion was complete. It was shown by Befus et al (1978) that the transfer of Peyer's patch lymphocytes to irradiated rabbits produced a proliferation of IgA containing cells in the intestinal lamina propria four days after cell transfer. It would therefore seem reasonable to expect that mucosal IgA secreting cell numbers would have been maximal at 13 DAI.

There may be several explanations for this apparent discrepancy. The technique used to detect IgA cell populations in the small intestine mucosa depended on the detection of IgA containing cells, i.e. mature immunoglobulin secreting cells rich in IgA. It may be that immature IgA secreting cells contribute significantly to immunoglobulin secretion at mucosal sites and yet may not easily be detected by the technique used in this experiment. It is also important to note that the results refer to the mean number of IgA-containing cells per VCU obtained by counting the numbers of cells in 200 VCU in one rat from each group on necropsy days.

A similar type of apparent discrepancy was highlighted by Woodbury, Miller, Huntley Newlands, Pallister and Wakelin (1984) in studies on intestinal mast cell responses to N. brasiliensis infection in the rat. These workers confirmed the well documented fact that the maximum mature granulated mast cell response in N. brasiliensis infections occurs after worm expulsion at about 16 DAI. In contrast to this, it was shown that the maximum systemic secretion of the enzyme rat mast cell protease secreted by mast

cells, was observed between 6 and 12 DAI when there was no proliferation of mature mast cells. These workers concluded that immature mast cells and their precursors may be as fully active as mature mast cells.

It has also been shown that biliary transport of serum derived IgA contributes substantially to intestinal IgA. In the upper small intestine as much as 90% of specific IgA antibodies can be derived from bile secretion (Lemaitre-Coelho, Jackson and Vareman, 1977). The appearance of such biliary IgA would not be reflected in corresponding increases in IgA secreting cells in the intestinal mucosa but could contribute to high anti-parasite antibody levels in the lumen.

It is also well established that there is a significant increase in intestinal mucosa permeability coinciding with the presence of adult N. brasiliensis in the intestine (see review by Ogilvie and Jones, 1971). The resulting plasma leak into the gut of infected animals provides another possible route for the transport of serum IgA into the intestine.

In conclusion, the results demonstrated that in irradiated rats there was a significant suppression of both systemic responses and local immune responses in the intestine to N. brasiliensis. The immunosuppression resulted in a severe depletion of Peyer's patches and IgA-containing cells in the small intestine lamina propria. There was also a virtual absence of any detectable parasite-specific IgA in mucosal extracts or of haemagglutinating antibodies in serum or mucosal extracts in the infected irradiated rats.

Moreover serum precipitating antibodies to nematode somatic antigens were not detected in the latter group of rats.

In the normal N. brasiliensis infections there was both a proliferation and an apparent hypertrophy of Peyer's patches coinciding with the presence of the nematodes in the intestine. Elevated haemagglutinating antibody levels in serum and mucosal extracts also correlated with the intestinal phase of infection. The observed hyperplasia of IgA-secreting cells in the intestinal mucosa correlated less closely with the other parameters measured. An obvious consequence of the presence of N. brasiliensis antigenic material in the intestine was a stimulation of Peyer's patches. These tissues are believed to play an important role in the sampling of antigen from the intestinal lumen and in the stimulation and transport of sensitised lymphoblasts, the precursors of the immunoglobulin secreting cells which subsequently appear in the small intestine lamina propria. Peyer's patches appeared to play a similar role in the 'sampling' of N. brasiliensis antigens and in the sensitisation of lymphoblasts prior to their transport to secreting sites in the intestine.

CHAPTER 2

Detection and Measurement of Coproantibodies to Parasite Antigens in Rats Infected with *Nippostrongylus brasiliensis*

Introduction

In Chapter 1 it was shown that N. brasiliensis infections in rats produce a strong local immune response in the small intestine. The experiment described involved examination of post-mortem material collected from the small intestine. In this chapter local immune responses in N. brasiliensis infected rats were examined by the detection and measurement of immunoglobulins and specific anti-parasite antibodies in faecal extracts. Copro-antibody levels were compared with immunoglobulin and anti-parasite antibody levels in small intestine mucosa and in serum collected at necropsy.

Experiment 1

Changes in immunoglobulin levels and in anti-parasite anti-bodies in faecal extracts were measured in rats following a primary infection with N. brasiliensis. These coproantibody measurements were compared with levels of immunoglobulins and anti-parasite antibodies in corresponding samples of small intestine mucosa and serum. Coproantibodies were also measured in a group of rats following second and third infections with N. brasiliensis.

Experimental Design

Fifty five rats were used. Thirty five were infected with 5,000 L3 N. brasiliensis. Five rats acted as non-infected controls. Six groups of 5 infected rats were used for evaluation of immune responses following a primary infection. A group of 5 rats acted as a hyperimmunised group. These rats were given infections of 5,000 and 10,000 L3 larvae on days 15 and 25 respectively after

Table 2.1

Experimental Design

	First Infection 5×10^3 L3			Second Infection 5×10^3 L3		Third Infection 1×10^4 L3	Worm-free Control Rats
	35 rats			5 rats		5 rats	5
	Days after First Infection						
Collection of faeces for coproantibody measurement	3	6	12	15	18	30	15
	18	24	30	21	24		
Necropsy for small intestine mucosa and serum samples	3	6	12			34	15
	18	24	30				

primary infection. A group of 5 rats was necropsied on each of the days shown in the experimental plan (Table 2.1). Overnight faecal collections (16 hour period) were taken from each group of 5 rats prior to necropsy.

Faecal extracts, small intestine mucosal extracts and serum samples were prepared as previously described. Serum and small intestine mucosal samples were collected from the worm-free control rats at necropsy. Rats from the hyperimmune group were killed 4 days after the third infection and serum samples and intestinal mucosal extracts were prepared.

Immunoglobulin Estimations

Faecal and mucosal extracts and sera were analysed for IgA, IgG and IgM by Radial Immuno Diffusion (Mancini et al, 1965) using sheep anti-rat IgA, IgG and IgM sera (alpha, gamma and mμ chain specific, Eivai Bios Laboratories Ltd.).

Gel Diffusion Tests

Gel double diffusion tests were performed as described in the General Materials and Methods (Ouchterlony, 1953).

Passive Haemagglutination Tests

The titration of antibodies was carried out by the Taketsy micromethod (Harbert, 1975). Suspensions of 1% tanned sheep erythrocytes were coated with either L3SE, ASE or ESP antigens. Heterophile agglutinins for red cells were removed by absorption of test serum, mucosal and faecal extracts as previously described. To confirm the removal of non-specific agglutinins, controls consisting of sera, faecal or mucosal extracts at the initial dilution were incubated with tanned but uncoated red cells. Coated tanned

erythrocytes were also incubated with serum, faecal and mucosal extracts during each group of tests. The results of the reaction were read at 2 hours and the reading confirmed 24 hours after adding the red cells.

Results

Worm Burdens

The mean number of worms recovered from the intestine of the rats on days 6, 9 and 12 after primary infection were 3,000, 1,800 and 833 respectively.

Immunoglobulin Levels in Faecal Extracts in Primary N. brasiliensis Infections

Changes in the levels of total IgA, IgG and IgM as measured by radial immunodiffusion and expressed as percentages of the total protein content of 1 g faeces are shown in Fig. 2.1. Although immunoglobulins represented only a small fraction of the faecal extract protein the concentrations increased significantly after infection.

Immunoglobulin A concentrations gradually increased after infection and reached a peak at 24 DAI. Levels of IgG were relatively much higher than IgA but showed a similar rapid rise after infection, also reaching maximum levels at 24 DAI. Immunoglobulin M levels were comparatively much lower but showed small increases on 6 and 12 DAI.

Immunoglobulin Levels in Mucosal Extracts and in Serum in Primary N. brasiliensis Infections

The results of changes in mucosal and serum immunoglobulin levels are shown in Fig. 2.1.

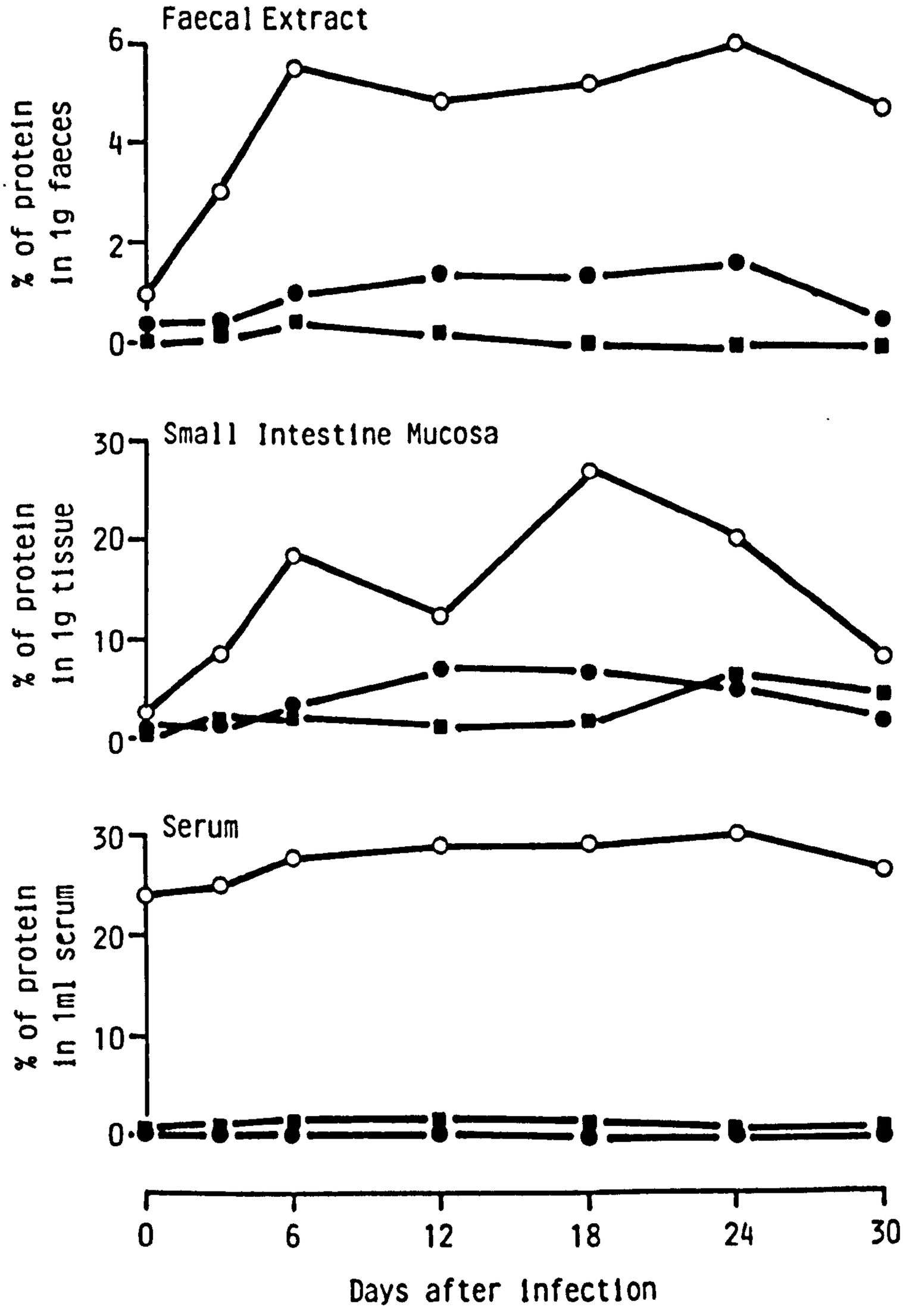

Fig. 2.1. Total immunoglobulin levels in faecal extracts, small intestine extracts and serum, of rats following infection with 5,000 N. brasiliensis

—●— IgA, —○— IgG, —■— IgM

Mucosal IgA levels rose steadily after infection and reached a plateau between 12 and 18 DAI and then declined steadily. Serum IgA showed a small but steady increase over the period monitored.

Levels of IgG in mucosal extracts rose rapidly to reach maximum at day 18 of infection and then declined sharply. Serum IgG showed a steady increase from a relatively high base-line to reach a peak at day 24.

Mucosal IgM concentration, after an initial post-infection increase remained at a comparatively low level before showing a large increase at 24 DAI. IgM serum values increased slightly after infection then fell back to pre-infection levels by 30 DAI.

Haemagglutinating Antibody Titres in Primary N. brasiliensis Infections

Haemagglutinating antibody titres in faecal and mucosal extracts and in serum are shown in Figure 2.2.

In this experiment faecal extracts were found to contain anti-worm antibodies as early as 3 DAI. Titres against ESP and L3 antigens were maximal at 12 DAI while titres against ASE antigens reached a peak at 18 DAI.

The haemagglutinin levels in mucosal extracts were remarkably similar to those observed in faecal extracts. Haemagglutinating antibodies to all three antigen preparations appeared later in sera, at day 6 of infection and reached maximum titres at 24 DAI, again later than maximum titres for faecal or mucosal extracts. The increase in serum antibody titres was slower and the final increased antibody levels were less marked.

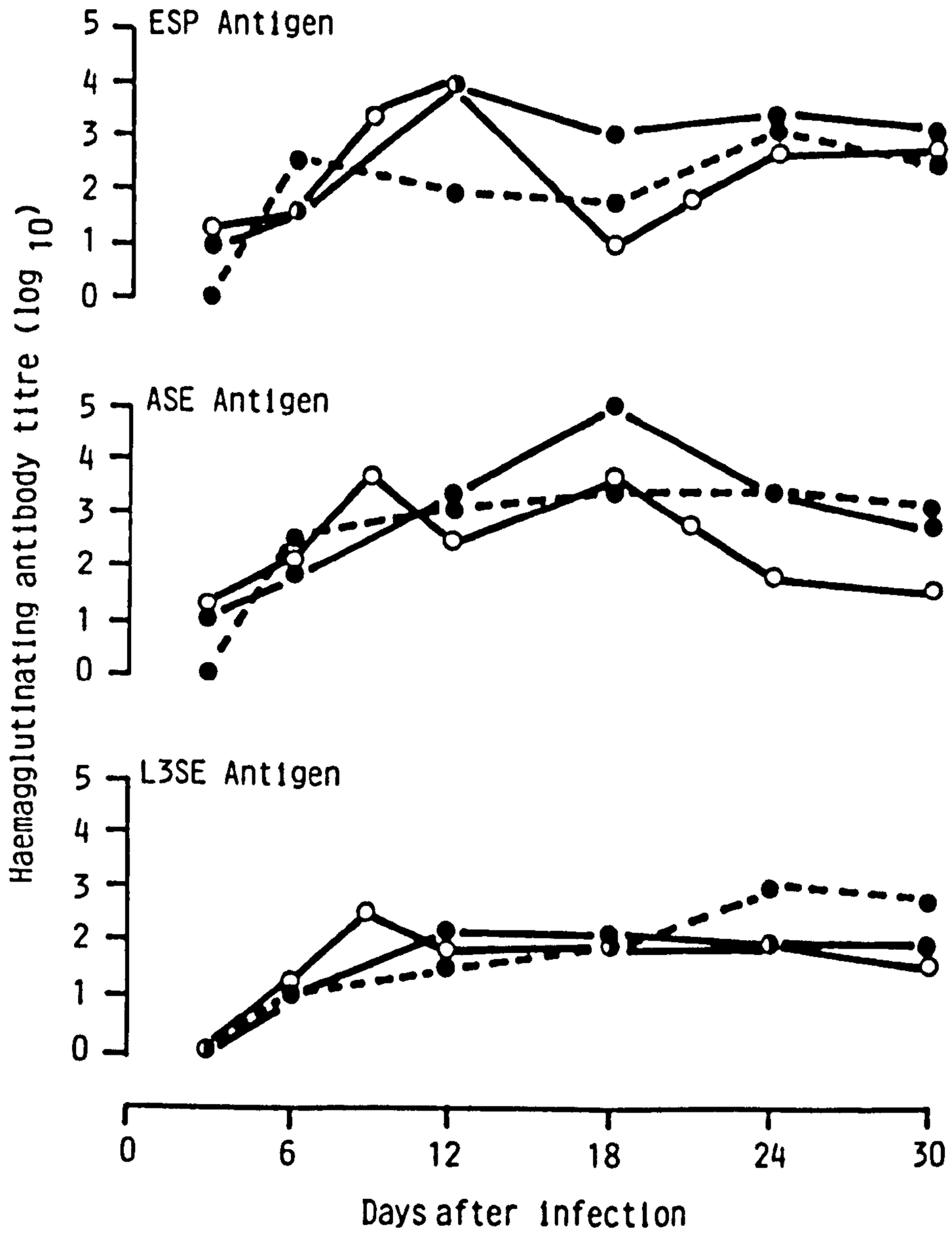

Fig. 2.2. Haemagglutinating antibody titres in faecal extracts, small intestine mucosal extracts and serum of rats infected with 5,000 N. brasiliensis.

—o— Faecal extracts

—●— Mucosal extracts

- - -●- - - Serum

Table 2.2

Numbers (mean of 5 samples) of Precipitin Arcs in Serum of Rats Following Infection with 5,000 N. brasiliensis and in Hyperimmune Rats

Sample Tested	Antigens Used	Days after Infection						
		3	6	12	18	24	30	Hyper-immune
Serum	ASE	0	0	0.8	1.8	1.8	1.4	2.6
	ESP	0	0.8	1.0	1.6	2.0	1.6	2.0
	L3SE	0	0	0.2	1.6	1.6	1.2	1.4

Faecal Haemagglutinating Antibody Titres in Hyperimmune Rats

Figure 2.3 shows the haemagglutinating titres in faecal extracts when tested with ASE, ESP and L3SE antigens following second and third infections with 5,000 and 10,000 N. brasiliensis larvae. Titres against ASE antigens increased between 3 and 15 days after the second infection whereas in the unchallenged animals (Fig. 2.2) titres showed reduced values during this period. Titres against ESP antigens showed a similar pattern and reached higher levels between 3 and 15 days after the second infection compared with the corresponding period for unchallenged rats (Fig. 2.2). Challenge with second or third infections did not enhance antibody titres against L3SE nor was there evidence of increased titres against ASE or ESP following third infections.

Serum Precipitating Antibodies in Primary N. brasiliensis Infections and in Hyperimmune Rats

Strong precipitating antibodies were found only in sera of infected rats after day 6 of infection (Table 2.2). Faecal and mucosal extracts produced very weak single arcs against ESP antigens only and these results were positive only when highly concentrated mucosal or faecal extracts from 24 or 30 DAI were used.

Experiment 2

Experiment 2 was designed essentially to corroborate the results obtained when changes in IgA, IgG and IgM levels and in anti-worm antibody levels were measured in faecal extracts following a single infection with 5,000 N. brasiliensis larvae. In this experiment the results recorded are mean values for a single group

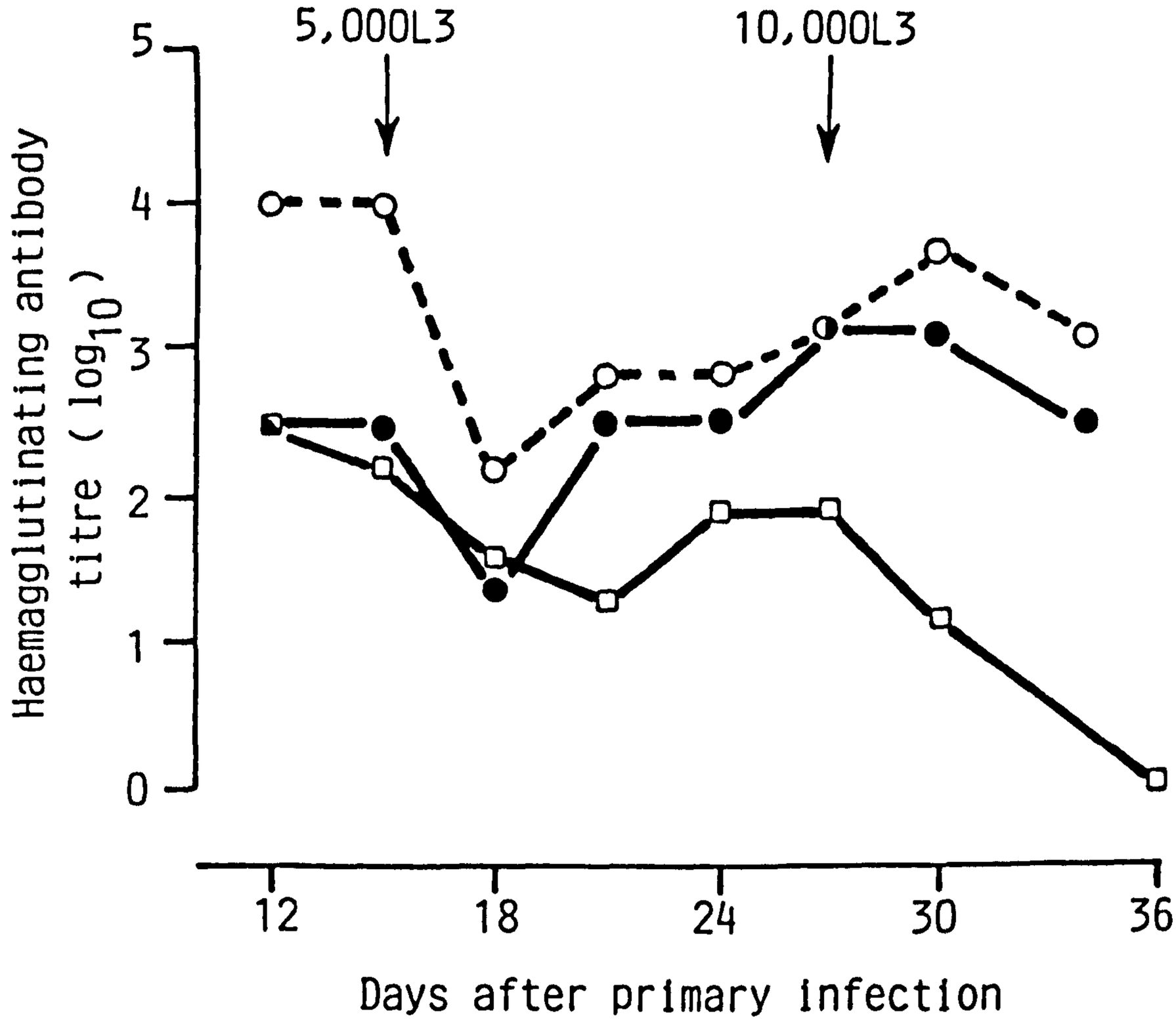

Fig. 2.3. Haemagglutinating antibody titres in faecal extracts of rats following first, second and third infections with N. brasiliensis.

——●—— ASE, -----o----- ESP, ——□—— L3SE

of 25 infected rats throughout the experimental period (0 - 30 DAI). This allowed changes in immunoglobulin levels and in anti-worm antibody levels to be compared each day in the same group of rats in contrast to Experiment 1 where different groups of five infected rats were used at three or six day intervals during the experiment.

Experimental Design

Thirty rats were used. Twenty five were infected with 5,000 L3 N. brasiliensis and the remaining 5 acted as non-infected controls. The infected rats were divided into 5 groups of 5 animals. Faeces from each group were collected each day for 22 DAI then every second day till 30 DAI.

Materials and Methods

Immunoglobulin levels and anti-parasite antibody titres against ASE, ESP and L3SE antigens were estimated as described in Experiment 1.

Results

Immunoglobulin Levels in Faecal Extracts

Changes in the levels of total IgA, IgG and IgM measured by radial immunodiffusion and exprossed as percentages of the total protein content of 1 g faeces are shown in Fig. 2.4

IgA levels increased steadily after infection and showed two peaks at 13 DAI and between 18 and 24 DAI, and declined to pre-infection level by 30 DAI. Concentrations of IgG in faecal globulins rose sharply after infection, reached maximum levels at 18 DAI, with an earlier peak at 9 DAI and declined sharply after the 24th day of infection. Only extremely low levels of IgM were recorded, except between 6 and 12 DAI.

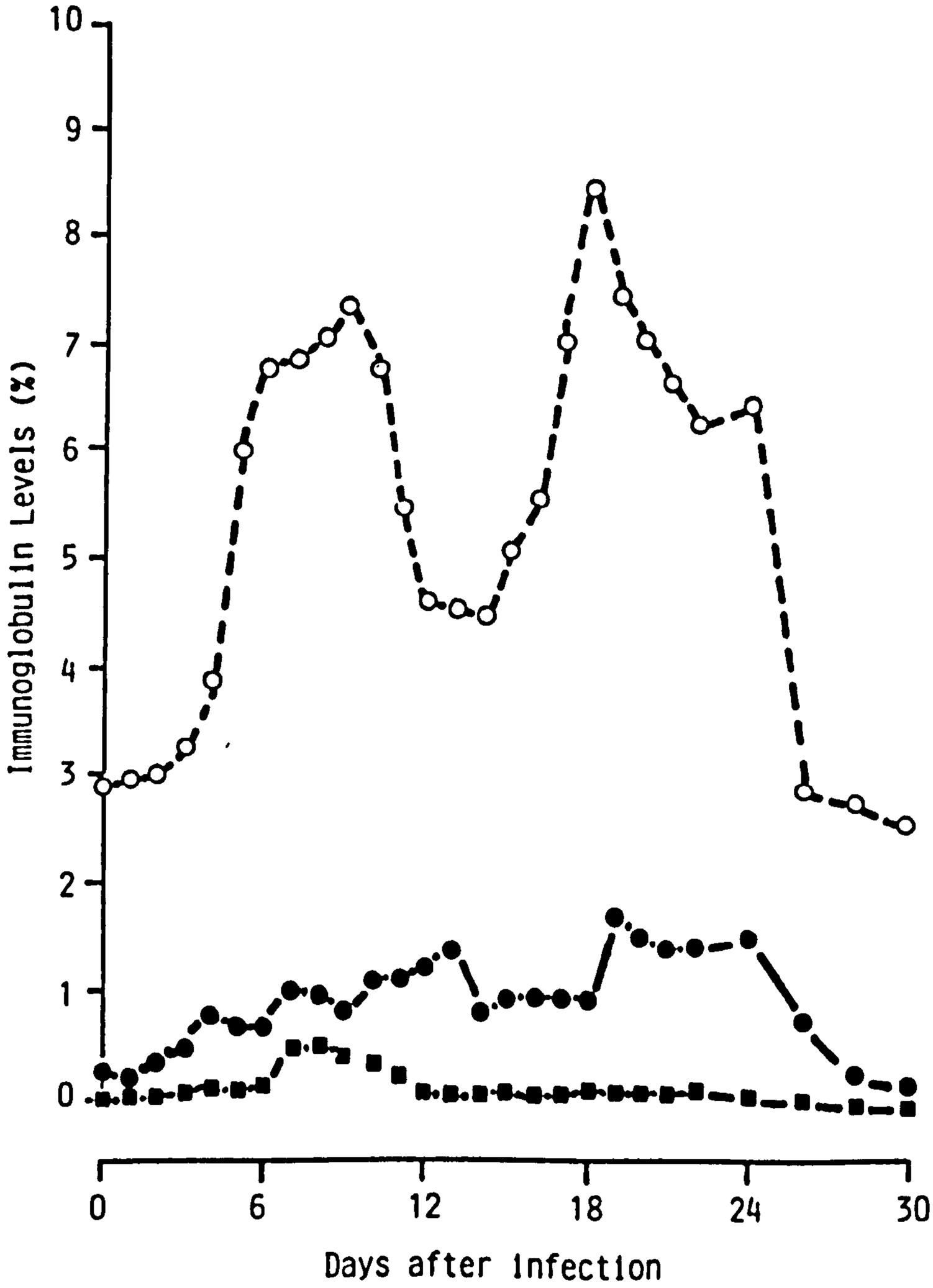

Fig. 2.4. Total immunoglobulin levels in faecal extracts of rats following infection with 5,000 N. brasiliensis.

—●— IgA, ------o------ IgG, ——■—— IgM

These results were very similar to those recorded in Experiment 1 (Fig. 2.1). The main difference was that higher IgG concentrations were recorded in Experiment 2.

Haemagglutinating Antibody Titres

The results of the haemagglutinating antibody measurements are shown in Figure 2.5. The anti-worm antibody levels in the faeces of the rats rose following infection when tested against all three antigens, ESP, ASE and L3SE.

The highest titres were recorded when ESP antigens were used. These were at maximum levels between 10 and 12 DAI. Titres against ESP showed two peaks, at 9 DAI and between 15 and 19 DAI. Antibodies to L3SE antigens were slower to develop and reached maximum level between 9 and 12 DAI at lower titres than antibodies to ESP or ASE antigens.

The results recorded were very similar to those shown in Experiment 1 (Fig. 2.2).

Experiment 3

The results from Experiments 1 and 2 showed that anti-worm antibodies were present in faeces and in serum collected from rats infected with N. brasiliensis. This experiment was designed to ascertain if the antibodies detected in faeces were produced against antigens similar to those stimulating serum antibodies.

Erythrocytes tanned and coated with ASE or ESP antigens were absorbed with faecal extracts collected in Experiments 1 and 2 on 6, 12, 18, 24 and 30 DAI. These absorbed coated cells were used to ascertain haemagglutinating antibody titres in serum samples

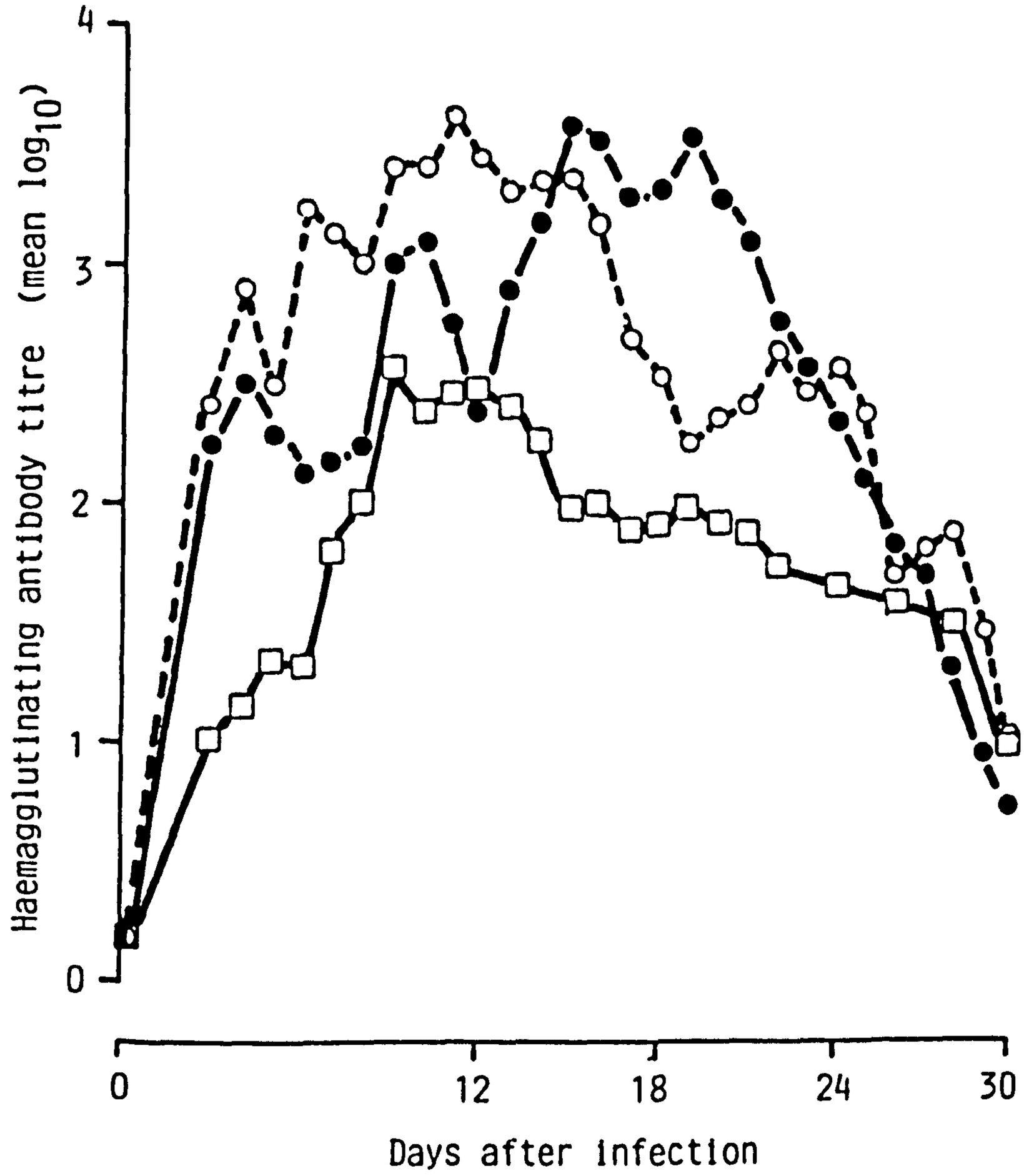

Fig. 2.5. Haemagglutinating antibody titres in faecal extracts of rats following infection with 5,000 N. brasiliensis.

-----o----- ESP, ——●—— ASE, ——□—— L3SE

Table 2.3

Haemagglutinating Antibody Titres Against ASE and ESP Antigens Absorbed with Corpoantibodies and with Unabsorbed Antigens in Serum of Rats Infected with 5,000 N. brasiliensis

Source of Antibody		Titres with Unabsorbed Antigens		Haemagglutinating antibody titres against ASE or ESP antigens absorbed with coproantibodies from infected rats on DAI											
				6		12		18		24		30			
		ASE	ESP	ASE	ESP	ASE	ESP	ASE	ESP	ASE	ESP	ASE	ESP		
Sera Expt.1															
6 DAI	a	320	320	4	2										
	b			4	2										
12 DAI	a	1024	80			2	2								
	b					2	2								
18 DAI	a	2560	60					2450	60						
	b							2348	60						
24 DAI	a	2560	1280							2304	1024				
	b									2400	980				
30 DAI	a	1280	512									1024	410		
	b											1280	512		

(a) Represents titres against coated erythrocytes absorbed with faecal extracts from Experiment 1, and
(b) represents titres with coated erythrocytes absorbed with faecal extracts from Experiment 2.

collected on 6, 12, 18, 24 and 30 DAI in Experiment 1. Faecal extracts prepared in Experiments 1 and 2 on 6, 12, 18, 24 and 30 DAI were also tested for haemagglutinating antibodies using coated red cells absorbed with coproantibody. Haemagglutinating antibody titres obtained against coproantibody absorbed antigens were compared with similar titres observed when unabsorbed antigen coated cells were used in the tests.

Materials and Methods

Absorption of Tanned Antigen Coated Erythrocytes with Coproantibody

Sheep red cells were tanned and coated with ASE or ES products antigens as previously described. The antigen coated red cells (0.7 ml in a universal bottle) were then washed three times with PBS containing 1% bovine serum albumin. These cells were then suspended in 50 ml PBS. One volume of this suspension was incubated with an equal volume of faecal globulin, prepared as described in General Materials and Methods. After 30 minutes, the cells were centrifuged and resuspended in the initial volume of PBS containing 1% bovine serum albumin. These cells were used in the tests.

Haemagglutination Tests

These tests were carried out as in the previous experiments.

Results

Results of haemagglutinating antibody titres in sera of infected rats against red cells coated with ASE or ESP antigens which had previously been absorbed with coproantibodies are shown in Table 2.3.

Table 2.4

Haemagglutinating Antibody Titres Against ASE and ESP Antigens Absorbed with Coproantibodies and with Unabsorbed Antigens in Faecal Extracts of Rats Infected with 5,000 N. brasiliensis

Source of Antibody		Antibody Titres with Unabsorbed Antigens *		Haemagglutinating antibody titres against ASE or ESP antigens absorbed with coproantibodies from infected rats on DAI									
				6		12		18		24		30	
		ASE	ESP	ASE	ESP	ASE	ESP	ASE	ESP	ASE	ESP	ASE	ESP
Faecal Extracts													
6 DAI	a	160	40	0	0								
	b	180	1650	0	0								
12 DAI	a	320	10240			0	0						
	b	576	7550			0	0						
18 DAI	a	5120	40			0	0						
	b	2300	555			0	0						
24 DAI	a	80	640					0	0				
	b	256	448					0	0				
30 DAI	a	40	380							0	0		
	b	10	20							0	0		

* Results from Experiments 1 and 2
(a) Represents samples from Experiment 1
(b) Represents samples from Experiment 2

Red cells absorbed with faecal extracts from 6 and 12 DAI did not show haemagglutination reactions with sera diluted more than four-fold while erythrocytes coated with the same antigens but not absorbed with coproantibodies gave positive titres with sera diluted up to 1 in 2,500. On the other hand the reactivity of antigen coated cells absorbed with faecal extracts from 18, 24 and 30 DAI were almost the same as with untreated cells. The results were almost identical when sera from Experiment 1 were tested against antigen coated erythrocytes absorbed with coproantibodies in faecal extracts from Experiment 1 or 2.

Table 2.4 shows haemagglutination titres recorded in Experiment 1 and 2 when faecal extracts from 6, 12, 18, 24 and 30 DAI were tested against red cells coated with ASE and ESP antigens and also shows the negative titres obtained when tested against coproantibody absorbed coated red cells, using the same antigens.

Occasionally weak non-specific reactions were observed between ASE coated erythrocytes and faecal extracts from uninfected rats.

In such cases maximum titres were 1:16.

Experiment 4

The results of Experiments 1 and 2 showed that coproantibodies can be detected in rats infected with N. brasiliensis. Experiment 4 was carried out in order to investigate more fully the immunochemical properties of these coproantibodies. Faeces collected from 10 rats on day 9 after infection was used in the investigations.

Materials and Methods

Experimental Animals

Ten rats were infected with 2,000 N. brasiliensis larvae.

Preparation of Faecal Globulins

Faeces was collected for a 24 hour period between day 8 and 9 of infection. Faecal globulins were precipitated from faecal extracts as described in General Materials and Methods.

Gel Filtration of Faecal Globulins

Gel filtration of faecal extracts from infected rats was carried out using Sephadex G200 (Pharmacia, Uppsala, Sweden) columns (10 cm x 2.5 cm) equilibrated at pH 8.0 with a phosphate-NaCl buffer (0.05M Na2HP04, 0.14M NaCl) containing 0.02% sodium azide to inhibit bacterial growth. Sixty mg of faecal globulins in 2 ml of phosphate-NaCl buffer was applied to a column. Four successive fractions of 40 drops were collected at a rate of 10 ml per cm^2 per hour to give four fractions, I, II, III and IV.

Preparation of Antisera

Antisera to normal rat serum, to unfractionated faecal globulins and to faecal IgA (fraction II of the fractionated faecal globulins) were produced by immunisation of adult rabbits weighing approximately 3.5 kg. A homogenate of 100 µg of the appropriate protein in 1 ml solution and 1 ml of Complete Freund's Adjuvant (Difco, East Mosley, Surrey, England) was prepared. This was injected intramuscularly followed 4 and 8 weeks later by booster injections of the same quantity of protein in a homogenate with incomplete Freund's Adjuvant.

Table 2.5

Immunoglobulin Classes and Anti-worm Antibody Activity in Fractions of Faecal Globulins Obtained after Filtration through Sephadex G-200

Fraction No.	Protein Concentration (mg/ml)	Immunoglobulins Detected	Antibody Titres Against Adult Worms ES Products
I	2.5	IgM	1:64
II	4.5	IgA	1:4096
III	14.5	IgG, IgA	1:16
IV	18.0	IgG	1:8
Unfractionated globulins	30	IgA, IgM, IgG	1:1024

The rabbits were bled one week after the last injection. After clotting, the serum was removed by centrifugation and the antiserum stored at -20°C till required.

In addition, a commercial goat antiserum against IgA (Miles Laboratories, USA) and sheep antisera against rat IgG and IgM (Eivai Bios, England) were used.

Protein Estimations

The total protein content of the four fractions prepared above and of normal rat serum and faecal IgA were ascertained by the method of Lowry, et al (1966).

Immunoglobulin Identification

Fractions I, II, III and IV and a sample of unfractionated faecal globulins were tested with anti-rat secretory IgA, anti-rat IgG and anti-rat IgM antisera by the Double Diffusion Method of Ouchterlony (1953).

Antibody titres against worm ESP antigens were measured in unfractionated faecal globulins, in fractions I, II, III and IV by the passive haemagglutination test (Herbert, 1975).

Immunoelectrophoresis Tests

Immunoelectrophoresis tests were carried out using 1% agarose gel in 0.05M barbitone buffer in an adaptation of the method described by Scheidegger (1955).

Results

Protein concentrations, immunoglobulins identified by gel double diffusion and antibody titres to worm ES products in unfractionated faecal globulins and in fractions I, II, III and IV are listed in Table 2.5.

The sample of unfractionated faecal globulins contained IgA, IgG and IgM. Fraction I, the fraction with the highest molecular weight proteins was shown to contain only IgM which has a molecular weight of 900,000. The molecular weight of the dimeric S-IgA is approximately 390,000 and S-IgA was the only immuno-globulin identified in fraction II. Immunoglobulin A and the lower molecular weight protein IgG were identified in fraction III and fraction IV contined IgG only.

Fraction II which gave a positive reaction against antiserum to rat secretory IgA gave the highest antibody titres when tested against adult nematode ESP antigens (Table 2.5). This fraction which contained IgA only was used to immunise rabbits for production of anti-S IgA serum. The results of the electrophoretic tests showed that the precipitin arcs produced between fraction II of the faecal globulins against both anti-sera to fraction II and goat anti-rat IgA monospecific serum appeared to be identical, i.e. fraction II showed electrophoretic mobility typical for IgA (Fig. 2.6).

The results indicated that while IgG was the most abundant immunoglobulin in the faecal globulins (18 mg/ml) it had a very low anti-worm antibody titre. In contrast while the concentration of IgA in faecal globulins was relatively low (4.5 mg/ml), the anti-worm antibody titres of fraction II was relatively high, i.e. the anti-worm antibody activity in the faecal globulins was mainly due to the presence of S-IgA.

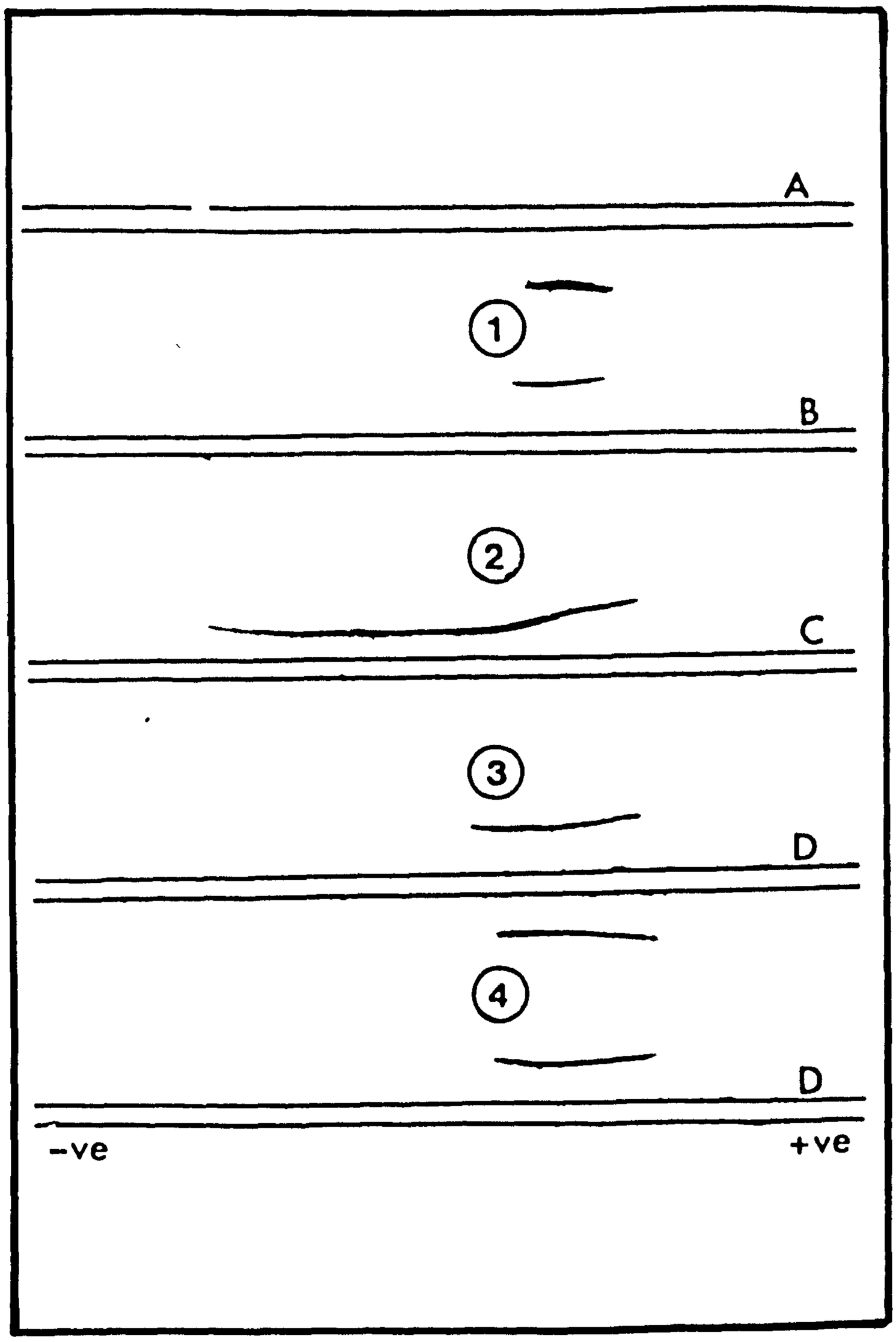

Fig. 2.6. Immunoelectrophoresis of faecal globulins Fraction II (wells 1 and 3), rat serum (well 2) and small intestine mucosal globulins (well 4) against:
goat anti-serum to rat IgA (A and B)
sheep anti-serum to rat IgG (C) and
rabbit anti-serum to faecal globulins Fraction II (D)

Discussion

The results of the experiments described in this chapter strongly suggest that the immunological evaluation of extracts prepared from faeces of N. brasiliensis infected rats can provide information on local immune responses. Changes in faecal haemagglutinating antibody levels against both larval and adult N. brasiliensis antigens were similar to changes in antibody titres in small intestine mucosal extracts following infection. In addition, changes in total faecal IgA, IgG and IgM closely matched changes in immunoglobulin concentrations in intestinal extracts.

Immunoglobulin levels in faecal extracts were calculated as a percentage of the total protein obtained from 1.0 g of faeces and mucosal immunoglobulin values were expressed as a percentage of the total protein in 1.0 g of mucosal tissue. Consequently absolute values of immunoglobulins measured in faecal and small intestinal globulins can not be directly compared but valid comparisons can be made between changes in the immunoglobulin levels in faecal and small intestine globulins.

In infected rats, changes in total IgA, IgG and IgM levels in faecal extracts observed in Experiment 1 (Fig. 2.1) and in Experiment 2 (Fig. 2.3) reflected closely changes in immunoglobulin concentrations observed in small intestine globulins during the same period after infection (Fig. 2.1). These results closely mirror those previously reported by Poulain et al, (1976a) and Sinski and Holmes (1977) who observed maximal levels of total and worm specific

intestinal mucosa IgA at 12 DAI and the highest mucosal IgG concentration on the 18th DAI.

Mucosal IgA levels reached maximum at 12 DAI and then decreased steadily during the remainder of the study. In contrast faecal IgA in the first experiment reached the highest concentration at 18 DAI and in the second experiment there were two concentration peaks, at 12 DAI and between 18 - 24 DAI. This was clearly seen in Experiment 2 where faecal globulin immunoglobulin levels were monitored daily (Fig. 2.3). The reason for this is not clear but it is interesting that recent studies on the origin of S-IgA have shown that considerable quantities of IgA are rapidly removed from the serum by the liver and after attachment of the secretory piece, are transported as S-IgA in bile to the intestine (Jackson et al, 1978; Lemaitre-Coelho et al, 1978 and Orlans, Peppard, Reynolds and Hall, 1978). Biliary transport of serum derived IgA may therefore represent a significant proportion of intestinal antibody and Lemaitre-Coelho et al (1977) have suggested that in the upper small intestine approximately 90% of the specific IgA antibodies originate from the bile.

Changes in IgG levels in faecal extracts and in mucosal extracts were remarkably similar over the period studied. Mucosal IgG levels showed two peaks, at 6 and 18 DAI, and faecal IgG concentrations showed peaks at 6 to 10 DAI and about 24 DAI.

Faecal IgM levels showed a slight rise at about the time of arrival of the worms in the small intestine. However the faecal IgM levels fell back to very low levels in contrast to the mucosal

IgM concentrations which rose steadily then showed a rapid rise after day 18 of infection, reaching a maximum at day 24. This difference may be explained by the fact that IgM is thought to be relatively susceptible to proteolytic degradation in the gut, compared with IgA. Antibody titres in faecal and mucosal extracts specific to adult worm somatic and metabolic antigens and to infective larval somatic antigens showed similar changes over the period from infection to 30 DAI. There was some evidence of a more rapid fall in antibody titres in faecal samples compared with mucosal samples when titrated against adult somatic antigens. Changes in serum antibody titres against larval or adult worm somatic antigens generally mirrored the changes seen in mucosal or faecal extract levels. However serum antibodies reacting with ESP antigens decreased between 6 and 18 DAI while titres of mucosal and faecal antibodies specific to these antigens rose rapidly between 6 and 12 DAI (Fig. 2.2). These differences might be explained by the transport of protective antibodies from serum through the mucosa into the gut (Urquhart et al, 1965).

The results of haemagglutination tests using erythrocytes absorbed with coproantibodies to estimate serum haemagglutination levels (Experiment 3) indicated that coproantibodies appearing in faeces during the first 12 days of infection were the same as or elicited by the same antigens as the antibodies detected in serum. In contrast, antibodies detected in faecal extracts later in infection were different from those circulating in serum at the same time.

The results from Experiment 4 showed that although not the most abundant immunoglobulin in faecal globulins, secretory IgA when tested against ESP antigen in haemagglutination tests proved to have the highest immunological activity.

The results discussed in Chapter 1 showed that the Peyer's patch response to N. brasiliensis infections was maximal at 9 DAI and IgA plasma cell proliferation in the intestinal mucosa was greatest between 13 and 16 DAI. In this chapter it was found that following N. brasiliensis infection, immunoglobulin levels in serum, intestinal mucosa and in faecal globulins increased in response to infection. Immunoglobulin A levels in mucosal and faecal extracts were at maximum levels between 12 and 24 DAI. These manifestations of immune responses to N. brasiliensis support the hypothesis that primary stimulation of immunoglobulin producing cells occurs mainly in Peyer's patches and that the immunoblasts subsequently migrate to the intestinal lamina propria where they secrete immunoglobulin into the lumen of the gut.

In view of the close correlation between changes in copro-antibody levels in rats following N. brasiliensis infections and the other immunological parameters reported above it would appear that coproantibody measurements can be used to monitor local antibody responses to intestinal parasite infections and that such measurements provide a useful index of local intestinal immunity. The results however do not give any indication as to how antibodies produced locally in the intestine act against the parasites.

The techniques used in this chapter are applied in experiments described in later chapters further to investigate the possible significance of local immune responses to N. brasiliensis infections in both the intestine and in the lungs.

Chapter 3

Local Antibody Responses in the Lungs and Intestines of Rats Following Infection with Normal or Irradiated Larvae of *Nippostrongylus brasiliensis*

Introduction

The experiments discussed in Chapter 2 showed that antibodies can be detected in the faeces of rats infected with N. brasiliensis. It was shown that haemagglutinin levels in faecal extracts detected by adult worm or infective larvae somatic extracts and adult worm excretory-secretory products rose markedly about the time of worm expulsion following primary N. brasiliensis infections. It seems reasonable to postulate that these coproantibody measurements provide a useful index of local intestinal responses to the parasite. In order to investigate further possible correlations between local immune responses and resistance to gastrointestinal helminths, the following experiment was conducted in which coproantibody measurements were made in rats following vaccination with irradiated N. brasiliensis larvae and later challenged with non-irradiated larvae.

Cobalt 60 irradiated N. brasiliensis have been previously used to stimulate immunity in rats against reinfection with N. brasiliensis (Prochazka and Mulligan, 1965). In addition, it has been shown that, in rats infected with irradiated larvae, the percentage of infective larvae destroyed in the lungs increases proportionately with increases in the irradiation dose (Jennings, Mulligan and Urquhart, 1963). Therefore, because of the possible importance of local humoral responses in the lungs, lung extracts were also assayed for anti-parasite antibodies.

Table 3.1

Experimental Plan

Group	Larval Irradiation (krad)	No. of Rats	No. Killed 10 DAI	No. Challenged 15 DAI	No. Killed 10 Days After Challenge
A	0	10	5	5	5
B	10	10	5	5	5
C	20	10	5	5	5
D	40	10	5	5	5
E	80	10	5	5	5
F	120	10	5	5	5
G	-	-	-	5	5

Materials and Methods

Experimental Design

Sixty five rats were used. Groups of 10 were infected with larval suspensions (2,000 larvae per rat) which had been irradiated as shown in Table 3.1. Ten days later 5 animals from each group were killed and the mean worm burdens per group (A - F) were calculated. The remaining 5 rats in each group were challenged with 5,000 normal (non-irradiated) N. brasiliensis larvae per rat 15 days after the initial infection, together with a group of 5 clean rats (G) which acted as challenge controls. Ten days post-challenge these rats were killed and the mean worm burdens after challenge in each group were determined.

Irradiation of Infective Larvae

Third stage N. brasiliensis larvae were irradiated using a Cobalt-60 source (Nuclear Engineering, Ltd., Reading, England) as described by Jennings et al (1963).

The 'Hot-Spot' type ^{60}Co source was accurately calibrated both by a simplified form of the Fricke method (Allen, 1961) and by Thermaluminescent Dosimetry (Scottish Reactor Research Centre, East Kilbride).

Parasitological Techniques

The methods used for recovering and counting adult worms and for conducting faecal egg counts were as already described.

Passive Haemagglutination Tests

Tests were carried out as already described on faecal extracts prepared from faeces excreted over a 24 hour period from each group of rats every third day of the experiment. Tests were

Table 3.2

Effect of dose of irradiation on development and immunogencitiy of *N. brasiliensis* larvae

	X-ray dose to larvae (krad)	Adult Worm Burdens on 10 DAI * (± SE)	P **	Challenge on 15 DAI	Worm burdens on 10th Day After Challenge * (± SE)	P **	% *** of Protection
A	0	258 ± 36		5,000 L_3	30 ± 15	N.S.	98.3
B	10	207 ± 24	N.S.	5,000 L_3	18 ± 8	N.S.	99.4
C	20	162 ± 29	N.S.	5,000 L_3	22 ± 8	N.S.	98.8
D	40	168 ± 53	N.S.	5,000 L_3	12 ± 5	N.S.	99.3
E	80	72 ± 28	< 0.01	5,000 L_3	6 ± 2	N.S.	98.7
F	120	5 ± 2	< 0.001	5,000 L_3	1 ± 1	N.S.	99.9
G	-	-	-	5,000 L_3	1780 ± 121		

* Mean of 5 rats

** Results of analysis by student's T-test; A vs. B, C, D, E, F

*** % of worm burden reduction compared to challenge control (G).

also carried out on lung extracts and on serum samples prepared from lungs and blood collected at necropsy. Infective larval somatic extracts (L3SE) and adult worm excretory/secretory products (ESP) were used as antigens in the haemagglutination tests.

Results

Worm Burdens and Faecal Egg Counts

N. brasiliensis worm burdens and the effect of irradiation dose on development and immunogenicity of *N. brasiliensis* larvae is shown in Table 3.2. Faecal egg counts are shown in Fig. 3.1.

The results in Table 3.2 indicate that very little attenuation was caused by an irradiation dose of 10 krad since the number of worms recovered on 10 DAI was almost the same as in rats infected with intact larvae.

Although lower worm burdens were found in groups infected with 20 or 40 krad irradiated larvae (Groups C and D) compared with the rats infected with intact larvae (Group A), the differences were not statistically significant.

However, with doses of 80 and 120 krad significant attenuation occurred, as manifested by a reduction in the worm burden of 62% and 98% in Groups E and F respectively compared to Group A.

Although the numbers of worms found on 10 DAI in Groups B, C and D were only slightly different from Group A, the nematode egg output in all these groups was markedly reduced. Thus, in rats infected with larvae irradiated at 20 krad (Group C) or 40 krad (Group D), maximal faecal egg counts were only 45% and 14% respectively of those recorded in Group A (Fig. 3.1). In faeces of rats infected

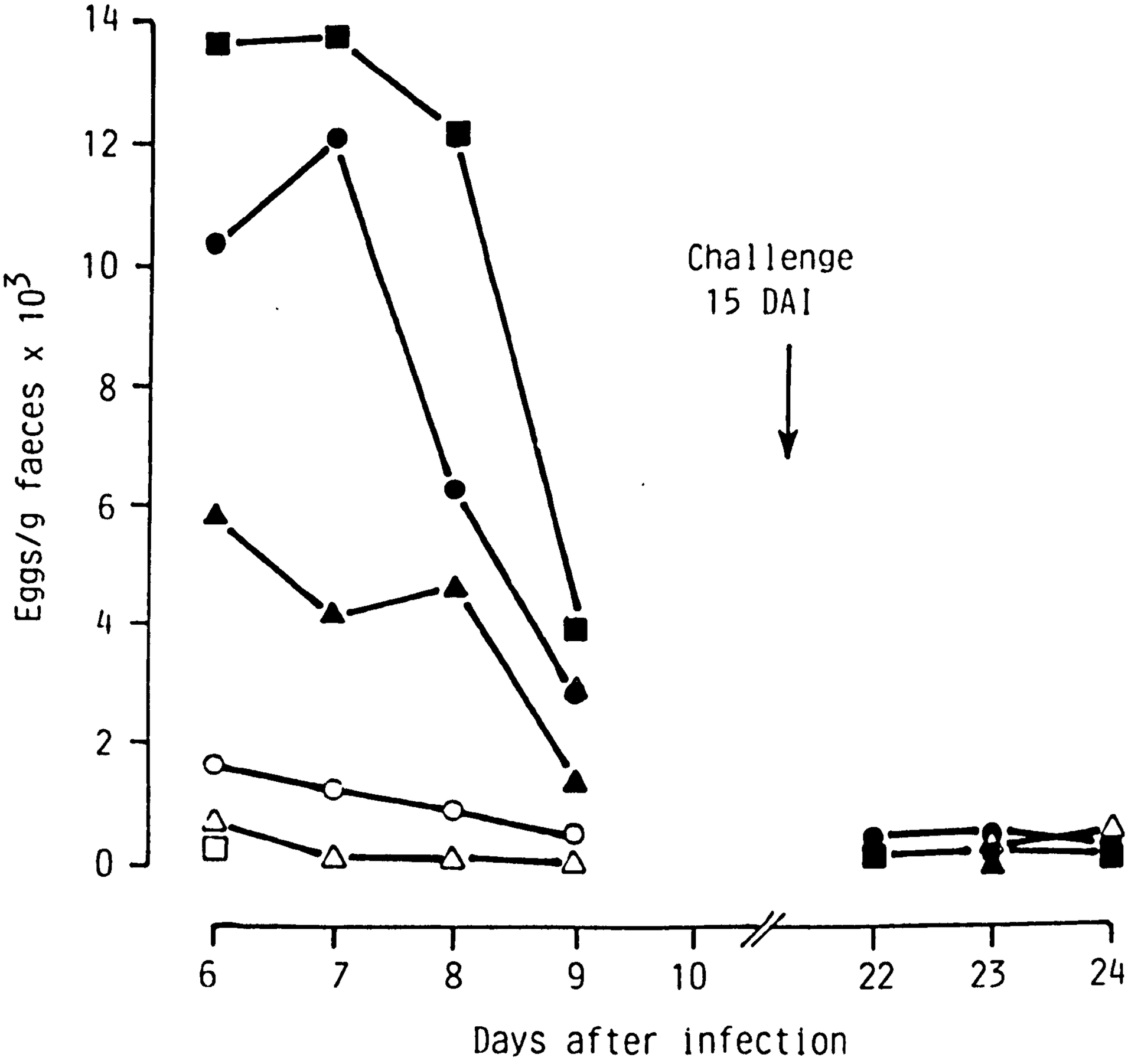

Fig. 3.1. N. brasiliensis faecal egg counts in rats following infection with normal or irradiated larvae

■ = group A (normal larvae;
● = group B (10 krad)
▲ = group C (20 krad)
○ = group D (40 krad)
△ = group E (80 krad)
□ = group F (120 krad)

with larvae irradiated with 120 krad nematode eggs were detected only on 6 DAI and their number/g of faeces was only 2% of those of Group A at that time.

Effect of Different Doses of Irradiation on Larval Immunogenicity

The number of worms detected in the small intestines of vaccinated rats 10 days after challenge with 5,000 normal larvae were very low in all groups and did not exceed 1% of the challenge dose, whilst in challenge control rats infected with the same dose of larvae (Group G), more than 35% of the infective dose became established. In the vaccinated rats, nematode eggs were only observed after challenge in Groups A, B and C and their number per gramme of faeces did not exceed 400, while in the challenge control group (G), e.p.g. values fluctuated between 64,000 and 84,000.

Haemagglutinating Antibody Levels in Lung Extracts and in Serum

The results are shown in Fig. 3.2.

In lung extracts obtained from rats killed 10 days after primary infection with normal or 10 and 20 krad irradiated larvae (Groups A, B and C) no adult worm ESP specific antibodies were detected. However, low and variable levels of infective larval specific antibodies were detected in lung extracts from Group D and in the groups infected with larvae irradiated at 80 or 120 krad (Groups E and F), haemagglutinins reacting with larval antigen were commonly present and showed high titres.

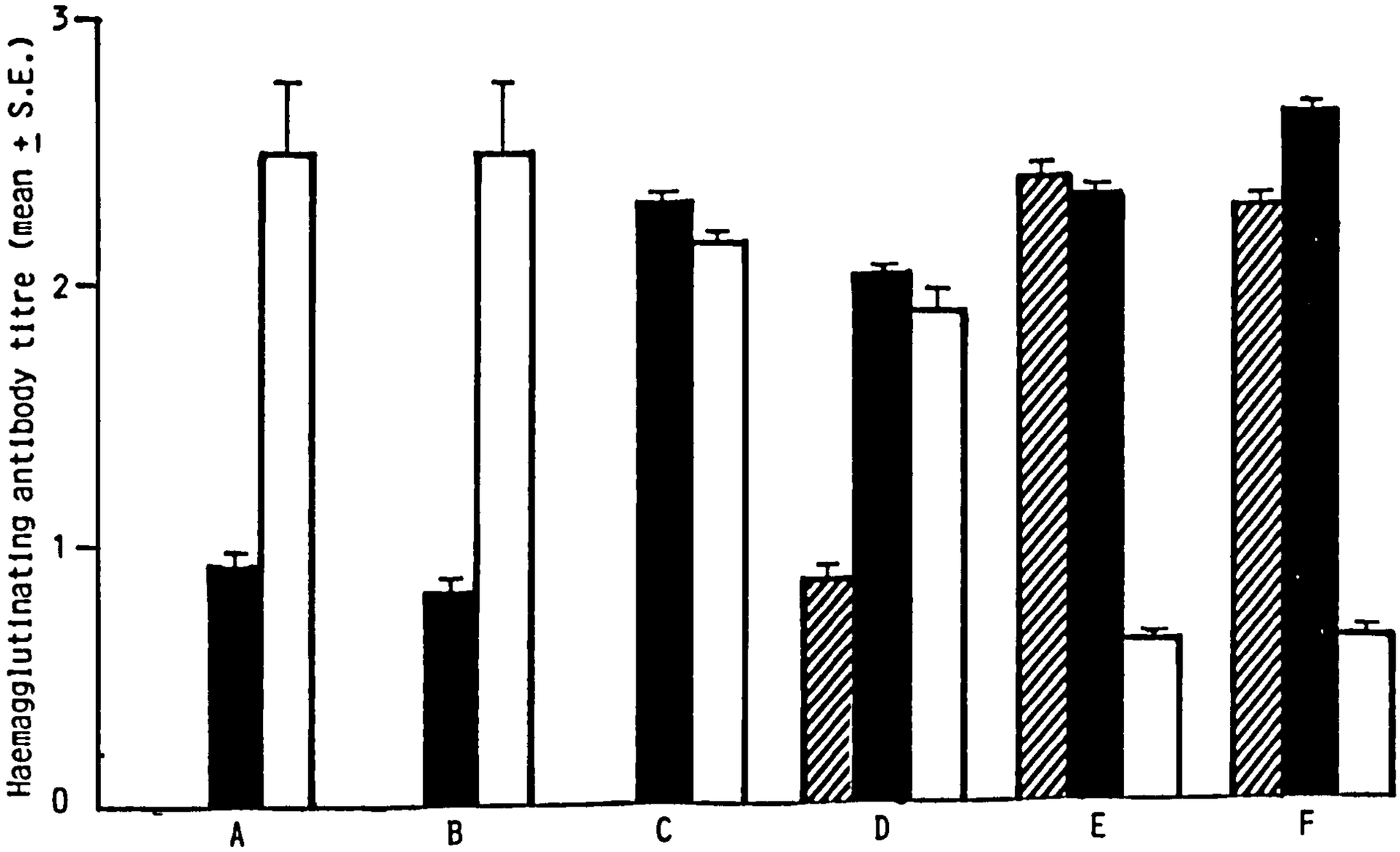

Fig. 3.2. Haemagglutinating antibody titres in serum and lung extracts detected with L3SE antigens. Open bars = serum 10 DAI, Hatched bars = lung extracts 10 DAI: Shadowed bars = lung extracts 10 days after challenge. A - F = groups of animals.

In marked contrast, the highest haemagglutinating antibody titres to larval antigens detected in the sera were found in rats infected with normal or 10 krad irradiated larvae. Anti-L3SE serum haemagglutinating titres were negatively correlated with larval irradiation dose rate.

After challenge, antibodies to larval antigens were observed in the lungs of all vaccinated rats with the highest levels in the group vaccinated with 120 krad irradiated larvae (Group F). However, at no time were antibodies to adult worm ESP antigens detectable in lung extracts. Results of lung extract and serum haemagglutinating antibody titres in individual groups are shown in Appendix C, Tables 2 and 3 respectively.

Haemagglutinating Antibody Levels in Faecal Extracts

The results are shown in Figures 3.3 and 3.4.

After primary infection, a clear correlation was observed between levels of antibody detected with adult worm ES products and the intensity of infection as measured by the numbers of recoverable adult worms and their fecundity. Thus, the highest coproantibody titres were observed in Groups A and B between 6 and 9 DAI. In groups infected with more highly attenuated larvae, maximal antibody levels were much lower and inversely proportional to the irradiation dose. The only possible exception was observed in Group D infected with larvae irradiated at 40 krad where haemagglutinin levels were higher than in the group infected with larvae attenuated with a dose of 20 krad. It should be noted, however, that in Group D the adult worm burden was also high (Table 3.2).

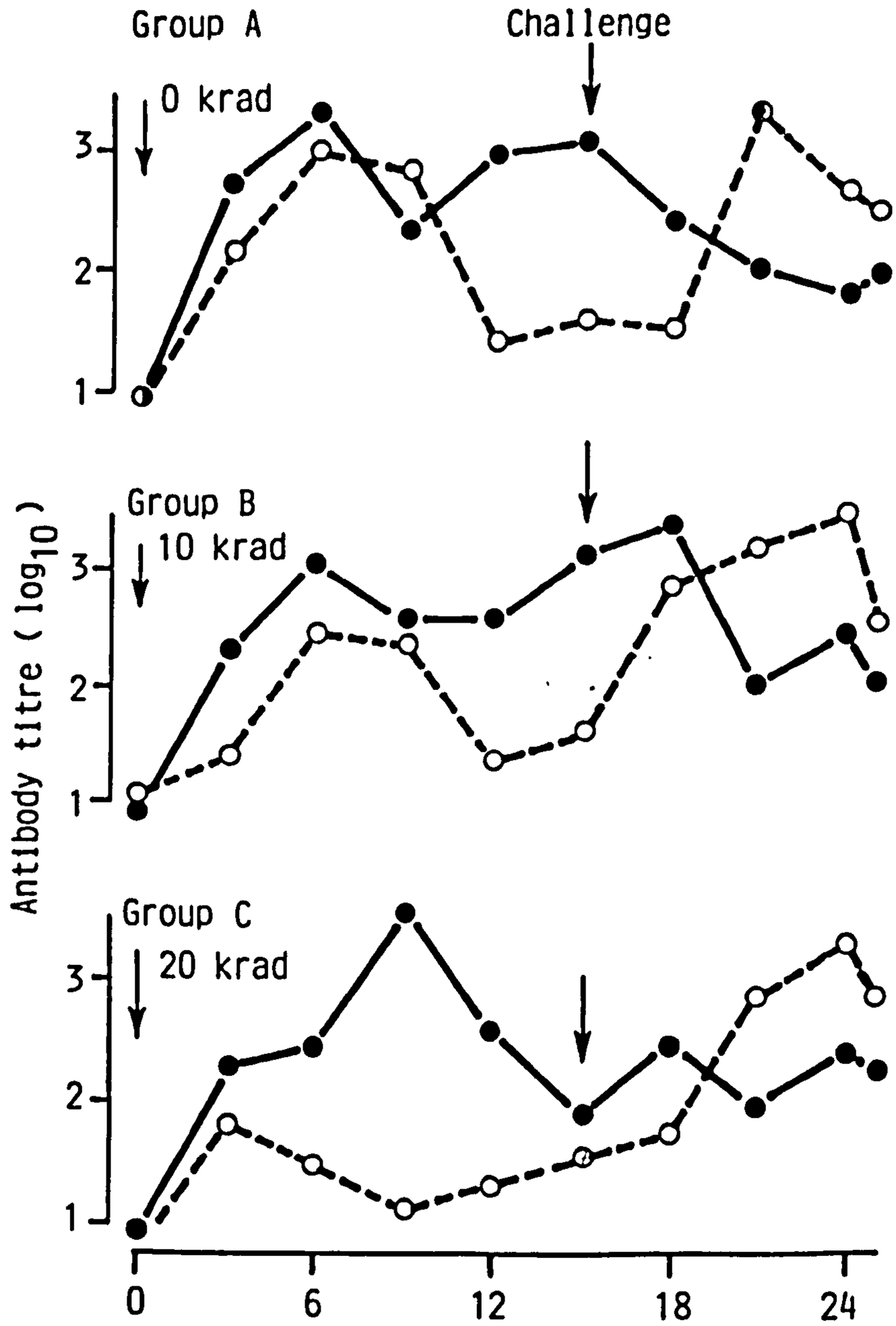

Fig. 3.3. Haemagglutinating antibody titres (log 10) in faecal extracts of rats following infection with normal or irradiated N. brasiliensis larvae.

-----o----- represents antibodies detected with adult worm ESP antigens

——●—— represents antibodies detected with larval somatic antigens, L3SE

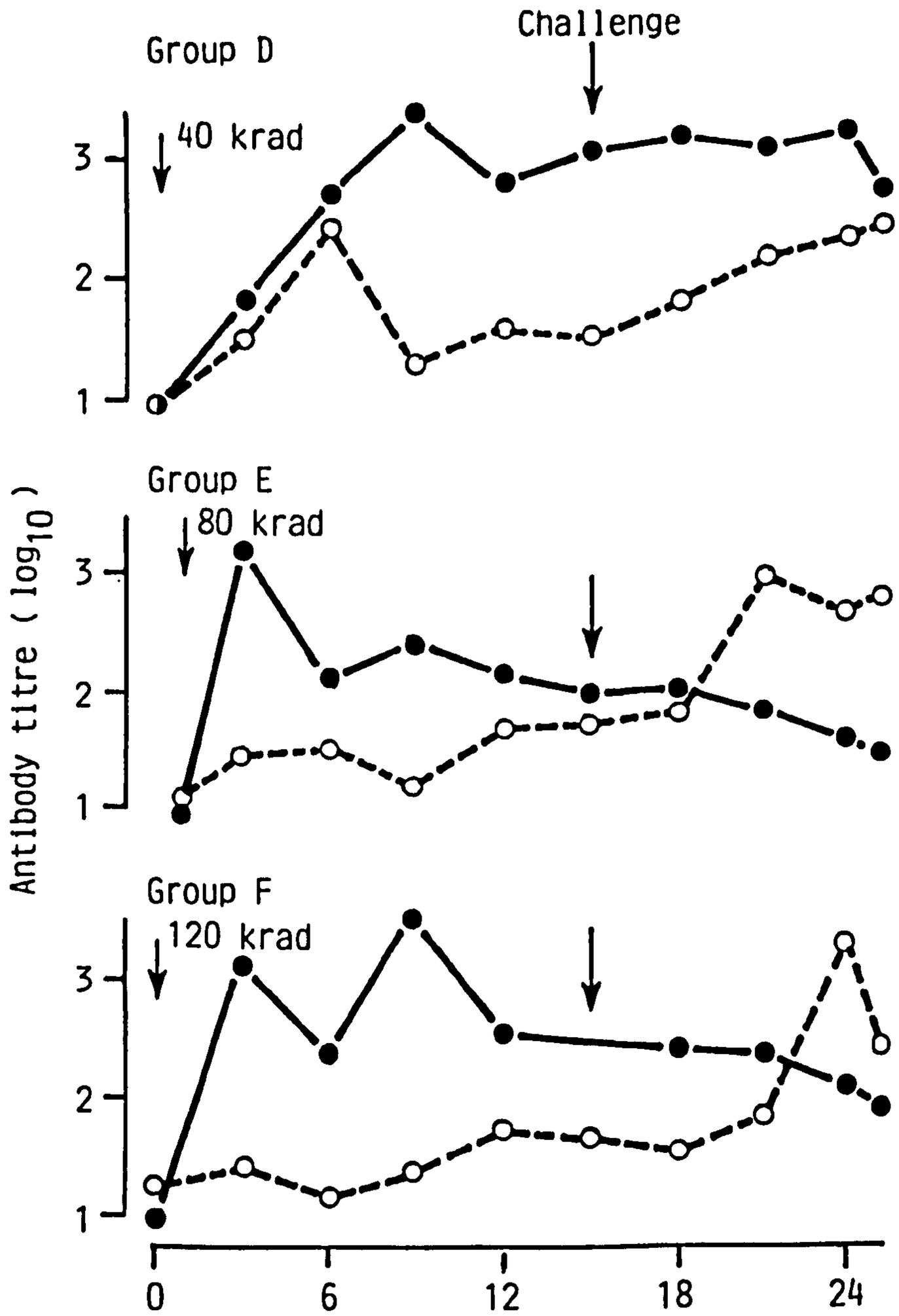

Fig. 3.4. Haemagglutinating antibody titres (log 10) in faecal extracts of rats following infection with irradiated N. brasiliensis larvae.

------o------ represents antibodies detected with adult worm ESP antigens

——————●—————— represents antibodies detected with larval somatic antigens, L3SE

After challenge, all groups showed a rapid and closely similar rise in anti-adult worm ESP antibodies.

Antibodies reacting with larval antigens in Groups A and B showed similar levels to those detected with adult worm ESP antigens during the first 9 days of the experiment and, until 18 DAI their titres remained high, but then declined.

In the remaining groups larval specific antibody levels were higher than those against adult worm ESP antigens until day 18 of the experiment and, in contrast to those reacting with adult worm antigens they did not increase after challenge infection (Figs 3.3 and 3.4).

Discussion

The data presented in this chapter clearly indicate that the exposure of third stage larvae of N. brasiliensis to Cobalt-60 gamma irradiation had a profound effect on their subsequent development in the host and caused changes in antigenic stimulation of the host's immunological system without altering their ability to elicit protective immunity.

As the level of irradiation to which the larvae were exposed was increased, the parasite egg output, as well as the recovery of adult worm burdens from the intestine at 10 DAI, were reduced. Immunogenic properties however, remained the same and sometimes slightly better than intact larvae even after 120 krad irradiation. Such results are closely similar to those previously reported by Prochazka and Mulligan, (1965). Also Hagan, Behnke

and Parish (1981) using Cobalt-60 radiation attenuated larvae of Nematospiroides dubius reported that levels of resistance to a subsequent challenge infection were often higher in mice immunised with irradiated larvae than after immunisation with intact larvae.

Haemagglutinating antibody titres against L3SE larval antigens in lung and faecal extracts indicated that irradiated larvae elicit a strong local humoral response. Moreover, the level of the response in each tissue was apparently related to its level of stimulation. This in turn was linked to the degree of irradiation used to attenuate the larvae. Thus the highest anti-L3SE copro-antibody titres were observed in rats following vaccination in groups inoculated with normal larvae or larvae irradiated with 10 krad in which large numbers of parasites had reached the intestine. In contrast, the highest titres in lung extracts were observed in rats vaccinated with larvae irradiated with 80 krad or 120 krad in which the majority of the parasites were apparently retained in the lungs. This latter finding is similar to that previously reported by Jennings et al (1963) that the percentage of the total worm burden of N. brasiliensis retained in the lungs increases in proportion to the attenuating dose of irradiation.

Following challenge, all the groups of vaccinated rats showed a very high level of protection (i.e. > 98%) regardless of the dose of irradiation (0 - 120 krad) used to attenuate the larvae.

Several aspects of these results are noteworthy. First, despite the differences in antigenic stimulation between the lungs and intestine which, as explained above, varied with the radiation

dose, the host's ability to develop a high degree of protective immunity was not altered. These findings tend to highlight the continuing uncertainty regarding the respective roles of the pneumonic and intestinal phases in stimulating protective immunity to Nippostrongylus brasiliensis. However, there is now considerable evidence that the intestinal phase is critical for the induction of protection. For example, Prochazka and Mulligan (1965) using irradiated larvae, showed that rats given larvae exposed to X-ray doses of 0, 50 or 100 krad had positive faecal egg counts and strong protection against challenge, whilst rats given larvae irradiated with 180 krad had negative egg counts and had no protection against challenge. Ogilvie (1965a),during a series of experiments in which rats were immunised with adult worms and challenged with subcutaneous L3, found that as few as 10 female worms could induce a level of protection of about 90 percent against a challenge infection of 500 larvae.

It is also worth noting that in the present experiment, there was a very close correlation between anti-ESP coproantibody levels and the number of worms recovered from the intestines following a primary infection. Thus, in rats receiving larvae irradiated with 120 krad in which very few worms reached the intestine, only low levels of anti-ESP coproantibody were detectable, whilst in groups with high worm burdens, coproantibody levels were correspondingly increased. Compared with anti-ESP coproantibody levels anti-L3SE faecal antibody titres following primary infection were high in all groups vaccinated with irradiated larvae. In particular Groups E and F had the highest anti-L3SE coproantibody

titres 3 days after vaccination. Sensitisation of lymphoblasts in the bronchial submucosa and their subsequent transport to other mucosal sites as immunoglobulin secreting cells via a common mucosal associated lymphoid tissue system could explain this phenomenon. The existence of such a common mucosal immunological system has been demonstrated by many workers (see review of literature in the General Introduction). In this respect it is significant that Groups E and F also had high lung anti-L3SE antibody titres 10 days after vaccination.

However, after challenge, levels of coproantibody to adult ESP antigens increased markedly and to a similar degree in all groups, clearly reflecting the high levels of protection against the challenge infection induced in all the immunised rats. These findings support the observations in Chapter 2 that coproantibody levels are closely correlated with protective responses in the intestine.

While anti-L3SE coproantibody levels in Groups B, C and D showed small increases immediately following challenge, titres decreased between 5 and 11 days after challenge. In contrast, anti-ESP coproantibody levels increased after challenge in all groups to reach broadly similar levels 9 days after challenge. These high anti-ESP antibody titres were related to the high degree of protection observed in all groups. This leads to the suggestion that the high anti-ESP antibody response is the result of strong local immunological memory since only small numbers of adult worms were recovered from the intestines of rats after challenge.

The detection of anti-parasite antibodies in lung extracts of rats vaccinated with larvae attenuated with 40, 80 or 120 krad and in all groups (A - F) following challenge is an interesting observation which does not appear to have been reported previously.

However, it has been previously reported that N. brasiliensis larvae do elicit a strong inflammatory response in the lungs of infected rats and these responses are particularly severe when inactivated larvae are administered (Salman and Brown, 1980). Furthermore, these workers reported the presence of IgA containing cells around parasite-related granulomata in the lung.

From the results described in this chapter, it is apparent that the antibody titres in lung extracts are negatively correlated with titres in serum. Such findings support the view that antibodies detectable in lung extracts are locally produced, possibly by the bronchus-associated lymphoid tissue (BALT) system, and not directly related to systemic antibody levels. Recent reports have indicated that stimulation of local immune responses is not restricted to gut-associated lymphoid tissues (GALT) as the administration of antigens to the lungs can induce local immunological reactions. Bronchus associated lymphoid tissue contains a high number of IgA precursor cells (Rudzik et al, 1975). These cells resemble similar cells in Peyer's patches and in the intestinal lamina propria. It is therefore significant that several workers have shown that the lungs are a major site of parasite destruction following N. brasiliensis challenge of hyperimmune rats (Love, Kelly and Dineen, 1974; Love, 1975; Salman and Brown, 1980).

The results of this experiment support the findings reported in Chapter 2 and show that coproantibody levels closely reflect humoral anti-parasite responses in the intestine. The results also show that low levels of antigenic stimulation in the intestine can elicit a high degree of protective immunity and that anti-parasite antibodies can be detected in the lungs of vaccinated and challenged rats.

The possible significance of bronchial and enteric antibodies in conferring protection against N. brasiliensis infections is investigated in greater detail in the next chapter.

Chapter 4

The Influence of *Trypanosoma brucei* Infection on Local and Systemic Immune Responses to *Nippostrongylus brasiliensis* Infection in the Rat after Primary Infection and Following Challenge

Introduction

The results of the experiments previously described in this study have shown that anti-parasite antibodies can be detected in the faeces of N. brasiliensis infected rats and it has been suggested that such coproantibody measurements are a convenient measurement of local intestinal responses. It has also been demonstrated that anti-parasite antibodies can be detected in the lungs of infected rats. However the significance of enteric and bronchial antibodies, both in the development of immunity to primary infections and in conferring protection against challenge, is unknown.

In order to investigate further the possible role of local intestinal immunity on the development of resistance in N. brasiliensis infected rats, experiments were conducted in which local and systemic humoral responses to N. brasiliensis infections were measured in normal and immunosuppressed rats.

Chemically induced immuno-suppression has been used by several workers in the study of immune mechanisms in rats infected with N. brasiliensis. Ogilvie (1965b)treated rats with cortisone derivatives to inhibit resistance to N. brasiliensis infection and Henney, Maclean and Mulligan, (1971) used cortisone treated rats to study N. brasiliensis metabolic activity in immune animals. However these substances were not considered to be appropriate immunosuppressive agents in the present study since corticosteroids are known to exert a number of non-specific effects on the host and to influence both humoral and cellular immune responses.

It is now well established that a number of protozoan infections exert important immunosuppressive effects on the host (see review by Terry, 1977). In particular, this has been investigated in considerable detail in animals infected with African trypanosomiasis. Urquhart, Murray, Murray, Jennings and Bate (1973) and Maclean (1977) demonstrated that trypanosome-induced immunosuppression of rats caused a significant delay in the expulsion of N. brasiliensis infections. Since it is known that trypanosome-induced immunosuppression particularly affects humoral immune responses (see review by Terry, 1977) it seemed that such a system would provide a useful model for examining the role of local humoral responses in rats infected with N. brasiliensis.

It has been shown that one of the most significant effects associated with trypanosome-induced immunosuppression is an elevation of total serum immunoglobulin levels, particularly IgM (Houba, Brown and Allison, 1969; Clarkson, 1976).

The role played by antibodies of different immunoglobulin classes in protecting rats against N. brasiliensis infections has not been elucidated. However, there is evidence that serum precipitating antibodies are mostly of the IgG class (Jones and Ogilvie, 1971; Sinski and Holmes, 1977, 1978), while after primary infection both total and anti-parasite intestinal mucosa IgA concentrations have been shown to increase (Sinski and Holmes, 1977). However while elevated intestinal IgA levels show a temporal relationship with developing host immunity to N. brasiliensis, the involvement of local IgA secretions in worm expulsion is still speculative.

There is some evidence that, especially during secondary and multiple infections with N. brasiliensis, the bronchial lymphoid system may play an important role in host immunity and may be a site of local antibody synthesis (Salman and Brown, 1980). However, until now, there has been no information on lung immunoglobulin levels following infection with N. brasiliensis. In order to investigate and compare changes in local and systemic humoral responses in N. brasiliensis infections in immunosuppressed and normal rats, two experiments were carried out. In Experiment 1 rats were given 1 x 10^5 T. brucei 3 days prior to infection with N. brasiliensis. Serum anti-L3SE precipitating antibody levels and haemagglutinating antibody responses to L3SE and ESP antigens in serum, in small intestine mucosal extracts and in faecal extracts were measured after primary infection and after challenge.

In Experiment 2, 8 x 10^5 T. brucei were inoculated into rats 7 days prior to infection with N. brasiliensis. In this experiment serum precipitating antibody responses to L3SE antigens were measured. The effects of primary and secondary N. brasiliensis infections on total immunoglobulin levels in serum, lung, small intestine mucosa, small intestine contents, large intestine contents and in faecal extracts were estimated and compared in normal and T. brucei-infected rats.

Table 4.1

Experimental Plan

Group	No. of Rats	T. brucei Infection on day	N. brasiliensis Infection on day	Necropsy of 5 rats on day
I	35	- 3	0, 28	14, 18, 24, 28, 32, 38, 42
II	35		0, 28	14, 18, 24, 28, 32, 38, 42
III	10		-	14, 28

Experiment 1

Materials and Methods

Experimental Animals

Eighty female hooded Lister rats weighing approximately 150 g were used.

Experimental Design

Three groups of rats were used. Group I was infected with 1 x 10^5 Trypanosoma brucei 3 days prior to infection with 5,000 L3 of N. brasiliensis. The second group (II) of animals received only N. brasiliensis infections. Twenty eight days after the first nematode infection, surviving rats in these groups were given a second infection of 5,000 L3 N. brasiliensis. Group III acted as uninfected controls (see Table 4.1).

During the experiment faecal samples for coproantibody measurements were collected from individual rats over a 24 hour period prior to necropsy. Groups of five rats were killed and dissected at intervals as shown in Table 4.1. At necropsy the number of worms in the intestine was ascertained. Blood samples for serum preparation, and small intestine mucosal samples, were also collected as previously described.

Trypanosoma brucei Infections

A stock of Trypanosoma brucei derived from TREU 667 was used in these experiments. This stock causes a chronic relapsing form of infection in rodents.

The presence of trypanosomes in the blood was estimated at frequent intervals after infection and immediately prior to necropsy by examination of wet blood smears.

Table 4.2

Numbers of adult N. brasiliensis recovered (mean ± S.E.) from Group I rats (N. brasiliensis + T. brucei infected) and from Group II rats (N. brasiliensis infected) after primary infection and following challenge on day 28 after primary infection

Group	Days after primary infection					
	14	18	24	28	32	42
I	140 (±29)	23 (±6)	25 (±7)	20 (±13)	853 (±205)	17 (± 9)
II	96 (± 35)	18 (± 5)	15 (± 6)	18 (±9)	151 (±36)	1 (± 1)

Sample Preparation

The preparation of mucosal samples, small intestine fluid, large intestine contents, faecal and lung extracts for antibody measurements were as described in the General Materials and Methods.

Double Diffusion Tests

Serum precipitating antibody responses to L3SE antigen were evaluated by the Ouchterlony technique previously described. Five serum samples were tested on 14, 18, 28, 32 and 42 days after primary N. brasiliensis infection (DAPI).

Passive Haemagglutination Tests

Passive haemagglutination tests were carried out on intestinal mucosa samples, faecal extracts and on serum. Two antigen preparations were used in the tests, L3SE and adult worm ESP.

Results

Trypanosome Parasitaemia

The trypanosome infections became patent by day 5 after T. brucei infection and they remained so until necropsy.

Influence of T. brucei Infection on N. brasiliensis Worm Burdens

The numbers of adult worms recovered from the small intestines of rats during the experiment are shown in Table 4.2. Worm numbers in T. brucei infected rats were higher than in rats exposed to N. brasiliensis infection alone. Statistically significant differences in worm burdens between Groups I and II were observed

Table 4.3

N. brasiliensis faecal egg counts in single random samples from Group I rats (N. brasiliensis + T. brucei infected) and in Group II rats (N. brasiliensis infected) after primary infection

Group	6 DAI	10 DAI	12 DAI	14 DAI
I	16,088	2,000	1,913	280
II	11,888	1,600	1,380	168

only on day 4 after reinfection. Results from individual rats are shown in Appendix D, Table 1.

N. brasiliensis Faecal Egg Counts

The results are shown in Table 4.3. Faecal egg counts were higher in rats infected with both T. brucei and N. brasiliensis (Group I) compared with rats infected with N. brasiliensis only (Group II).

Double Diffusion Tests

The results are shown in Table 4.4. In non-trypanosome infected rats(Group II) the highest serum responses against L3SE antigens were observed on 14 and 18 DAPI when the serum of all animals produced at least one precipitation arc. Approximately 60% of all sera from this group showed three arcs at 18 DAPI. High antibody levels were again found in the non-trypanosome infected rats at 18 days after reinfection when 80% of the sera showed three arcs.

In contrast, in the trypanosome-infected rats (Group I) precipitating antibody responses were much weaker. All samples showed two arcs at 14 DAPI and at 18 DAPI 40% produced one arc and 20% gave two arcs. All tests were negative in this group after reinfection.

Haemagglutinating Antibody Response Against L3SE Antigens

Levels of intestinal mucosal, faecal and serum antibodies reacting with N. brasiliensis L3SE in rats from Group I (T. brucei + N. brasiliensis infection) and Group II (N. brasiliensis infection only) are shown in Fig. 4.1. Antibody levels in general were lower in Group I compared with Group II, particularly serum antibodies

Table 4.4

Evaluation of serum precipitating antibody levels against L3SE antigens in Group I rats (*N. brasiliensis* + *T. brucei* infected) and in Group II rats (*N. brasiliensis* infected) after primary infection and following challenge on day 28 after primary infection

Group	Days after primary infection				
	14	18	28	32	42
I (5 samples)	+ +	+	-	-	-
	+ +	-	-	-	-
	+ +	+	-	-	-
	+ +	+ +	-	-	-
	+ +	-	-	-	-
II (5 samples)	+ +	+	+	+	+ + +
	+ +	+ + +	+	+	+ + +
	+ + +	+ + +	-	+	+ + +
	+ +	+ + +	+	+	+

+ = 1 precipitation arc

- = negative result

All uninfected (Group III) sera were negative

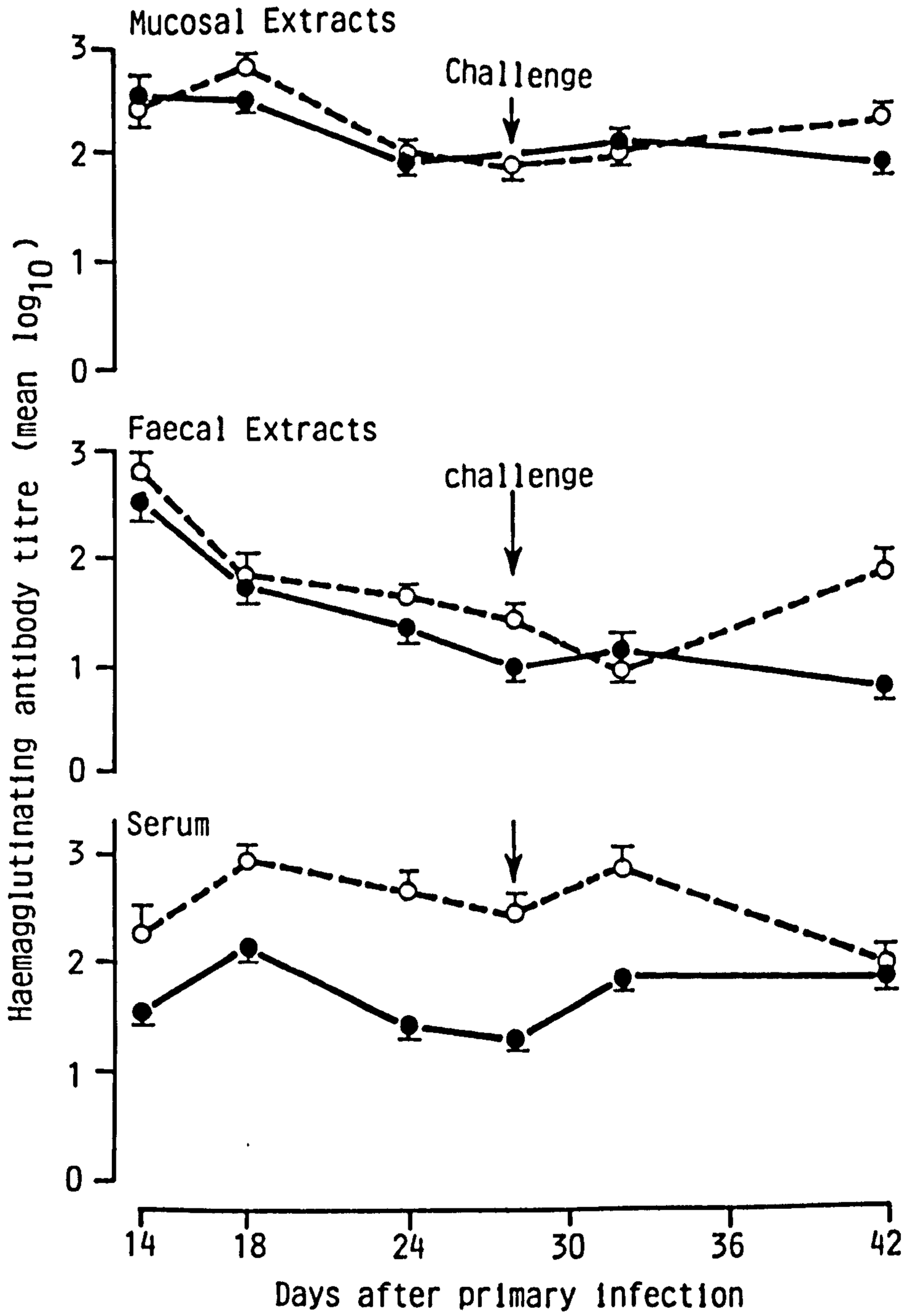

Fig. 4.1. Haemagglutinating antibody titres against L3SE antigens (mean log 10 ± S.E.) in small intestine mucosal extracts, faecal extracts and serum of Group I rats (infected with N. brasiliensis + T. brucei) and Group II rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI.

——●—— Group I, -----o----- Group II

prior to reinfection on 28 DAPI. Serum antibody levels between Groups I and II were statistically different on days 14, 18, 24 and 28 DAPI, whereas there were no statistical differences in either intestinal mucosal or faecal antibodies in the same period. After reinfection with N. brasiliensis haemagglutinin levels of Group I were significantly lower than Group II in intestinal mucosa and faecal extracts 14 days after challenge. In uninfected control rats (Group III) antibody titres to L3SE in intestinal mucosa, faecal extracts, and serum were either negative or barely detectable. Individual results are shown in Appendix D, Tables 2, 3 and 4.

Haemagglutinating Antibody Responses Against ESP Antigens

Levels of antibody responses to N. brasiliensis ESP antigens in intestinal mucosal extracts, faecal extracts and in serum from rats in Group I and Group II are shown in Fig. 4.2. In general antibody levels in Group I were lower than those observed in Group II. Serum antibody levels were statistically different in the two groups on 14, 18, 24 and 28 DAPI. In faecal extracts there were significant differences on 24 and 28 DAPI. Differences in intestinal mucosal antibodies during this period were not statistically significant. After reinfection on 28 DAPI antibody levels in mucosa, faeces and serum in the rats infected with T. brucei and N. brasiliensis (Group I) remained at lower levels than in the rats infected with N. brasiliensis only (Group II). Faecal antibodies were significantly different on days 4 and 14 after challenge but there were no significant differences in intestinal mucosa or serum.

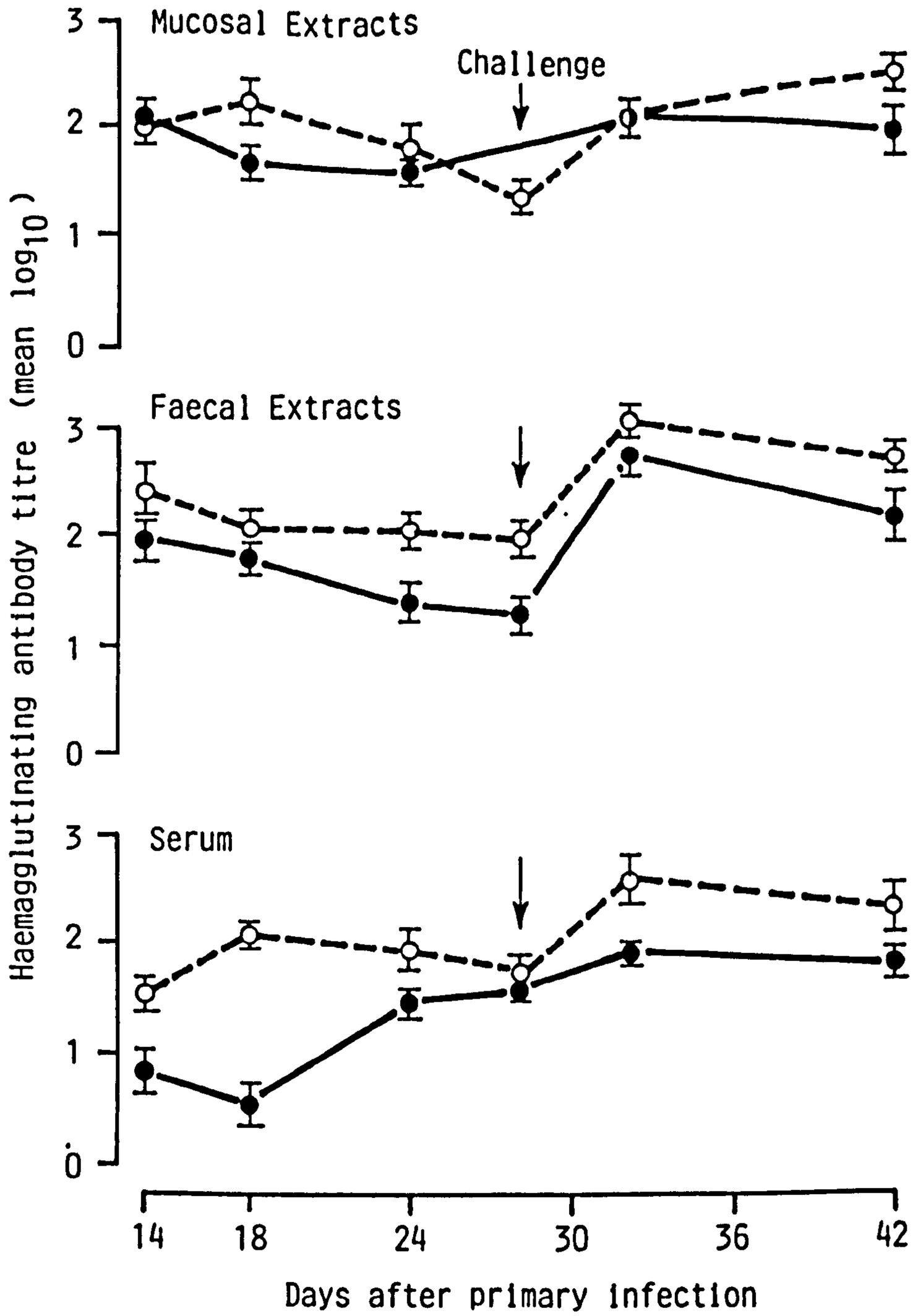

Fig. 4.2. Haemagglutinating antibody titres against ESP antigens (mean log 10 ± S.E.) in small intestine mucosal extracts, faecal extracts and serum of Group I rats (infected with N. brasiliensis + T. brucei) and of Group II rats (infected with N. brasiliensis) after primary infection and following challenge at 28 DAPI.

——●—— Group I, ------o------ Group II

Table 4.5

Experimental Plan

Group	No. of Rats	T. brucei infection - 7 DAPI	N. brasiliensis infection 0 DAPI	N.brasiliensis challenge infection 28 DAPI	Faecal Egg Counts DAPI	Faecal Collection DAPI	Necropsy of 5 rats on DAPI
A	35	8×10^5	5×10^3	5×10^3	6,7,8,9, 11,12,14	Every 3rd day to 42 DAPI	8,12,14, 18,28,32, 42
B	35	-	5×10^3	5×10^3	6,7,8,9, 11,12,14	As above	8,12,14, 18,28,32, 42
C	5	-	-	5×10^3	-	32	32
D	5	-	-	-	-	42	42

Intestinal mucosal, faecal and serum antibody titres in the uninfected control rats (Group III) when tested against ESP antigens were either negative or barely detectable. Individual results are shown in Appendix D, Tables 5, 6 and 7.

Experiment 2

Materials and Methods

Experimental Animals

Eighty female hooded Lister rats of approximately 150 g in weight were used.

Experimental Design

Four groups of rats were used. Group A was infected with 8×10^5 *T. brucei* 7 days prior to infection with 5×10^3 *N. brasiliensis*. Group B was infected with 5×10^3 *N. brasiliensis* only. Some rats in these groups were given a second infection of 5×10^3 *N. brasiliensis* on 28 DAPI along with five rats, Group C, which acted as challenge controls. Five rats, Group D, acted as uninfected controls (see Table 4.5).

During the experiment faecal samples for coproantibody measurements were collected from groups of five rats over a 24 hour period prior to necropsy. Groups of five rats were killed and dissected at intervals shown in Table 4.5. At necropsy the numbers of worms present in the intestines were ascertained and samples of serum, small intestine mucosal extracts, lung extracts, small and large intestine contents and faecal extracts were prepared as previously described.

Table 4.6

Numbers of adult *N. brasiliensis* recovered (mean ± S.E.) from Group A rats (*N. brasiliensis* + *T. brucei* infected), from Group B rats (*N. brasiliensis* infected) and in Group C rats (challenge control rats) following primary infection and following challenge 28 days after primary infection

Group	Days after primary infection						
	8	12	14	18	28	32	42
A	3957 ± 425	367 ± 184	9 ± 4	13 ± 10	20 ± 12	2709 ± 298	9 ± 5
B	1894 ± 219	26 ± 5	30 ± 24	27 ± 9	15 ± 5	854 ± 106	0.4 ± 0.4
P Value A/B	< 0.01	N.S.	N.S.	N.S.	N.S.	< 0.001	N.S.
C	-	-	-	-	-	3463 ± 241	-

Trypanosoma brucei Infections

Trypanosoma brucei from the same TREU 667 stock as that used in Experiment 1 was used in this experiment and the presence of trypanosomes in the blood was ascertained as in Experiment 1.

Passive Haemagglutination Tests

These were carried out on serum, lung extracts, intestinal mucosa extracts, small and large intestine contents and on faecal extracts. L3SE and ESP antigens were used in these tests.

Total IgA, IgG and IgM Measurements

These were evaluated as already described by the method of Mancini et al (1965).

Results

Trypanosome Parasitaemia

Trypanosoma brucei were detectable in the blood of Group A rats from day 5 after trypanosome infection. Tests for T. brucei were also positive on days 6, 12, 14, 28 and 42 days after primary N. brasiliensis infection.

Influence of T. brucei Infection on N. brasiliensis Worm Burdens

Worm recoveries on necropsy days are shown in Table 4.6. In general the number of worms found in rats infected with T. brucei (Group A) were higher than in rats only infected with N. brasiliensis (Group B). Differences in the worm burdens were statistically significant on 8 DAPI and on day 4 after reinfection with N. brasiliensis, when the number of worms recovered from Group A was

Table 4.7

Evaluation of serum precipitating antibody levels against L3SE antigens in Group A rats (N. brasiliensis + T. brucei infected) and in Group B rats (N. brasiliensis infected) after primary infection and following challenge on 28 days after primary infection

Group	Days after primary infection				
	14	18	28	32	42
A (5 samples)	-	+	+	-	-
	-	-	+	+	+
	-	-	+	+	-
	-	+	+	-	-
	-	-	+	-	+
B (5 samples)	+	+	+	+	+
	+ +	+ +	+	+	+ + +
	+	+ +	+	-	+
	+	+ + +	+	+	+ + +
	+ +	+	+	-	+ + +

+ = 1 precipitin arc

- = negative result

All uninfected (Group D) sera were negative

more than three times higher than in Group B. When worm burdens in Group A were compared with those of Group C, the challenge control rats, it was observed that approximately 22% fewer worms were recovered from Group A rats, while similar comparisons of Groups B and C showed that there were approximately 75% fewer worms in Group B. Results from individual rats are shown in Appendix D, Table 8.

Double Diffusion Tests

The results are shown in Table 4.7. In rats infected only with N. brasiliensis (Group B), the highest antibody responses were observed on 18 DAPI, when 60% of the samples tested produced at least two arcs. After reinfection Group B sera again showed the highest antibody response when 60% of the samples produced three arcs. In the trypanosome-infected rats, the antibody response was both delayed and weaker. By 28 DAPI all sera tested produced one precipitation arc and after reinfection only 40% of the samples produced any response at 4 or 14 days after challenge.

Haemagglutinating Antibody Titres to L3SE Antigens

Levels of antibody detected with larval somatic antigen (L3SE) in lung extracts, small intestine mucosa and in serum are shown in Fig. 4.3.

Lung extract titres were significantly lower in Group A animals on each necropsy day after infection, compared with Group B. Individual results are shown in Appendix D, Table 9.

When intestinal mucosa titres in Groups A and B were compared a pattern similar to that observed in serum antibody titres was noted. Although the differences, in mucosal extracts, between

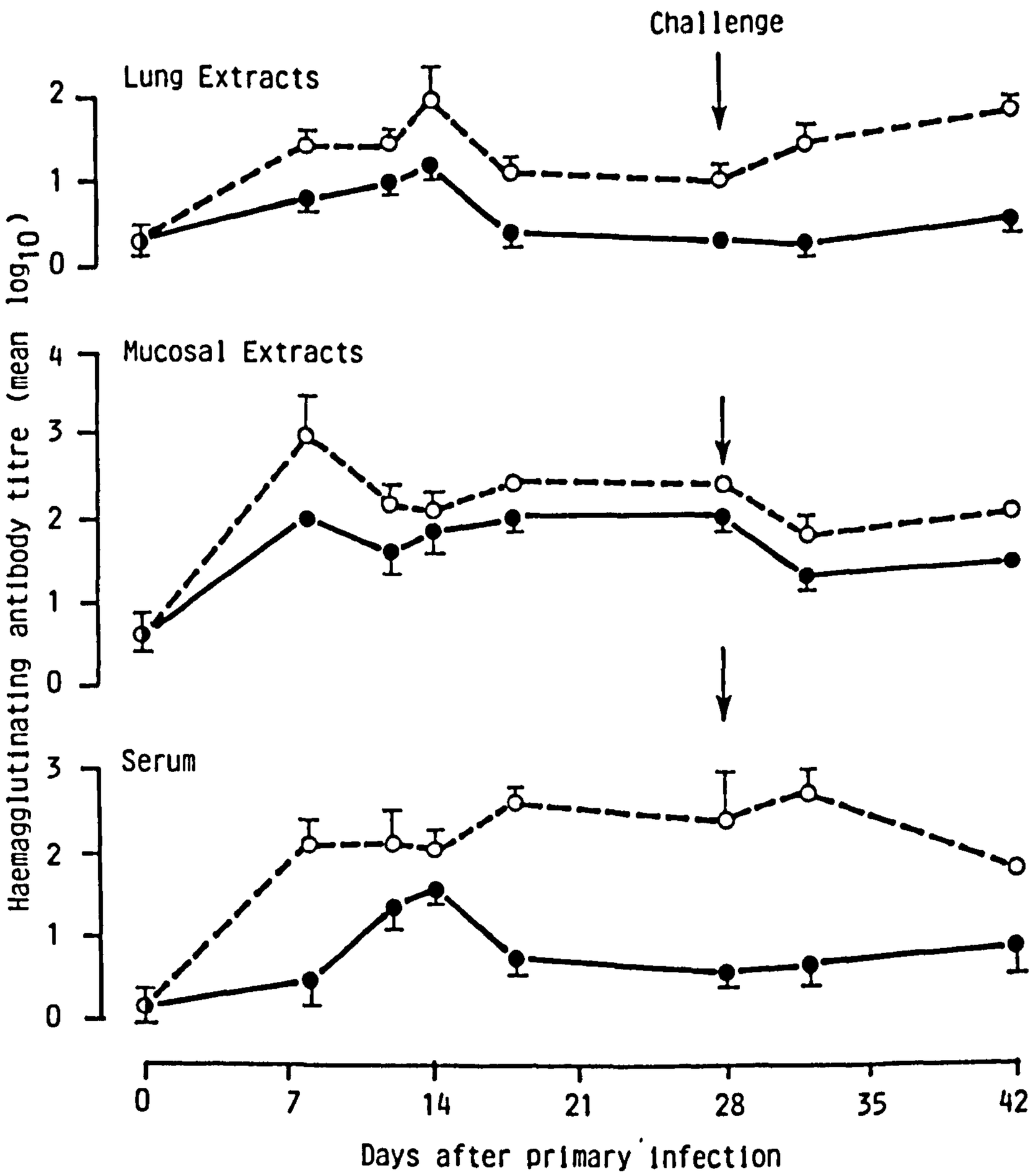

Fig. 4.3. Haemagglutinating antibody titres against L3SE antigens (mean log 10 ± S.E.) in lung extracts, small intestine mucosal extracts and serum of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis) after primary infection and following challenge at 28 DAPI.

——●—— Group A, -----o----- Group B

the two groups were smaller, Group A titres were significantly lower on 8, 18 and 28 DAPI and on days 4 and 14 after challenge. Individual results are shown in Appendix D, Table 10.

Serum antibody titres on each test day were lower in Group A rats (T. brucei + N. brasiliensis) compared with Group B rats (N. brasiliensis only). These differences were statistically significant on 8, 18 and 28 DAPI and on days 4 and 14 after challenge. Individual results are shown in Appendix D, Table 11.

Levels of antibody detected with L3SE antigen in the contents of the small intestine and large intestine, and faecal extracts are shown in Fig. 4.4.

Comparisons of antibody titres in small intestinal contents in Groups A and B show that on each necropsy day Group A titres were lower than Group B. These differences were statistically different on 8 and 18 DAPI and 14 days after challenge. Individual results are shown in Appendix D, Table 12.

Antibody titres in large intestine contents against L3SE antigens in Group B were higher than in Group A throughout the experiment. The differences were significant on 8 DAPI and on day 14 after challenge. Individual results are shown in Appendix D, Table 13.

Antibody titres in faecal extracts were lower in Group A than in Group B throughout the experiment and the difference was most marked during the first week following primary infection and following challenge. Since these tests were carried out on faecal samples pooled from 5 rats for each test statistical analysis of the results was not possible.

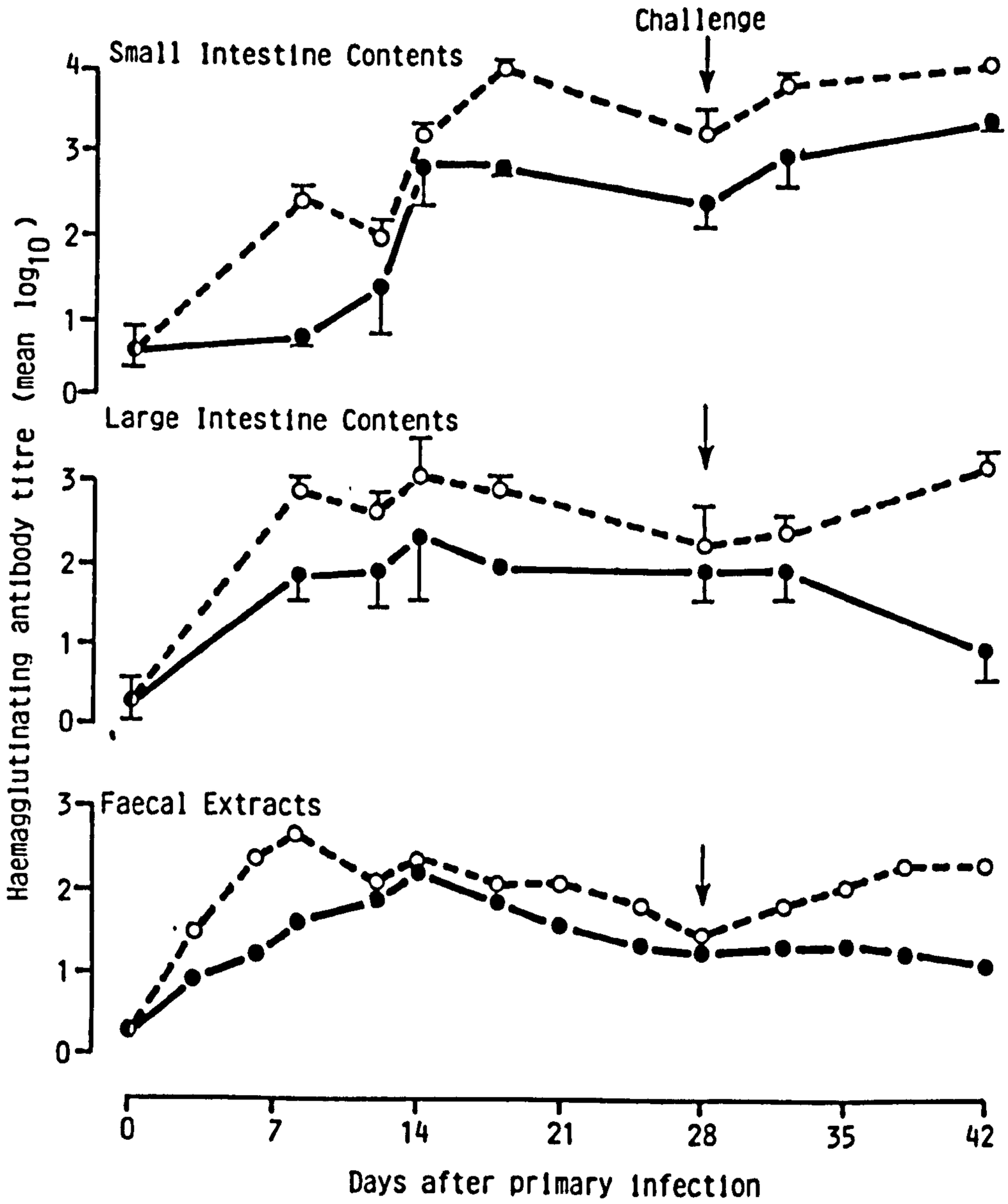

Fig. 4.4. Haemagglutinating antibody titres against L3SE antigens (mean log 10 ± S.E.) in small intestine contents, large intestine contents and faecal extracts of Group A rats (infected with N. brasiliensis + T. brucei)and of Group B rats (infected with N. brasiliensis) after primary infection and following challenge at 28 DAPI.

——●—— Group A, ------o------ Group B.

Haemagglutinating Antibody Titres to Adult N. brasiliensis ESP Antigens

Antibodies to ESP antigens were not detectable in any of the lung extracts from either group of experimental rats. Levels of antibody detected by ESP antigens in serum and small intestine mucosal extracts are shown in Fig. 4.5.

Small intestine mucosal antibody titres were lower in Group A than Group B on days 8, 14 and 28 DAPI and also after reinfection. On 14 DAPI the titres in Group A were slightly higher than in Group B. Individual results are shown in Appendix D, Table 15.

An examination of serum antibody titres to ESP antigens in rats in Groups A and B showed that titres in Group A were significantly lower than in Group B, on days 8 and 18 DAPI and on days 4 and 14 after reinfection. However, on 12 and 14 DAPI the titres in Group A were slightly above those in Group B. Individual results are shown in Appendix D, Table 16.

Antibody titres to ESP antigens in small intestine contents, large intestine contents and in faecal extracts are shown in Fig. 4.6. When titres in Groups A and B were compared there were no statistical differences between the groups until days 4 and 14 after challenge when both small and large intestine extract titres in Group A were significantly lower than in Group B. Individual results are shown in Appendix D, Tables 17 and 18.

Faecal extract titres in Group B showed a steady increase between 7 and 14 days after challenge whereas titres in Group A remained at about their pre-challenge level. Individual results are shown in Appendix D, Table 14.

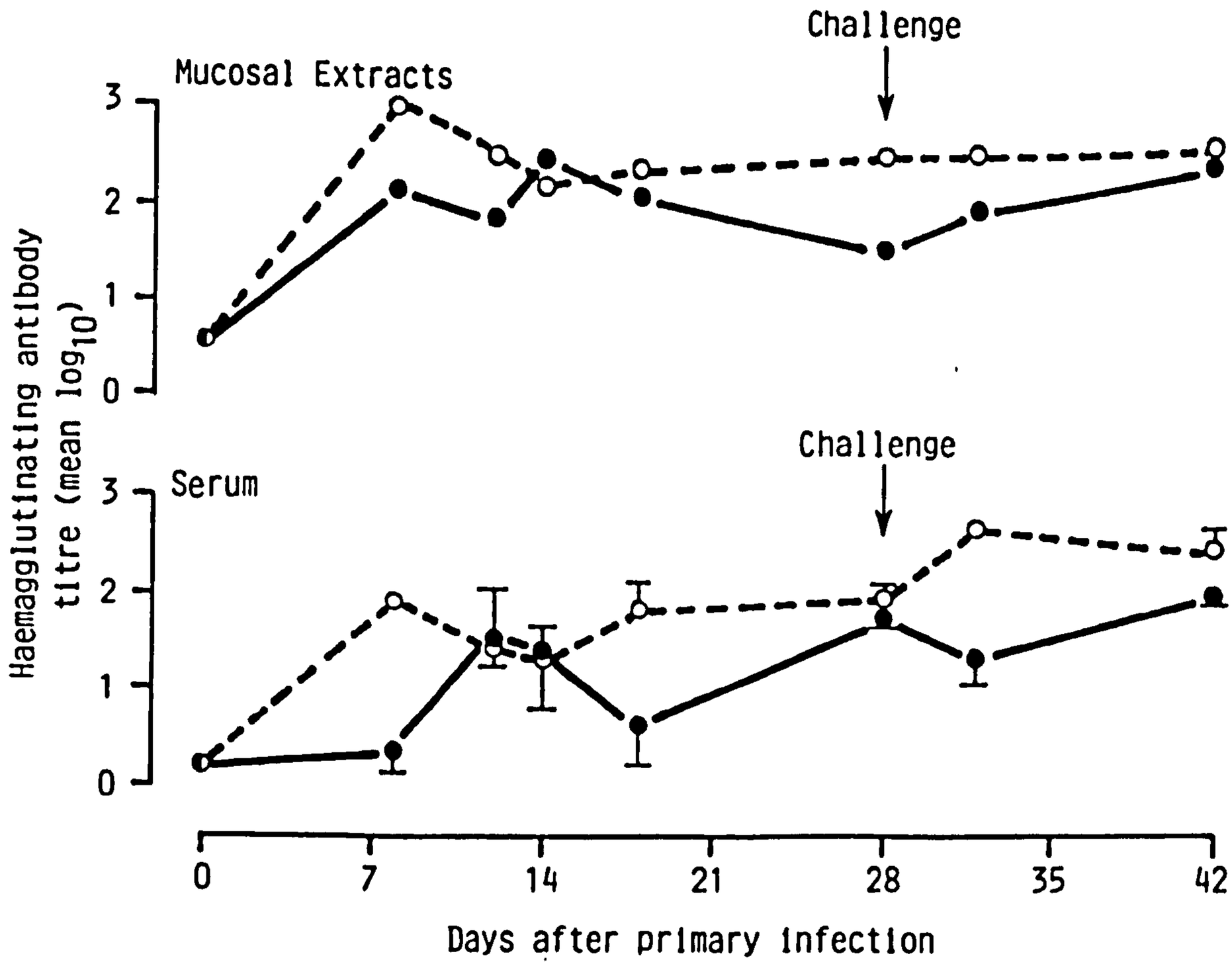

Fig. 4.5. Haemagglutinating antibody titres against ESP antigens (mean log 10 ± S.E.) in small intestine mucosal extracts and serum of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis) after primary infection and following challenge at 28 DAPI.

——●—— Group A, ------o------ Group B

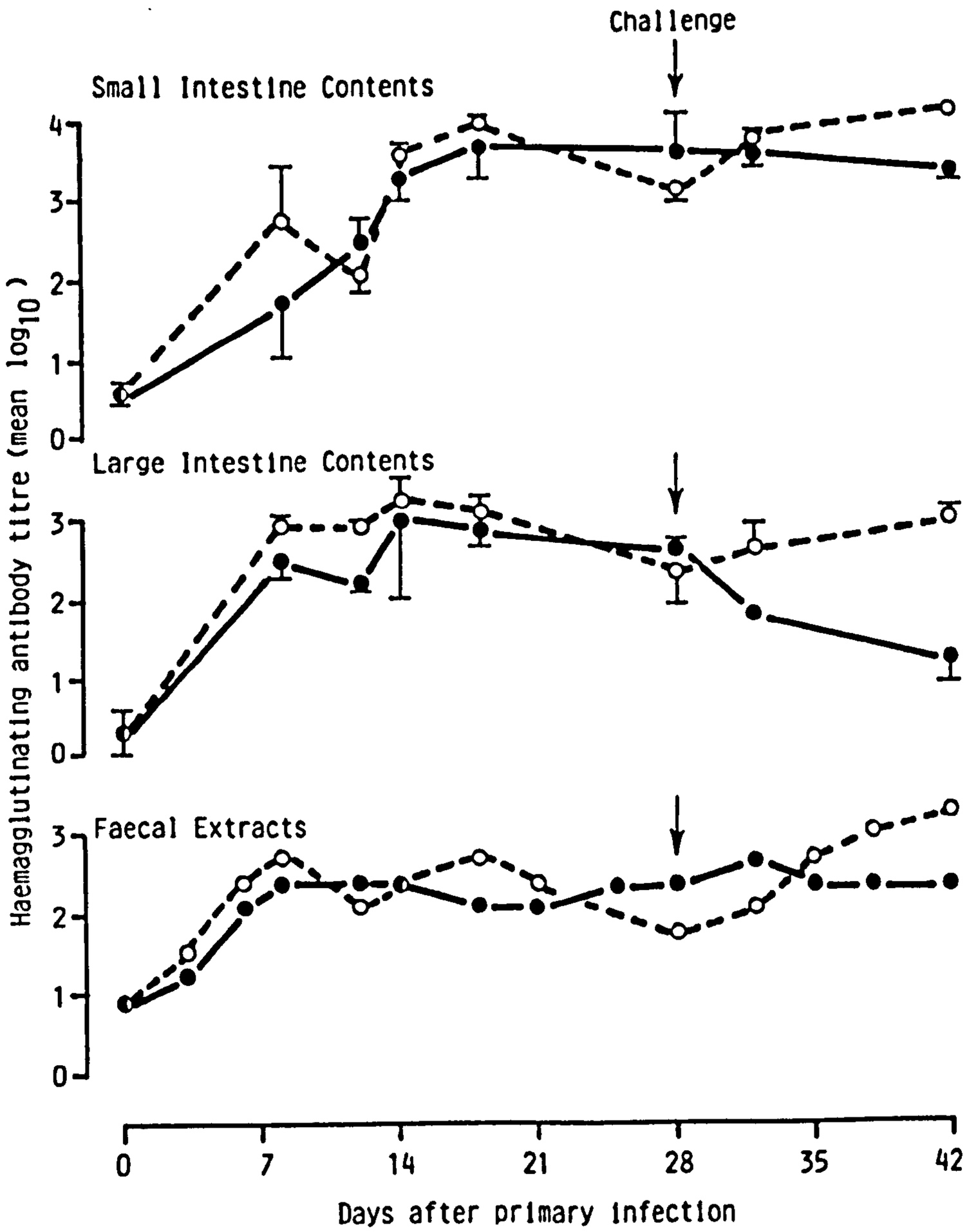

Fig. 4.6. Haemagglutinating antibody titres against ESP antigens (mean log 10 ± S.E.) in small intestine contents, large intestine contents and faecal extracts of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis) after primary infection and following challenge at 28 DAPI.

——●—— Group A, -----o----- Group B.

Immunoglobulin Levels

Changes in IgA, IgG and IgM levels, expressed as percentages of total globulin concentrations, in serum, lung extracts, small intestine mucosa, small and large intestine contents and in faecal extracts are presented in Figures 4.7, 4.8 and 4.9.

Immunoglobulin A Concentrations

Changes in total IgA concentrations are shown in Fig. 4.7.

Serum IgA increased slowly in Group B rats (*N. brasiliensis* alone) until 18 DAPI. In Group A rats (*T. brucei* and *N. brasiliensis*) IgA concentration in serum on 8 DAPI was very low and though the level subsequently increased slowly mirroring changes in Group B serum it remained lower than in Group B until 42 DAPI.

The most dramatic difference in IgA concentration following infection in Group A and B rats was observed in lung extracts. In Group B the lung IgA level had increased by approximately 47 times by 18 DAPI. In Group A rats the maximum IgA level occurred at 8 DAPI and this was only about 10 times the preinfection value. By 18 DAPI the IgA concentration in the latter group had fallen below the uninfected value and was about 100 times lower than in the rats infected with *N. brasiliensis* alone.

Small intestine mucosal extracts in Group B showed maximal IgA concentrations between 12 and 14 DAPI whilst in Group A rats on day 14 the levels of IgA were relatively depressed. After reinfection on 28 DAPI only Group B rats showed an increased IgA concentration.

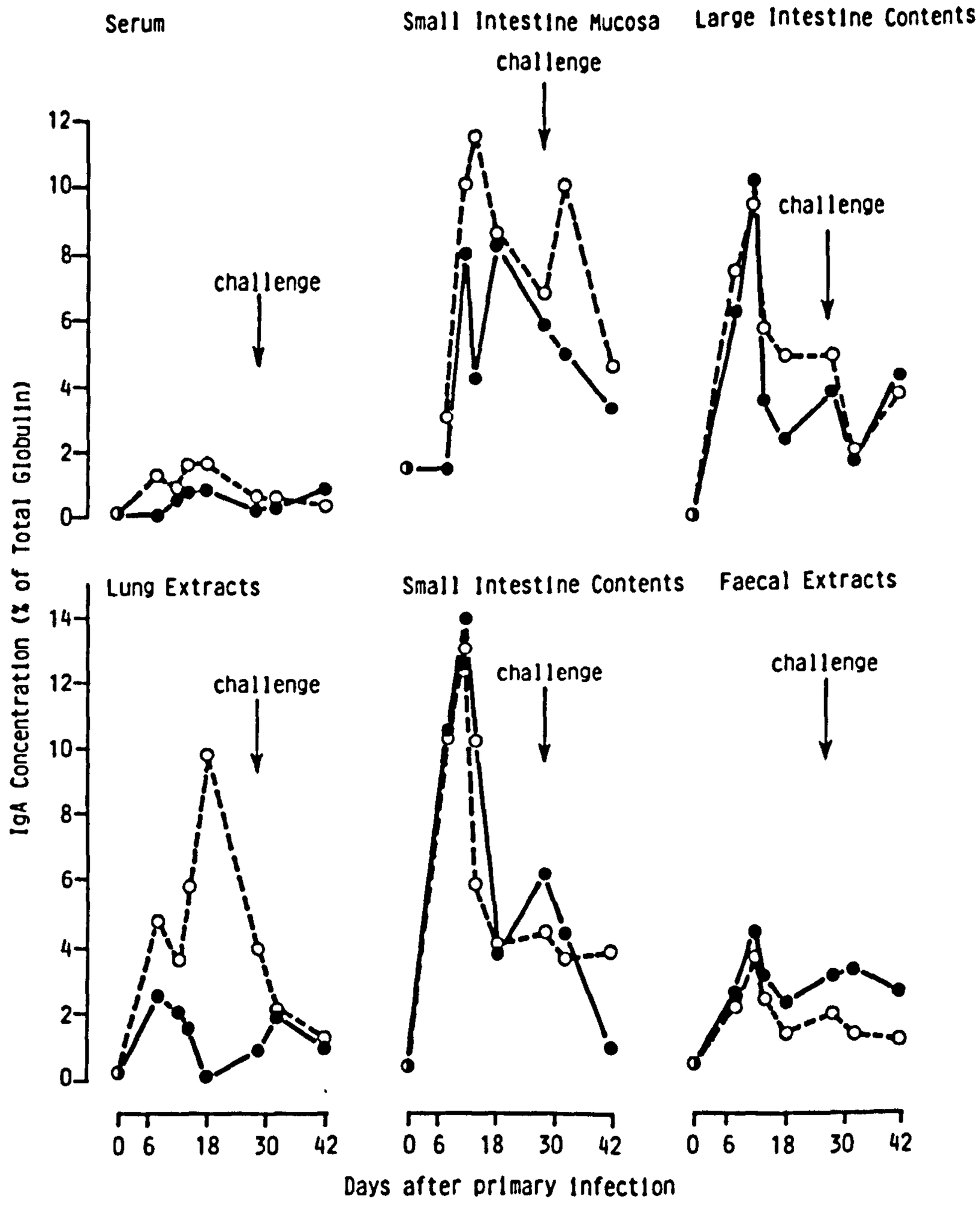

Fig. 4.7. Immunoglobulin-A concentrations expressed as % total globulins in serum, lung extracts, mucosal extracts, small and large intestine contents and in faecal extracts of rats in Group A (N. brasiliensis + T. brucei) and in Group B rats (N. brasiliensis only) after primary infection and following challenge at 28 DAPI

——●—— Group A, -----o----- Group B.

Immunoglobulin A levels in small and large intestinal contents were similar in both experimental groups, the maximum values occurring at 12 DAPI. Changes in faecal IgA in both Groups A and B closely reflected changes observed in the intestinal fluids and reinfection did not lead to an increase in faecal IgA.

Immunoglobulin G concentrations

Changes in total IgG levels in serum, lung and small intestinal mucosa extracts, small and large intestinal contents and in faecal extracts are presented in Fig. 4.8.

Serum IgG concentrations increased in both groups of experimental rats following infection with N. brasiliensis. In Group A, the T. brucei infected animals, the maximum level was reached at 12 DAPI. In Group B (N. brasiliensis only) serum IgG levels rose to a higher level than in Group A and reached a maximum on 18 DAPI. Between 18 and 28 DAPI IgG levels in the serum of Group B animals was at least twice the level recorded in Group A sera. Following challenge N. brasiliensis infections on 28 DAPI both Groups A and B showed rises in serum IgG between 4 and 14 days after challenge.

The total IgG levels in lung extracts increased markedly following infection in both groups but in contrast to the changes in serum IgG, the T. brucei infected animals, Group A, showed a higher IgG concentration than in Group B rats at 18 DAPI.

Group A rats showed slightly lower IgG levels in small intestine mucosal samples on 8 and 12 DAPI and on day 14 after reinfection compared with Group B.

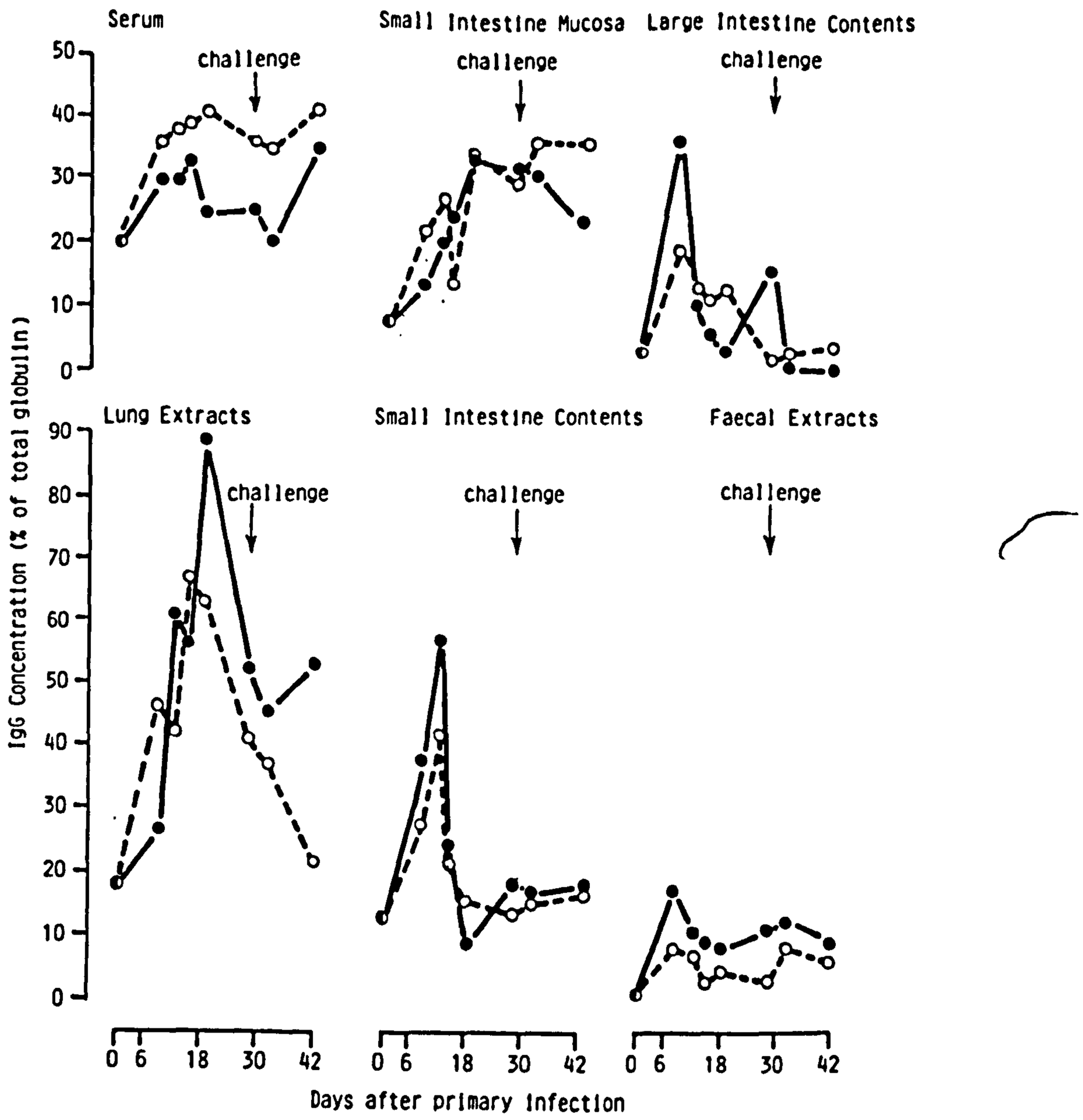

Fig. 4.8. Immunoglobulin-G concentrations expressed as % total globulins in serum, lung extracts, small intestine mucosal extracts, small intestine contents, large intestine contents and in faecal extracts of rats in Group A (*N. brasiliensis* + *T. brucei*) and in Group B rats (*N. brasiliensis* only) after primary infection and following challenge at 28 DAPI.

———●——— Group A. -----o------ Group B

Levels of IgG in small intestine contents reached maximum values on 12 DAPI, Group A rats showing higher values than Group B. After a sharp fall between 12 and 18 DAPI, IgG levels in small intestine contents were similar in both groups and both showed a slight increase after reinfection on 28 DAPI.

While IgG levels in large intestine contents increased after infection in both Group A and B rats, the concentrations were higher in Group A at 8 and 28 DAPI.

Throughout the experiment faecal IgG levels were higher in Group A rats than in Group B. Changes in IgG concentrations correlated with those observed in both small and large intestine contents.

Immunoglobulin M Concentrations

Changes in total IgM levels in serum, lung and small intestinal mucosa extracts, small and large intestinal contents and faecal extracts are presented in Fig. 4.9.

IgM in the serum of animals infected with N. brasiliensis only (Group B), increased slowly until 18 DAPI and afterwards declined. There was a slight rise on day 14 after reinfection. Levels of IgM in the T. brucei infected rats (Group A) reached a concentration two-fold higher than in Group B on days 8 and 18 DAPI. Unlike Group B, IgM levels in Group A sera did not show an increase after reinfection.

Total IgM in lung extracts of Group B rats showed maximal levels at 8 DAPI. In Group A rats, T. brucei infected, the highest bronchial IgM concentrations were observed at 14 DAPI and were 20 times higher than the value in Group B at the same time.

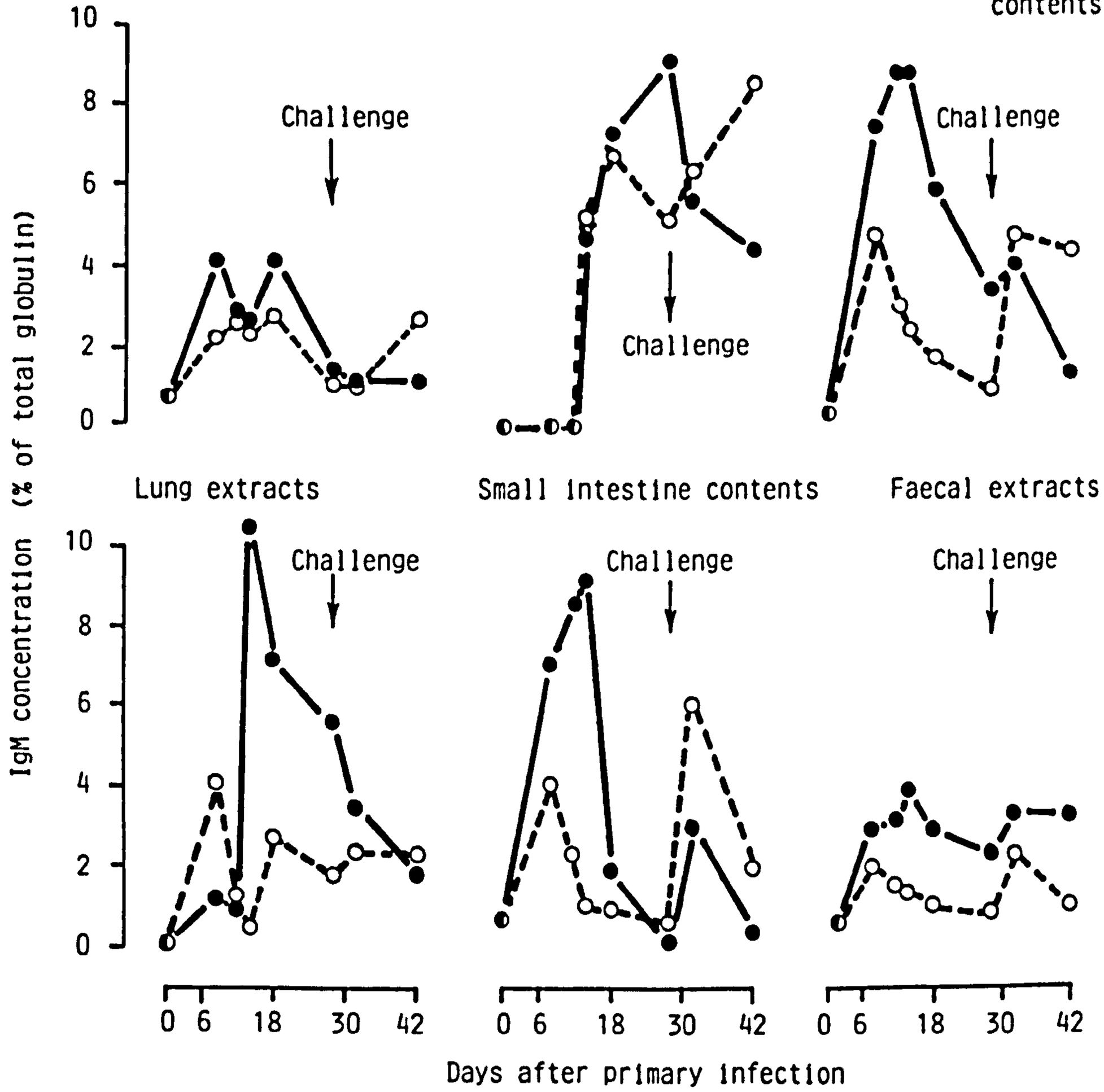

Fig. 4.9. Immunoglobulin-M concentrations expressed as % total globulins in serum, lung extracts, small intestine mucosal extracts, small intestine contents, large intestine contents and in faecal extracts of rats in Group A (N. brasiliensis + T. brucei) and in Group B rats (N. brasiliensis only) after primary infection and following challenge at 28 DAPI.

——●—— Group A. ------o------ Group B.

Significant amounts of IgM were detected in small intestine mucosa extracts only after the 12th day of infection, when an increase occurred in both groups, to reach a maximum in Group A at 28 DAPI and in Group B at 18 DAPI. After reinfection only Group B rats showed an increase in intestinal mucosal IgM.

Levels of IgM in small intestinal contents reached higher values in Group A than in Group B, the maximum value occurring at 14 DAPI. Group B rats showed two peaks in IgM concentration. The maximum value, detected 4 days after challenge in Group B was 6% of the total globulin concentration compared with a value of 9.2% in Group A at 14 DAPI.

Levels of IgM in large intestine contents in T. brucei infected rats, Group A, reached a peak between 12 and 14 DAPI and the values were two-fold higher than the maximum value observed in Group B at 8 DAPI. After reinfection, IgM values in Group B increased to reach a maximum value at 4 days after challenge.

Changes in faecal IgM levels following N. brasiliensis infection reflected changes in IgM concentrations in both small and large intestinal contents. A similar correlation was observed between faecal IgA and IgG levels and the concentrations of IgA and IgG in small and large intestinal contents. Higher faecal IgM levels were observed throughout the experiment in the T. brucei infected rats (Group A) compared to the rats infected with N. brasiliencis only (Group B).

Results of immunoglobulin estimations in serum, lung extracts, small intestine mucosal extracts, small and large intestinal contents and faecal extracts, expressed in μg/ml are shown in Appendix D, Tables 19 - 24.

Discussion

Previous studies have suggested that the mechanisms involved in the expulsion of adult N. brasiliensis are evoked by both humoral and cellular factors (Jones and Ogilvie, 1971), Ogilvie et al, 1977 and Sinski, 1981). Several workers have suggested that locally produced antibodies play a potentially important role in immunity to N. brasiliensis (Poulain et al, 1976a; Sinski and Holmes, 1977).

The experiments described in this chapter examined local antibody responses in rats infected with N. brasiliensis and evaluated changes in these responses resulting from the immunosuppressive effect of T. brucei infection to unrelated parasite antigens.

The results of Experiments 1 and 2 showed no clear difference in the time of N. brasilinesis expulsion between rats infected with trypanosomes and N. brasiliensis and rats infected with only N. brasiliensis. Nevertheless in Experiment 1 the mean number of adult worms in the small intestines of rats 14 days after N. brasiliensis infection in the T. brucei infected rats and in rats infected only with N. brasiliensis were 140 and 96 respectively. In Experiment 2 on 8 DAPI the numbers of adult worms in the T. brucei infected rats were more than two-fold higher than in the rats infected with N. brasiliensis alone. The results obtained in both experiments might be connected with suppression of serum and lung antibody responses to nematode antigens since titres of antibody detected with larval antigens on the 8th day after primary infection were significantly lower in T. brucei infected rats. Investigations of immunity to pre-intestinal and intestinal larval stages of N.

brasiliensis led Love et al (1974) to conclude that immune reactions to migrating larval stages are mediated by humoral antibodies. An increase in the number of worms established in the intestines of primary N. brasiliensis infected rats similar to that shown in the T. brucei infected rats in Experiments 1 and 2 has also been observed in N. brasiliensis infected rats treated with cyclophosphamide (Sinski, 1981).

In Experiment 1, four days after reinfection 17% of the injected larvae had become established in the T. brucei infected rats, compared to 3% in the rats infected with N. brasiliensis only and comparable results in Experiment 2 were 54% and 17% respectively.

Using a variety of approaches several workers have demonstrated that the ability of T. brucei infected rodents to respond to unrelated antigens may be impaired. Goodwin, Green, Guy and Voller (1972) showed that mice and rabbits, infected with T.brucei developed significantly lower serum agglutinating antibody levels to sheep erythrocytes than in uninfected animals. Murray, Jennings, Murray and Urquhart (1974) investigated the immunosuppressive effect of trypanosomiasis in mice and found that T cell function was relatively normal but that B cell response to antigens considered to be speicific for B cells was almost completely absent, despite considerable plasma cell proliferation. Hudson and Terry (1979) showed that in mice infected with T. brucei the IgG response to heterologus antigen was absent at 21 and 31 days after infection and the IgM antibody response was less than 5% of normal at the same stages of infection.

Urquhart et al (1973) observed that in rats infected with T. brucei 21 days prior to infection with 1,000 N. brasiliensis larvae, the normal process of adult worm expulsion had not occurred by the 18th day after nematode infection and the production of serum protective antibodies was grossly impaired. When rats were infected with 5,000 N. brasiliensis larvae 3 days after T. brucei infection the onset of T. brucei expulsion was delayed for about 5 days (Maclean, 1977).

Immunosuppression of responses to a clostridial vaccine in cattle infected with T. congolense was demonstrated by Holmes, Mammo, Thomson, Knight, Lucken, Murray, Murray and Urquhart (1974) and the suppressive effect of T. congolense was later shown in cattle when responses to both foot-and-mouth and clostridial vaccines were found to be diminished in trypanosome infected cattle (Scott, Pegram, Holmes, Pay, Knight, Jennings and Urquhart (1977).

Results of the experiments described in this chapter indicate that the immunosuppressive effects of T. brucei infections to unrelated parasite antigens may depend on the interval between trypanosome infection and the introduction of other antigens. Precipitating antibodies in serum against larval somatic antigens showed different patterns in Experiments 1 and 2. When T. brucei infection was given three days before N. brasiliensis administration, precipitating antibody responses measured on 14 DAPI were almost equal in both groups of rats. However, when the rats were infected with trypanosomes 7 days before the nematode infection, precipitins were absent on 14 DAPI. These results seem to support other

observations that the immunosuppressive effects of trypanosome infections depend upon the interval between infection and antigen administration (Hudson, Byner, Freeman and Terry, 1976; Hudson and Terry, 1979).

In Experiment 1 intestinal mucosa and faecal haemagglutinating antibodies to both L3SE and ESP antigens were marginally lower in the immunosuppressed rats following infection compared with the normal N. brasiliensis infections, although after challenge the differences were greater. In the second experiment only antibodies detectable with L3SE antigens were significantly depressed in intestinal mucosa, intestinal contents and in faecal extracts of the trypanosome infected rats after primary infection. However after reinfection the haemagglutinating antibody titres against ESP antigens were significantly lower in the immunosuppressed rats.

During investigations of rat local immune responses to bacterial infections it has been found that the nature of antibodies appearing in the intestinal tract greatly depend on the route of immunisation (Jackson and Cooper, 1981). Thus, if the first presentation of antigen occurred via the intestine, anti-bacterial agglutinating antibodies appeared to be solely of IgG and/or IgA classes. A second stimulation by the same route enhanced antibody titres and in contrast to the primary response, IgM provided a significant component of the response over the first 4 days. Systemic presentation (intravenous injection) of antigen led to a more rapid response and during the early stages

of the primary response, the antibodies were mostly of the IgM class, It may be that during N. brasiliensis infection a similar mechanism is involved. Thus larval antigens may stimulate both systemic and local immune systems, whilst adult worm antigens primarily provoke local immune responses in the intestinal tract (Sinski and Holmes, 1978).

When changes in immunoglobulin levels are examined following N. brasiliensis infections in both groups of rats in Experiment 2, it is obvious that trypanosome infection modifies not only systemic but also local immune responses of rats to N. brasiliensis antigens.

Changes in serum, small intestine mucosa and in faecal immunoglobulin levels observed after primary infection in Group B (N. brasiliensis infections only) were similar to those recorded in Chapter 2 and also similar to those reported by Jarrett and Bazin (1977) and Sinski and Holmes (1977). Immunoglobulin concentrations in small intestinal contents however only partially reflected those in mucosal extracts. For example, the immunoglobulin M level increased in mucosal extracts between 12 - 18 DAI and at the same time its concentration in the intestinal lumen declined. One explanation for this phenomenon could be that IgM is susceptible to proteolytic breakdown in the gut. This result might indicate that locally synthesised IgM may not be able to participate in worm expulsion after primary infection. Furthermore, Sinski (1982) observed a significant increase in the number of IgM producing plasma cells only on day 18 after N. brasiliensis infection. The results obtained in the present experiment from T. brucei infected rats also support

the view that intestinal IgM detected during the first 12 days after nematode infection is probably systemic in origin.

After reinfection significant increases in IgA and IgM concentrations were observed in small intestine mucosal extracts of Group B. In the intestinal lumen however only IgM and IgG increased and elevation of the former immunoglobulin class was proportionally much more significant. These results would suggest that IgM is synthesised and released into the intestinal lumen during anamnestic responses to intestinally presented antigens. There are suggestions that the function of IgM at secretory sites may be similar, in part, to that of IgA (Baklien and Brandtzaeg, 1976; Brantzaeg, 1981).

In Chapter 3, when rat immune responses to radiation attenuated N. brasieliensis larvae were investigated it was observed that a single dose of 2,000 larvae irradiated with 80 or 120 krad induced the production of anti-parasite antibodies in the lungs of infected animals. However in the lungs of rats infected with normal larvae, anti-larval haemagglutinating antibodies were detected only after challenge with 5,000 larvae. Results obtained in Experiment 2 show that infection with 5,000 N. brasiliensis larvae also resulted in a rapid increase in immunoglobulin concentration in the lungs after nematode infection. Immunoglobulin A concentration per gramme of lung tissue reached the same level as in intestinal mucosa and the IgM concentration increased several times after infection. In Experiment 1 tests failed to detect any lung haemagglutinating antibodies to red cells coated with ESP antigens. These results suggest that lung responses are directed against larval antigens.

Such a possibility is supported by a recent finding that surface antigens of parasitic L3 which is undergoing a moult to the 4th stage are different from those on the surface of intestinal L4 (Maizels, Meghji and Ogilvie, 1983). However the precise function of locally produced antibodies in the lungs is unclear. Much of our understanding of local immunity has come from studies of intestinal IgA (see reviews by Bienenstock and Befus, 1980; Befus and Bienenstock, 1982; and Bienenstock and Befus, 1983). Studies of immunity in the respiratory track have also been reviewed by McDermott, Befus and Bienenstock (1982).

Although IgA is generally reported to play a dominant role at mucosal surfaces results obtained in the investigations of primary and secondary antibody response in the intestinal secretions of rats immunized intravenously or orally suggest that IgG antibodies are also a significant component of secretions associated with intestinal epithelium (Kenrick and Cooper, 1978). Increases of total and worm specific IgG in intestinal mucosa following N. brasiliensis infection has already been demonstrated in Chapter 2 and by Sinski and Holmes (1977). There are indications that antibodies of IgG class operating in mucosal sites are partly derived by transudation from the serum and partly synthesised locally (Tomasi and Bienenstock, 1968; Platts-Mills, 1978). However the exact role of IgG in mucosal secretions is unclear but IgG may provide a second line of defence against infection. It has been shown in the respiratory tract that IgG agglutinates bacteria and promotes their phagocytosis by alveolar macrophages (see review by McDermott et al, 1982).

It is known that in man and in experimental animals there is a significant rise in serum IgM which is largely non-specific following infection with trypanosomes (see review by Clarkson, 1976). In Experiment 2 the IgM concentrations in serum, lung and intestinal contents were significantly higher in T. brucei infected rats than normal animals after primary N. brasiliensis infection. After reinfection however IgM values of the former rats were lower than in rats infected only with N. brasiliensis. Faecal IgM levels before and after reinfection were higher in the T. brucei infected rats, although the differences were not great. Serum IgA and IgG were lower in the T. brucei infected rats and in intestinal and faecal globulins similar to those observed in trypanosome-free rats.

In Experiment 2, the mean number of worms recovered from the intestines of the T. brucei infected rats on 8 DAPI was two-fold greater than in the normal N. brasiliensis infection. This phenomenon coincided with lower IgA and IgM levels in the lungs of the T. brucei infected rats compared with Group B rats, thus suggesting that immunodepression of local antibody response in the lungs may play a part in enhanced worm establishment. There are also indications that depression of systemic antibody responses could have a role in greater worm burdens in the trypanosome-infected rats. On 8 DAPI there was an absence of circulating precipitating antibody response to larval somatic antigen.

Several aspects of the results are noteworthy. First, it appeared that the immunosuppressive effects of T. brucei infections depend on the route of N. brasiliensis antigenic stimulation, the

nature of the antigen and the interval between T. brucei infection and nematode infection. Secondly, both systemic and local immune responses were modified as a result of trypanosome infection. Following primary nematode infection antibodies detectable with larval somatic antigens were depressed to a much greater degree as a result of the trypanosome immunodepression than were antibodies stimulated by ESP antigens. Thirdly, the results support the view that a multiantigenic stimulation of the host's humoral responses occurs in the course of a N. brasiliensis infection. Fourthly, they indicate that depression of systemic antibody responses and local IgA and IgG responses in the lungs may enhance parasite establishment in the intestine.

Finally it is important to note that the results recorded in this chapter show that coproantibody levels closely follow changes in antibody levels both in small intestine mucosa and small intestine contents. These results again point to the value of coproantibody measurements as an in vitro technique for monitoring local immune responses to intestinal antigens.

Chapter 5

Intestinal Antibody Responses to Different Antigens of *Nippostrongylus brasiliensis*

Introduction

A number of investigations have been conducted on the systemic immune responses of rats to Nippostrongylus brasiliensis infection (reviewed by Kassai, 1982) and on the possible role of local antibodies in establishing host immunity against this parasite (Poulain et al, 1976a; Sinski and Holmes, 1977 and 1978). However there is no information as to which antigens are the most significant in stimulating intestinal antibody responses, particularly IgA responses. Moreover the precise role of locally produced antibodies has not been adequately investigated.

Reports of the ability of worm metabolic or somatic antigens to induce protection against reinfection are somewhat conflicting. The most widely held opinion is that successful induction of protective immunity requires living parasites, and adult nematodes are thought to be the most effective in this respect (Ogilvie, 1965a). However, there are reports that somatic extracts of larval and adult nematodes and their excretory-secretory products are capable of stimulating various degrees of protection (Denham, 1969; Murray, Robinson, Gierson and Crawford, 1979; Poulain, Pery and Luffau, 1976b; Katiyar, Govil and Sen, 1972 and Govila, Katiyar and Sen, 1973).

The experimental work in this chapter was designed to investigate and compare the ability of living intestinal stages of N. brasiliensis with adult nematode somatic and metabolic antigens in their ability to induce intestinal antibody responses. In

Table 5.1

Experimental Plan

Group	Antigen	Route of Administration	Necropsy Days	
			9 Days after Vaccination	9 Days after Challenge
I	2,000 L3	Subcutaneous	5 rats	5 rats
II	1,500 L4	Intragastric	5	5
III	1,500 D5	Intragastric	5	5
IV	ESP Antigens (1.7 mg/rat)	Intragastric	2	5
V	ASE Antigens (1.7 mg/rat)	Intragastric	2	5
VI	Challenge Controls	-	-	5
VII	Uninfected Controls	-	5	-

particular intestinal IgA responses to the various antigens were compared. Systemic antibody responses were also measured and compared with local antibody responses.

Materials and Methods

Experimental Plan

Fifty-four rats in seven experimental groups were used (Table 5.1). On day 0 of the experiment, rats allocated to Group I were infected subcutaneously with 2,000 infective larvae. Using an intragastric intubation technique, Group II were given 1,500 fourth stage larvae and Group III were given 1,500 adult parasites. Another two groups received intragastrically 1.7 mg of adult nematode excretory-secretory products (Group IV) and 1.7 mg adult worm somatic protein (Group V) respectively. On day 9 after infection or antigen administration, five animals from Groups I - III and two rats from Groups IV and V were killed and dissected. The remaining rats were challenged subcutaneously with 5,000 infective larvae on day 14 of the experiment. Five naive rats (Group VI) were also infected with the same dose of larvae and acted as challenge controls. Five rats remained uninfected and served as parasite-free controls (Group VII). All the remaining animals were killed and dissected nine days after challenge.

During the experiment faecal samples from groups of rats were collected every third day. At necropsy the number of worms in the intestines was estimated. Samples of serum, small intestinal mucosa and small intestinal contents were also collected for antibody estimation.

Parasitological Techniques

Fourth stage larvae and young nematodes were obtained from intestines of donor rats 50 hours (L4) or 5 days (D5 worms) infection with 5,000 L3.

After sacrifice the small intestines were removed, opened and placed on sieves suspended in PBS at 37°C. After 30 minutes incubation, the nematodes were collected and counted. The infective doses were then suspended in 1.5 ml of warm PBS and administered intragastrically to the experimental rats using 2 ml disposable plastic syringes attached to a 3 cm length of intravenous catheter (Red Luer, 5 FG O/D 1.65 mm, Portex Ltd., Kent, England) to function as a stomach tube. Adult worm somatic extracts and ESP antigens were similarly administered.

Sampling and Extract Preparation

Sampling and preparation of small intestinal contents, mucosal and faecal extracts and parasite antigens were as previously described.

Preparation of ^{125}I-labelled L4SE Antigens

Washed 4th stage larvae were centrifuged and resuspended in PBS in the ratio of 1 volume packed larvae to 2 volumes of PBS and incubated with 1 mCi ^{125}I-NaI (Amersham International, IMS 30) and 50 mg/ml Chloramine-T for 5 minutes at room temperature. Excess ^{125}I was then removed by the addition of 50 µl of a saturated tyrosine solution after which 10 ml of 0.01 M Tris buffer pH 8.3 containing protease inhibitors was added. The protease inhibitors were N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)* 50 mg/ml, Nalpha-p-tosyl-L-lysine chloromethyl ketone (TLCK)* 25 mg/ml and phenylmethylsufonyl fluoride 174 mg/ml.

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* Sigma Chemical Co. Ltd., Poole, England.

Larvae were washed repeatedly in the 0.01M Tris buffer with gentle centrifugation (3 minutes at 300g).

The washed larvae were resuspended in the minimum volume of the Tris buffer containing the protease inhibitors and sodium deoxycholate was added to a final concentration of 1%. After standing the tube in ice for 15 minutes, the homogenate was centrifuged at 4° C for 30 minutes at 15,000 g to recover the soluble fraction.

Antibody Measurements

Three techniques were used for antibody measurement, namely Haemagglutination Tests, ELISA and Immunoprecipitation.

1. Haemagglutinating antibody tests

Haemagglutinating antibody measurements were carried out as previously described. Titres were measured in faecal extracts, small intestine contents and in serum. Two N. brasiliensis antigens were used in these tests, L4SE and ESP antigens.

2. ELISA tests

Flat-bottomed microtitration plates (Dynatech-Microelisa R) were coated with 100 µl of L4SE or adult nematode ESP antigens in concentrations of 20 µl/ml of coating solution. The antigens had been previously pre-absorbed on red cells coated with antisera to rat immunoglobulins in order to remove any host globulin contamination. The test samples were diluted to 500 µg protein/ml with 0.1% bovine serum albumin in phosphate buffer solution. The plates were coated with 100 µl antigen solution per well (18 hours, 4°C), washed 3 times with PBS-Triton solution and incubated

at room temperature for 1½ hours with 100 µl of diluted sample per well. Following incubation the plates were washed three times with Triton-PBS and 100 µl of enzyme conjugated anti-rat immunoglobulin per well was then added and the plates were incubated again for 1½ hours at room temperature. The excess of the second antibody was then removed and washed out (tap water followed by three washes with PBS-Triton solution) and 100 µl/well of enzyme substrate solution with TMB (3,3^{1} 5,5^{1}-tetramethylbenzidine) as a chromogen was added (Bos, van der Doelen, van Rooy and Schuurs, 1981). After 1 hour incubation the enzyme reaction was stopped by the addition of 100 µl/well of 2M sulphuric acid. The optical density was then estimated using an ELISA reader (MR 580 Microelisa Autoreader, Dynatech Lab., Alexandria, Virginia, U.S.A.).

Sheep anti-rat IgM, IgG and goat anti-rat IgA sera were labelled with horseradish peroxidase, as described by Avrameas and Ternynck (1971) with modifications employed by MacDonald, Nicol, Belfield, Shah and Mack (1980).

Ten mg of horseradish peroxidase was dissolved in 0.2 ml of 0.1Mphosphate buffer pH 6.8 containing 1.25% gluteraldehyde and allowed to stand for 18 hours at room temperature. This preparation was then dialysed four times against distilled water and finally against isotonic saline. Following dialysis 0.3 ml of 0.001 M carbonate-bicarbonate buffer pH 9.6 and a volume of either anti-rat IgA, IgG or IgM containing 10 mg of protein was added to the peroxidase solution. After storing for 24 hours at 4°C 0.1 ml of 0.2 M lysine solution was added and the mixture stored for a further 2 hours at 4°C. The antibody-enzyme conjugate was isolated by

passing the mixture through a 100 x 2.5 cm column of Sephadex G 100. The fractions containing the first protein peak were pooled and divided into 1 ml aliquots and stored at -20°C. Before use these aliquots were diluted 12 fold with isotonic saline with the addition of 0.1 ml 1 M sodium citrate, pH 7.4 to each diluted 1 ml aliquot.

3. Immunoprecipitation tests

(a) Anti-L3SE and anti-ASE secretory IgA levels in small intestine contents and in faecal extracts

Anti-L3SE and anti-ASE secretory IgA levels in small intestine contents and in faecal extracts were measured by immunoprecipitation tests using rabbit anti-rat secretory IgA serum radiolabelled with ^{125}I, as described by Greenwood, Hunter and Glover (1963). Small intestine and faecal globulins from infected and control animals were diluted to 100 µg of protein/ml with 10 mM Tris buffer, pH 8.3. Aliquots of 100 µl of these preparations were dispensed into test tubes and then 100 µl of ASE or L3SE (1 mg protein/ml) was added and the mixtures were incubated for 24 hours at room temperature. The antigen-antibody complexes were then separated by centrifugation, washed three times with cold PBS and 100 µl of ^{125}I anti-SIgA was added. After 4 hours incubation the precipitates were washed again, transferred to the new tubes and their radioactivity determined. The results were calculated by expressing the bound radioactivity as a percentage of the total radioactivity.

Table 5.2

Nippostrongylus brasiliensis burdens in rats 9 days after vaccination and 9 days after challenge with 5 x 10^3 N. brasiliensis

Group	Antigen	Route of Administration	Mean Worm Counts ± S.E. 9 Days After Vaccination	Mean Worm Counts ± S.E. 9 Days After Challenge	Percentage of Protection
I	2000 L3	Subcutaneous	519 ± 41	29 ± 16	98.7
II	1500 L4	Intragastric	468 ± 33	41 ± 8	98.6
III	1500 D5 Worms	Intragastric	144 ± 22	9 ± 13	99.6
IV	ESP Antigens (1.7 mg/rat)	Intragastric	-	866 ± 68	69.1
V	Adult Somatic Antigens (1.7 mg/rat)	Intragastric	-	2317 ± 525	17.3
VI	Challenge Controls	-	-	2801 ± 178	-

(b) Antibodies against late L4 surface antigens

Samples of serum, small intestine and faecal globulins were diluted to 50 µg protein/ml. Five µl of ^{125}I-labelled L4 surface antigen was added to 100 µl of 0.01 M Tris buffer followed by a 5 µl volume of the test sample. These tubes were incubated at 37°C for 3 hours followed by the addition of 50 µl of anti-rat IgA, IgG or IgM. After a further incubation of 3 hours at 37°C the precipitated antigen-antibody complexes were washed three times in cold PBS and the radioactivity of the precipitates was determined in a gamma spectrometer.

The results were expressed as the percentage of the radioactivity of the trichloroacetic acid precipitation of the added labelled antigen.

Results

Immunity to Reinfection

The mean worm counts are presented in Table 5.2. Worm burdens found after challenge in groups immunised with viable parasites (I - III) were very low and the rats showed 98.7 - 99.6% reduction in challenge establishment. In Group IV, immunised with adult ESP antigens, 17.3% of the challenge infection became established in the intestines compared with 56% in challenge controls. The mean number of intestinal worms detected in rats immunised with adult somatic antigens (Group V) was slightly lower than in controls but the difference was not statistically significant.

Serum Haemagglutinating Antibody Titres Against L4SE and ESP Antigens

The results of these tests are shown in Fig. 5.1.

Serum Antibody Titres Against L4SE

Nine days after vaccination, the highest titres were observed in Group I (vaccinated subcutaneously with L3 larvae), in Group III (vaccinated intragastrically with L3 larvae) and in Group IV (vaccinated intragastrically with ESP antigens). After challenge the highest titres were recorded in Group IV and Group V (vaccinated intragastrically with ASE antigens. Results from individual rats are shown in Appendix E, Table 1.

Serum Antibody Titres Against ESP

The serum antibody responses to ESP antigens were poor, both after vaccination and after challenge infection. Nine days after vaccination no antibodies could be detected in serum from Group V rats. Results from individual rats are shown in Appendix E, Table 2.

Serum Immunoglobulin Responses to L4SE and ESP Antigens

Serum immunoglobulin levels specific to L4SE and ESP antigens measured by the ELISA method are shown in Fig. 5.2.

IgA Levels

Immunoglobulin A responses to both antigens were very low in all groups of rats. In the vaccinated groups serum IgA levels barely exceeded the level detected in the uninfected control group (Group VII)

IgG Levels

Serum IgG levels were significantly higher than those recorded for IgA. Immunoglobulin G levels against L4SE antigens in Group I

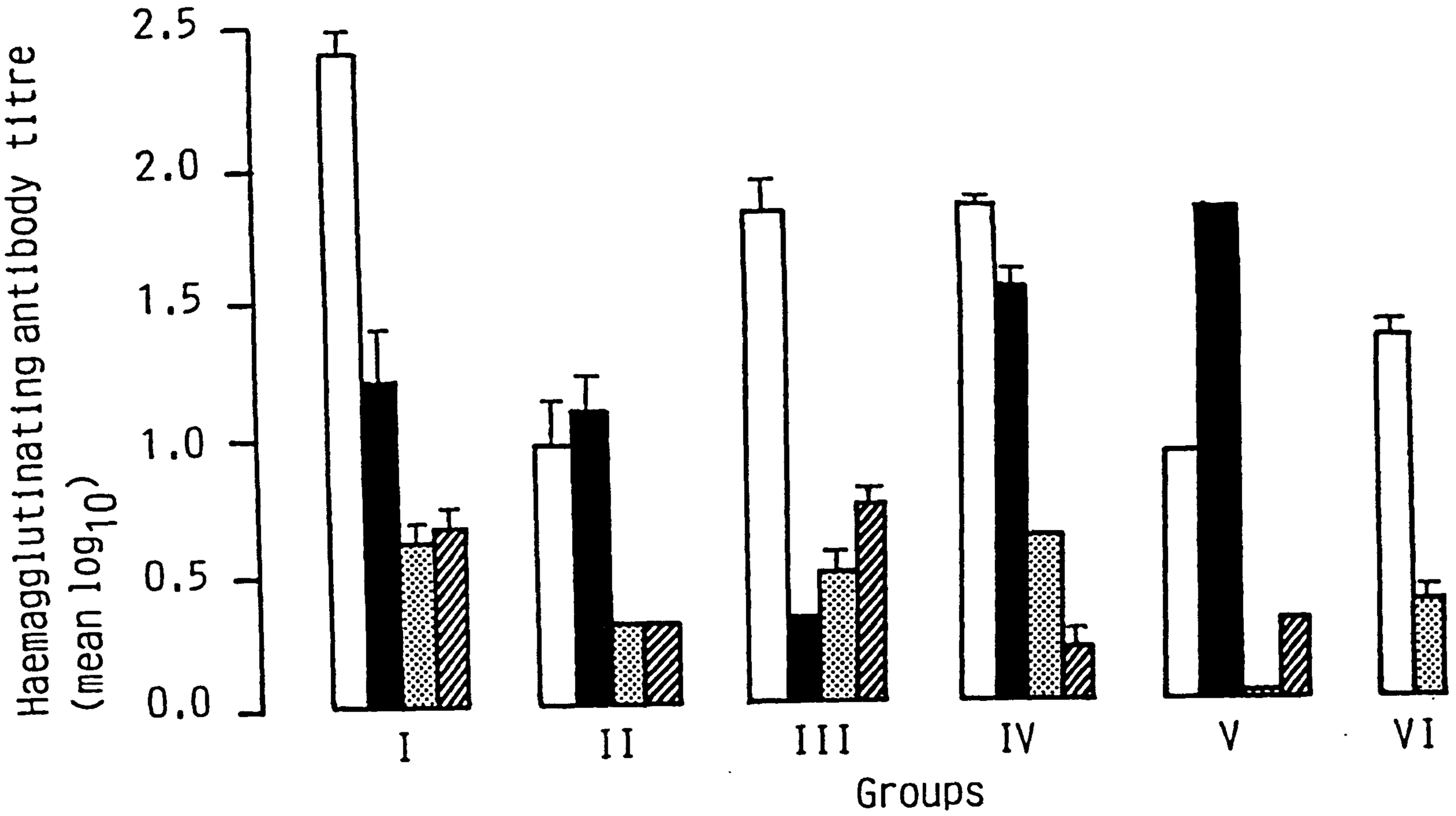

Fig. 5.1. Haemagglutinating antibody titres against L4SE and ESP antigens in serum of rats after vaccination with N. brasiliensis antigens and after challenge with 5 x 10^3 N. brasiliensis. The histograms represent the mean for 5 animals (± S.E.). (I = 2000 L3, II = 1500 L4, III = 1500 adults, IV = ESP, V = ASE, VI = challenge controls)
□ serum anti-L4SE IgA on day 9 after vaccination, ■ serum anti-L4SE IgA day 9 after challenge, ▣ serum anti-ESP IgA on day 9 after vaccination, ▨ serum anti-ESP IgA on day 9 after challenge.

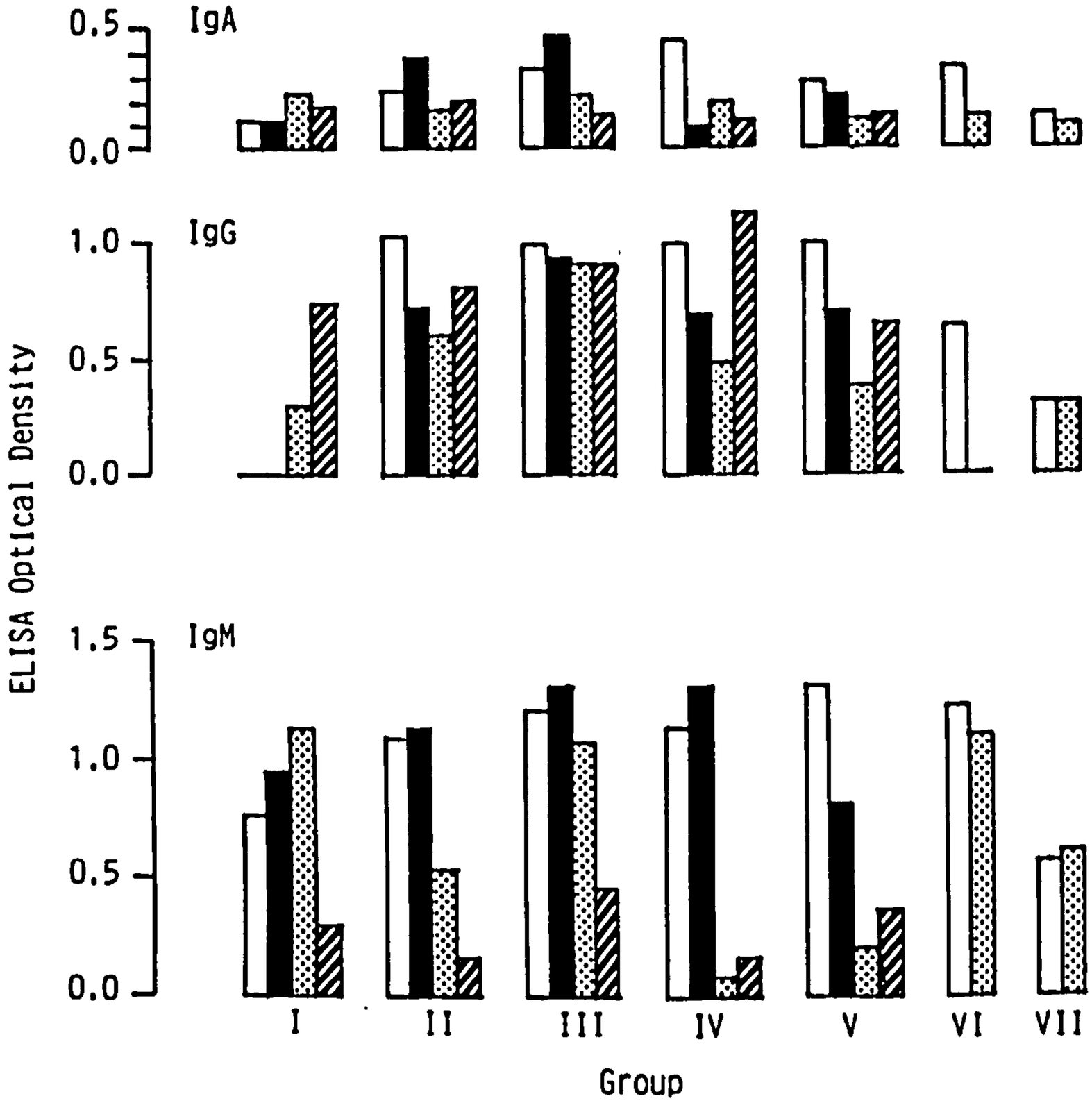

Fig. 5.2. Anti-L4SE and anti-ESP IgA, IgG and IgM levels in serum of rats after vaccination with N. brasiliensis antigens and following challenge with 5×10^3 N. brasiliensis. Histograms represent results (optical density) from pooled samples from each group. (I = 2000 L3, II = 1500 L4, III = 1500 adults, IV = ESP, V = ASE, VI = challenge controls, VII = worm-free controls.

□ anti-L4SE 9 days after vaccination

■ anti-L4SE 9 days after challenge

▩ anti-ESP 9 days after vaccination

▨ anti-ESP 9 days after challenge

and against ESP antigens in Group VI were not measured. Similar patterns of serum IgG responses to L4SE were recorded in the remaining vaccinated groups on day 9 after vaccination and after challenge infection. After vaccination serum IgG responses to ESP antigens were lower than against L4SE, and except in Group III anti-ESP antibody levels after challenge infection showed increases.

IgM Levels

Serum immunoglobulin M responses to L4SE antigens were higher than worm-free controls in all groups.

In Groups I - IV, small increases in IgM levels were observed on day 9 after challenge infections. Group V showed higher serum IgM levels at day 9 after vaccination and a lower level after challenge.

Groups I and III showed high levels of serum IgM against ESP antigens. After challenge infection IgM serum levels decreased in Groups I, II and III. Serum IgM levels against ESP in Groups IV and V were very low and only showed small increases after challenge infection. However the IgM levels at all times in these groups were lower than in the uninfected control rats, Group VII.

Mucosal Antibody Isotypes Against L4SE and ESP Antigens

Anti-L4SE and anti-ESP, IgA, IgG and IgM levels in mucosal extracts measured by the ELISA method on day 9 after vaccination or antigen administration and on day 9 after challenge are presented in Fig. 5.3.

Anti-L4SE Immunoglobulin Levels

On day 9 after vaccination, anti-larval IgA reached the highest values in Groups I, II and III. After challenge, an increase

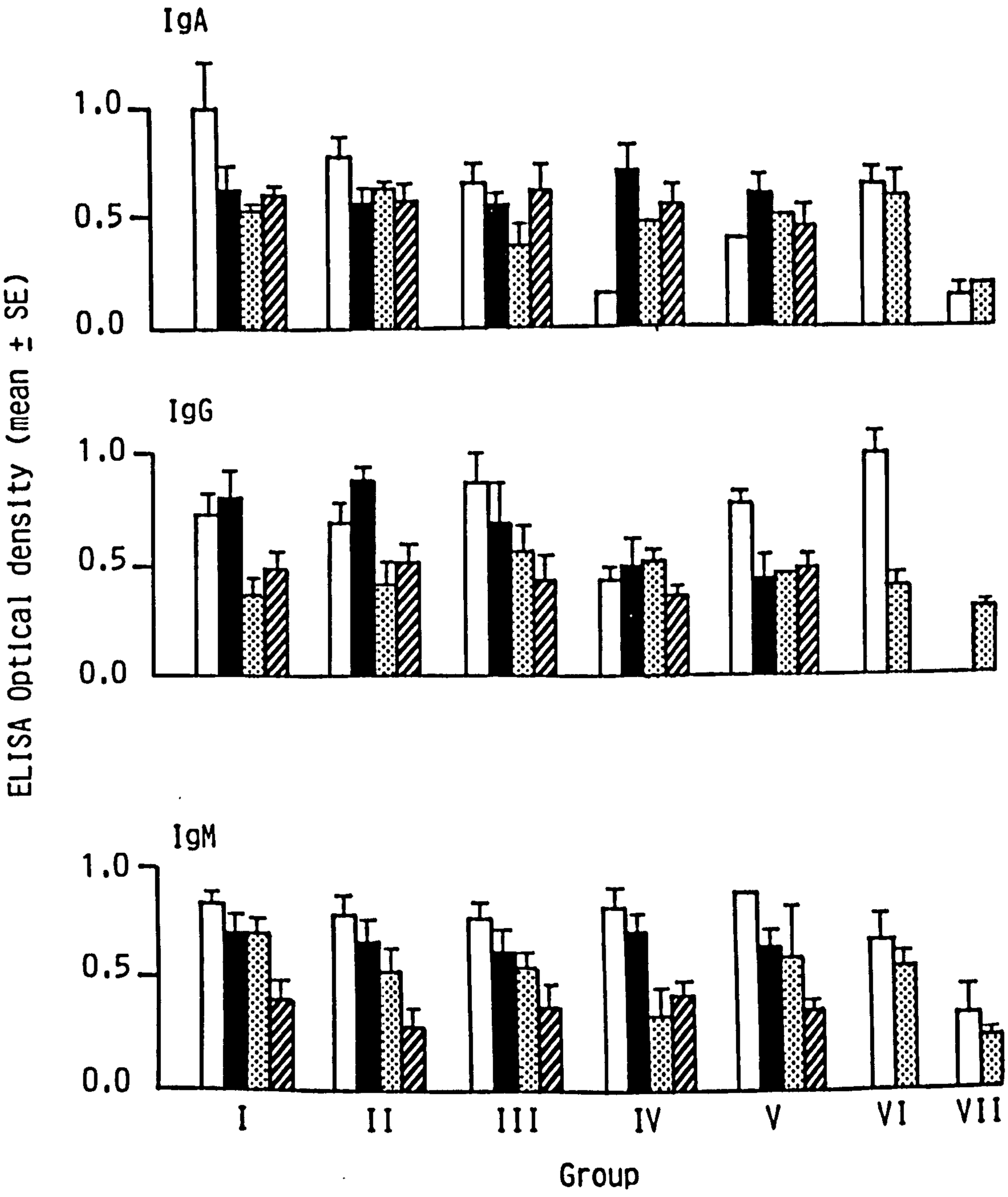

Fig. 5.3. Anti-L4SE and anti-ESP IgA, IgG and IgM levels in small intestine mucosal extracts of rats after vaccination with N. brasiliensis antigens and after challenge with 5×10^3 N. brasiliensis. Histograms represent mean optical densities for 5 rats ± S.E.
(I = 2000 L3, II = 1500 L4, III = 1500 adults, IV = ESP, V = ASE, VI = challenge controls, VII = worm-free controls).

□ anti-L4SE 9 days after vaccination

■ anti-L4SE 9 days after challenge

▨ anti-ESP 9 days after vaccination

▨ anti-ESP 9 days after challenge

in anti-larval IgA was noticed in Groups IV and V but the antibody levels observed at that time were no higher than in challenge controls (Group VI).

Post-challenge IgG and IgM levels were in general equal to or lower than those of the challenge controls (Group VI).

Anti-ESP Immunoglobulin Levels

Mucosal anti-ESP immunoglobulin levels were, in general, at lower levels on day 9 after vaccination than those detected with larval antigens. After challenge, the IgA and IgG values were similar and IgM levels slightly lower than in challenge controls

Haemagglutinating Antibody Titres Against L4SE and ESP Antigens in Small Intestine Contents

Haemagglutinating antibody titres against L4SE and ESP antigens in small intestine contents are shown in Fig. 5.4.

Antibody Titres Against L4SE Antigens

Titres against L4SE on day 9 after vaccination and in the challenge controls 9 days after challenge were almost identical. After challenge infection antibody levels against L4SE were similar to those detected 9 days after vaccination except in Group II where the post challenge level was 50% lower.

Results from individual rats are given in Appendix E, Table 4.

Antibody Titres Against ESP Antigens

Levels of haemagglutinating antibody against ESP antigens 9 days after vaccination showed highest titres in Groups II and III. in Groups I and V antibody levels showed increases after challenge

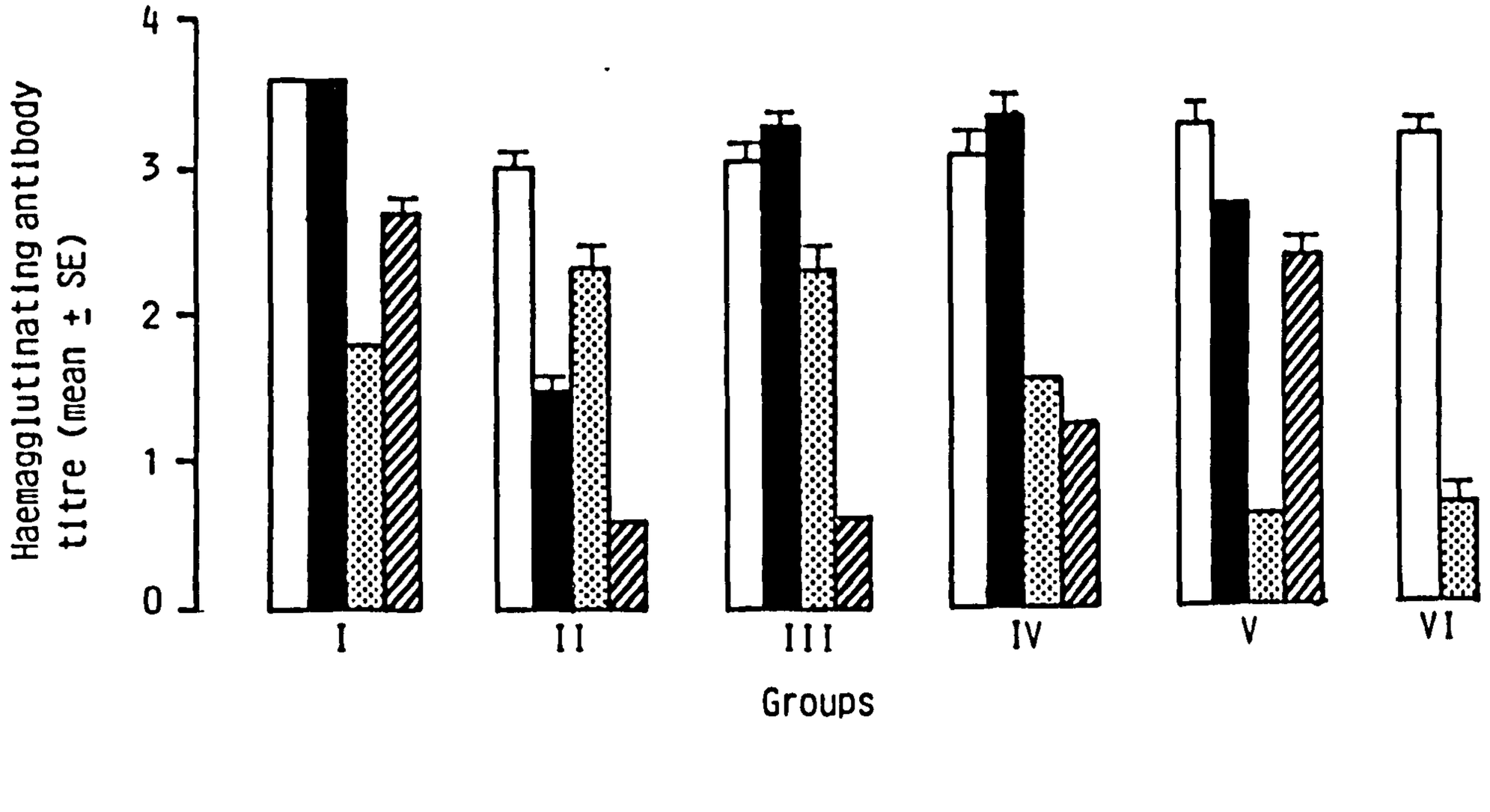

Fig. 5.4. Haemagglutinating antibody titres against L4SE and ESP antigens in small intestine contents of rats after vaccination with N. brasiliensis antigens and following challenge with 5×10^3 N. brasiliensis. Histograms represent mean log 10 ± S.E. from 5 rats. (I = 2000 L3, II = 1500 L4, III = 1500 adults, IV = ESP, V = ASE, VI = challenge controls, VII = worm-free controls.)

- □ anti-L4SE 9 days after vaccination
- ■ anti-L4SE 9 days after challenge
- ▨ anti-ESP 9 days after vaccination
- ▨ anti-ESP 9 days after challenge

infection. In the other groups there was a decrease after challenge infection. In all groups small intestine antibody levels to ESP antigens were lower than similar measurements against L4SE antigens.

Results from individual rats are shown in Appendix E, Table 5.

Anti-L4SE and Anti-ESP Immunoglobulin Levels in Small Intestine Contents

Anti-L4SE and anti-ESP, IgA, IgG and IgM levels in small intestine contents are shown in Fig. 5.5.

Immunoglobulin A levels against L4SE antigens on day 9 after vaccination were highest in Group II rats, which had been vaccinated with L4 larvae. In Group III the IgA titre was similar to the value in the uninfected control group. Nine days after challenge the highest intestinal IgA level was recorded in Group I rats.

Highest IgA levels against ESP antigens were recorded in Group III rats.

No clear pattern of IgG responses to these antigens was apparent.

Small intestine IgM responses specific to L4SE were similar in all vaccinated groups and the levels were significantly higher than in uninfected controls. The highest values were found in Group V rats which were vaccinated with ASE antigens.

Groups I and IV showed very low intestinal IgM responses to ESP antigens at 9 days after vaccination. The values were similar to levels in uninfected control animals. At this time the highest values for intestinal IgM were found in Groups III and V. Nine days after challenge high IgM concentrations were found in

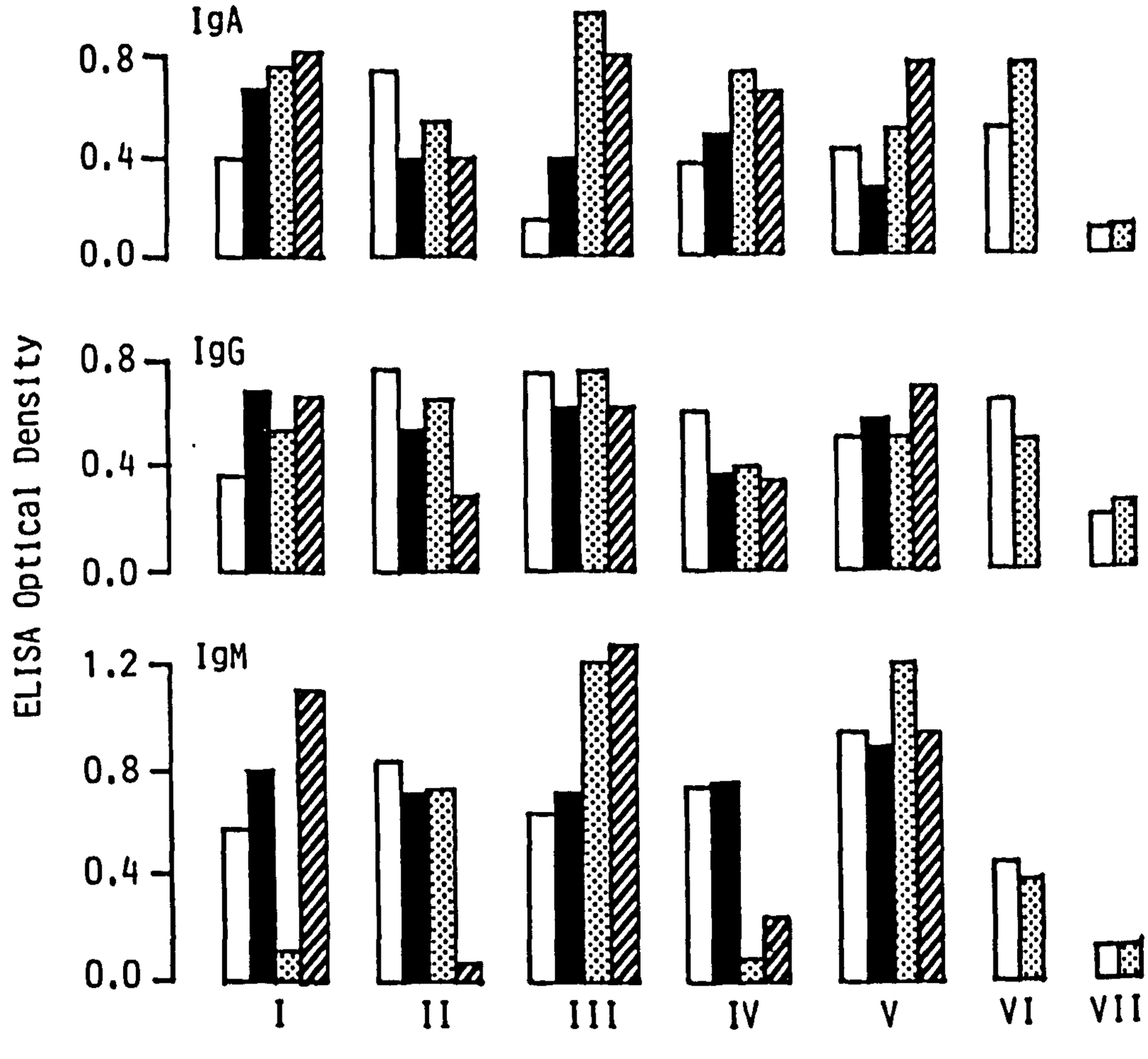

Fig. 5.5. Anti-L4SE and anti-ESP IgA, IgG and IgM levels in small intestine contents of rats after vaccination with N. brasiliensis antigens and after challenge with 5×10^3 N. brasiliensis. Histograms represent results (optical density) from pooled samples for each group. (I = 2000 L3, II = 1500 L4, III = 1500 adults, IV = ESP, V = ASE, VI = challenge controls, VII = worm-free controls.

- □ anti-L4SE 9 days after vaccination,
- ■ anti-L4SE 9 days after challenge
- ▨ anti-ESP 9 days after vaccination
- ▨ anti-ESP 9 days after challenge

intestinal contents of Groups I, III and V. In contrast similar measurements in Groups II and IV showed IgM values similar to uninfected controls.

Intestinal IgM and IgG antibodies also showed different levels depending upon the antigen used for immunisation and the route of the antigens administration. For example, considerable increase in anti-ESP IgM was observed after challenge in Group I whilst in Group II at the same time the IgM level decreased significantly.

Haemagglutinating Antibody Titres in Faecal Extracts

Haemagglutinating antibody titres against L4SE and ESP antigens in faecal extracts of Groups I - IV rats are shown in Fig. 5.6.

Faecal Antibody Titres Against L4SE Antigens

Following infection or vaccination the sharpest increases in titres were observed in Groups I, II and III, which had been given infective (L3) larvae, L4 larvae or day 5 adult worms, respectively. Faecal antibody responses were much slower in Groups IV and V, although between day 9 and 12 after vaccination all groups, except II, showed the same antibody titres. Titres in Group II were higher during this period. Nine days after challenge infection, the highest titres were shown in Groups II and III and the lowest in Groups I and V.

Faecal Antibody Titres Against ESP Antigens

Faecal antibody titres in Groups I, II and III showed similar increases following vaccination and after challenge infection.

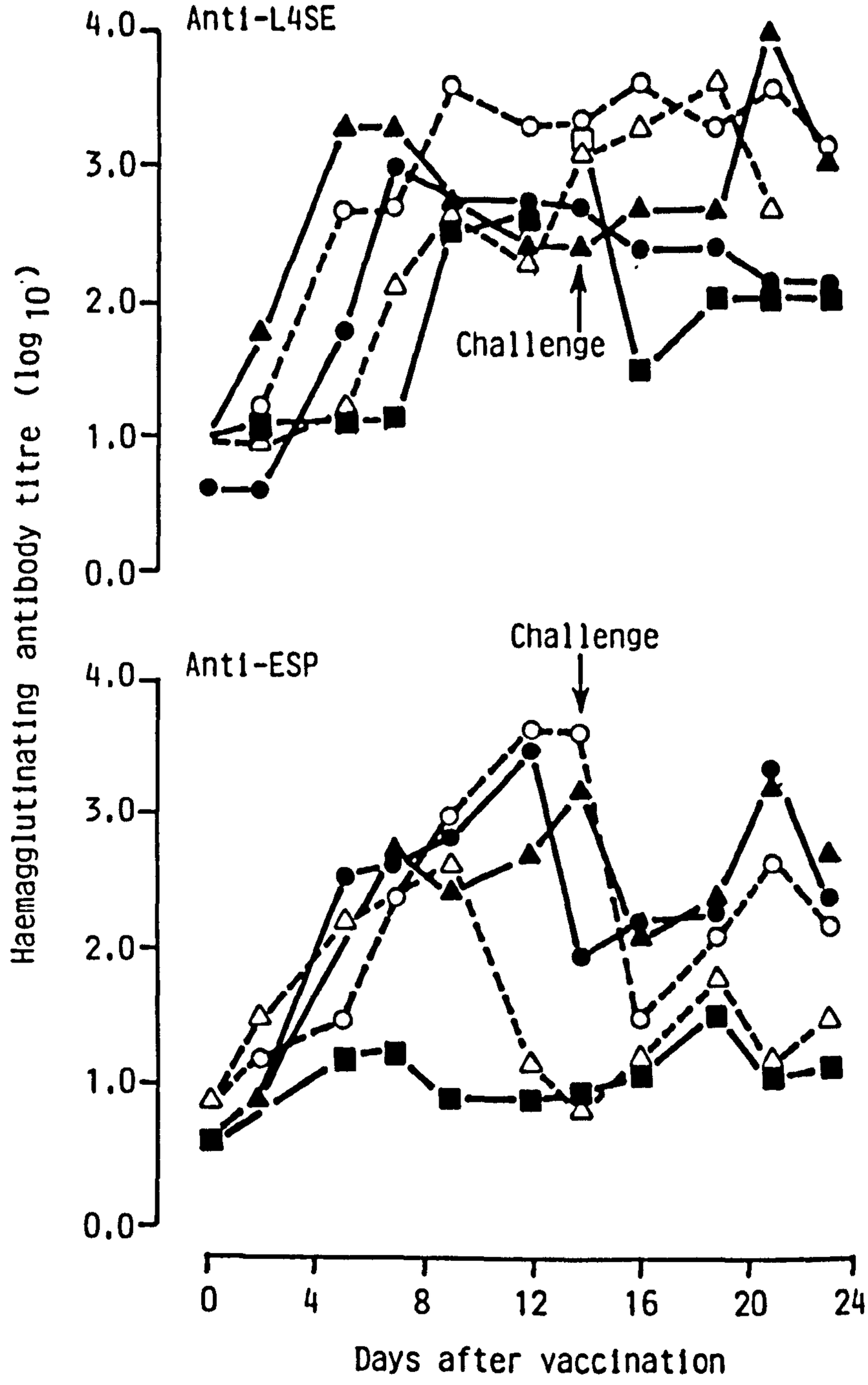

Fig. 5.6. Haemagglutinating antibody titres (log 10) against L4SE and ESP antigens in faecal extracts of rats after vaccination with N. brasiliensis antigens and after challenge with 5×10^3 N. brasiliensis. Points represent pooled samples for each group.

—●— 200L3 - -o- - 1500L4

—▲— 1500 adults - -Δ- - ESP

—■— ASE

Group IV rats, vaccinated with ESP antigens responded in a similar way as in Groups I - III, but showed a significant decline between 9 days after vaccination and challenge on day 14. Group V rats showed only slight faecal antibody responses to ESP antigens. However after challenge, titres in Groups IV and V increased in a similar manner as in the other groups.

Intestinal and Faecal IgA Against L3SE and ASE Antigens

The intestinal and faecal IgA responses to infective larvae and adult nematode somatic antigens were measured by immunoprecipitation tests using ^{125}I radiolabelled secretory IgA. The results are presented in Fig. 5.7.

Anti-L3SE IgA Levels

Levels of IgA specific to L3SE in both small intestine contents and faecal extracts were low. On day 9 after vaccination the highest IgA level was detected in intestinal extracts of Group I and in faecal extracts of Group III. After challenge a small increase was detected only in faecal extracts of Group I.

Anti-ESP IgA Levels

Intestinal IgA reacting with adult somatic antigens (ASE) showed slightly higher levels than those against L3SE and faecal IgA showed 2 - 3 times higher activity than intestinal IgA. On day 9 after vaccination the highest level of faecal IgA was measured in Group III and after challenge faecal extracts of Groups II, III and V all showed high levels of anti-ASE IgA.

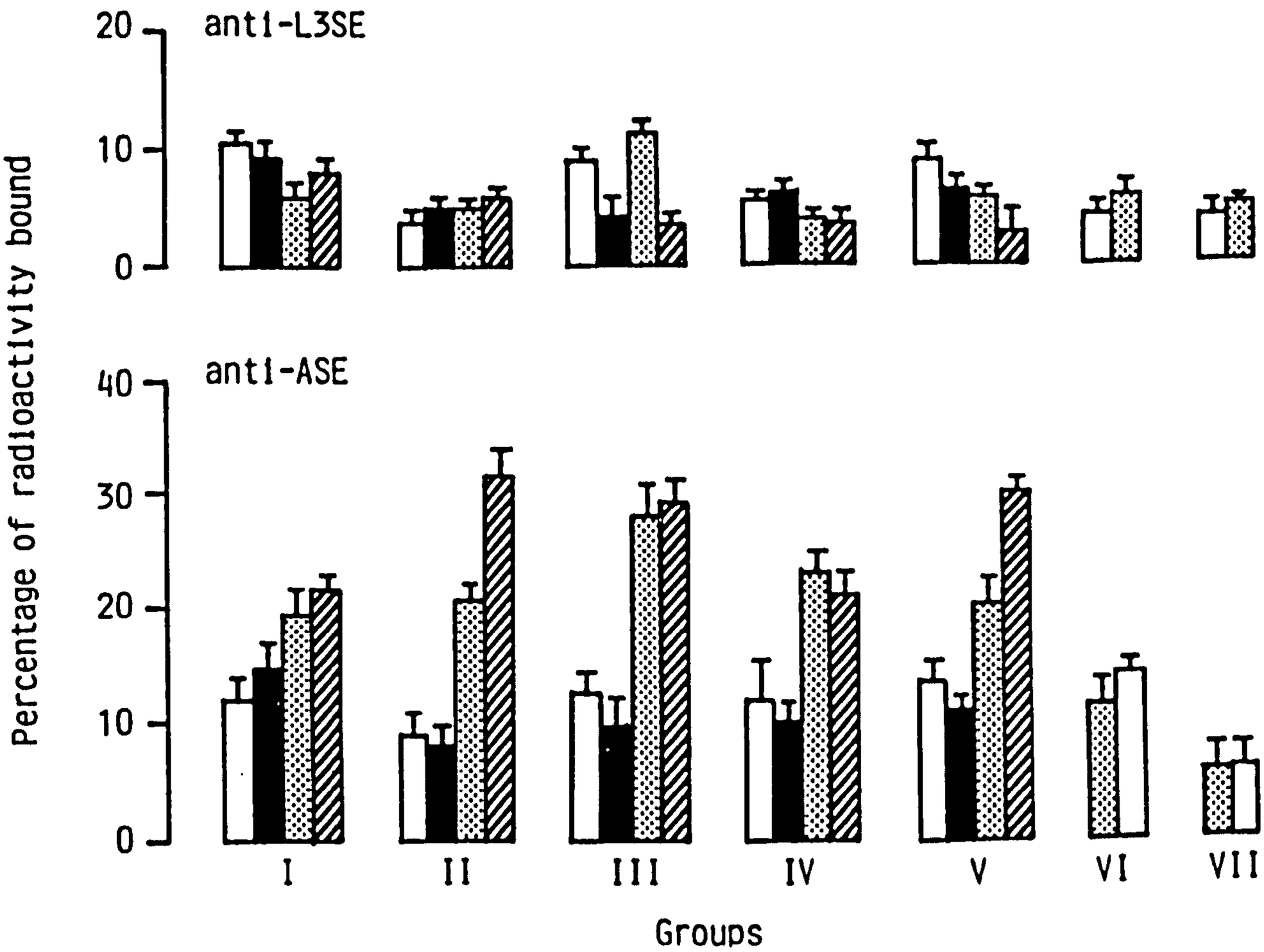

Fig. 5.7. Anti-L3SE and anti-ASE IgA levels in small intestine contents and in faecal extracts of rats after vaccination with N. brasiliensis antigens and following challenge with 5×10^3 N. brasiliensis. Histograms represent mean of % radioactivity bound for 5 rats ± S.E. (I = 2000 L3, II = 1500 L4, III = 1500 adults, IV = ESP, V = ASE, VI = challenge controls, VII = worm-free controls.

□ intestinal IgA 9 days after vaccination

■ intestinal IgA 9 days after challenge

▩ faecal IgA 9 days after vaccination,

▨ faecal IgA 9 days after challenge

Faecal IgA Antibodies Against L4SE and Adult ESP Antigens

The levels of IgA antibodies detected by the ELISA method in faecal extracts are presented in Fig. 5.8.

Anti-L4SE Antibodies

Antibodies detected with larval antigens showed a different pattern to that observed when adult excretory-secretory products were used. In faecal extracts of Group I the anti-L4SE IgA increased until day 7 of the experiment but later the concentration decreased and until the end of the experiment fluctuated at low levels. In Group II anti-L4SE IgA increased in a similar manner to Group I, reaching a maximum on day 9 after vaccination. Prior to challenge the highest anti-L4SE IgA responses were also observed on day 9 after vaccination in Groups III and IV. In contrast to the other groups, Groups III and IV showed a significant increase in ELISA values after challenge. Levels of anti-L4SE in faecal extracts of Group V rats, vaccinated with ASE antigens,were low throughout the study.

Anti-ESP IgA Antibodies

In Group I rats, anti-ESP IgA was maximal between days 12 - 14 after vaccination and then decreased steadily. In Group II faecal anti-ESP antibody increased until day 5 after challenge when the level observed was twice as high as that of anti-L4SE IgA. Group III showed maximum anti-ESP IgA response on day 9 after vaccination and there was no post-challenge increase in anti-ESP IgA. Group IV which showed the highest anti-ESP IgA level on day 9 after vaccination also showed a relatively large anamnestic increase in anti-ESP IgA. Anti-ESP faecal IgA concentration in Group V rats reached maximum

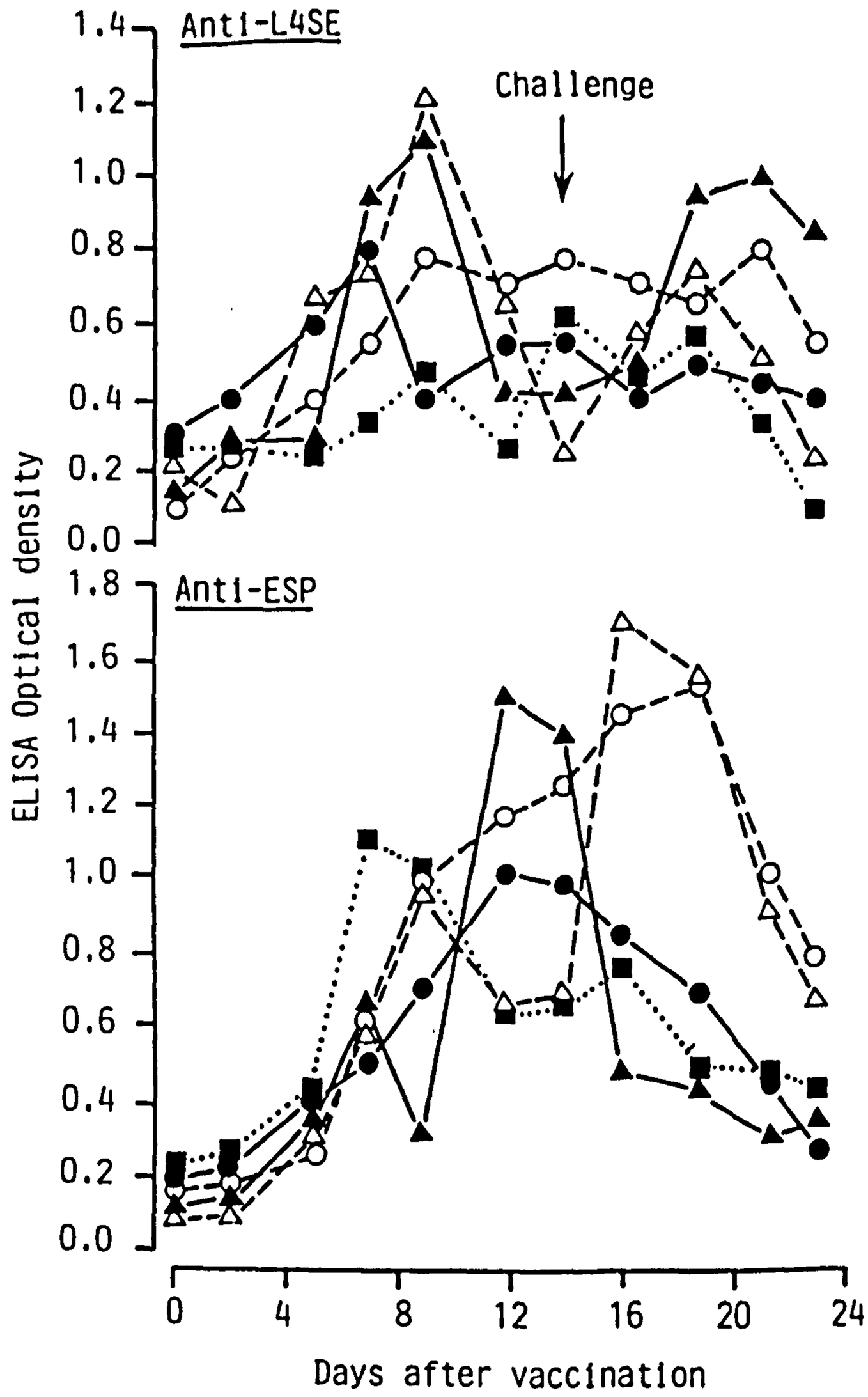

Fig. 5.8. Anti-L4SE and anti-ESP IgA levels in faecal extracts of rats after vaccination with N. brasiliensis antigens and following challenge with 5 x 10³ N. brasiliensis. Points represent mean of three optical density readings per group.

● = 2000 L3

○ = 1500 L4

▲ = 1500 adults

△ = ESP

■ = ASE

value on day 7 after vaccination and quickly declined to relatively low levels.

Intestinal Faecal and Serum Antibodies Against Surface Antigens

The levels of antibodies against late (90 hour) L4 surface antigens measured on day 9 after vaccination and on day 9 after challenge are presented in Fig. 5.9.

In small intestine contents the highest reactions on day 9 after vaccination were observed in Groups II, III and also V. After challenge, a significant increase in the antibodies was observed in Group IV. In faecal extracts the highest reactivity against L4 surface antigens was observed in Groups II and V. In the former group the percentage of bound activity at that time was 3 times higher than in Group I. In the sera, antibodies against surface antigens reached the highest levels in Groups I and II.

Discussion

The investigations described in this study represent an attempt to identify *N. brasiliensis* antigens capable of inducing protection against reinfection. The ability of these antigens to stimulate systemic and locally induced antibodies was also examined.

Protection was measured in terms of reduced worm burdens after challenge in vaccinated rats. Reduction in establishment of the challenge infection was almost identical in groups infected subcutaneously with infective L3 or intragastrically with L4 or adult parasites.

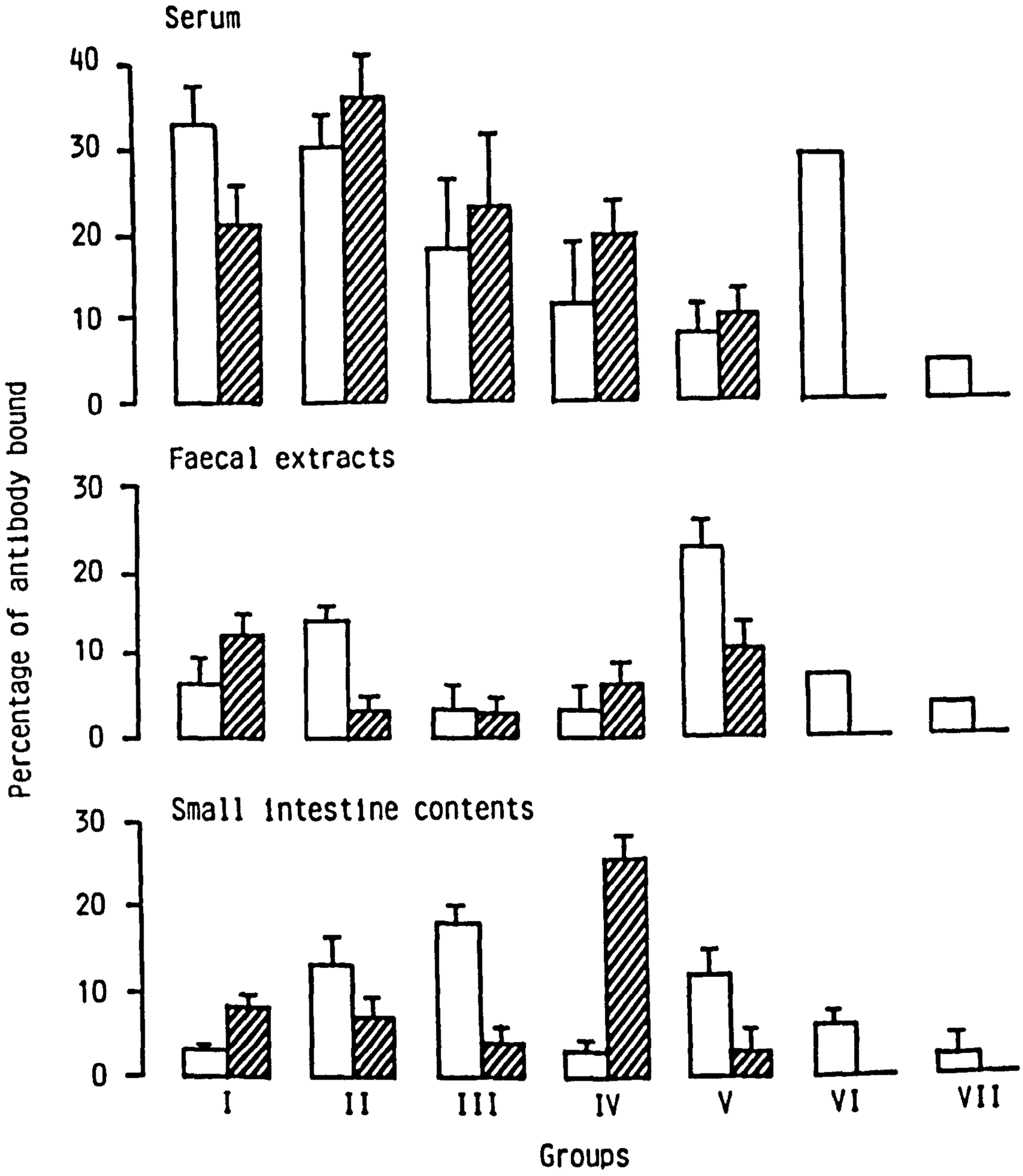

Fig. 5.9. Antibody levels against late L4 surface N. brasiliensis antigens in small intestine contents, faecal extracts and in serum of rats after vaccination with N. brasiliensis antigens and after challenge with 5×10^3 N. brasiliensis. Histograms represent means from 5 rats ± S.E. (I = 2000 L3, II = 1500 L4, III = 1500 adults, IV = ESP, V = ASE, VI = challenge controls, VII = worm free controls.)

These results support previous findings (Ogilvie, 1965) which showed that immunity to N. brasiliensis in rats is stimulated primarly by adult worms and that an infection consisting solely of adult worms terminates at the same time as an infection consisting of all stages of the parasite.

Govila, Katiyar and Sen (1972) showed that N. brasiliensis ASE antigens were not effective in inducing protection to infection. These workers found that early L4 (lung) ESP antigens offered the best protection against pre-patent stages of N. brasiliensis. Various N. brasiliensis antigens were tested for protective properties by Poulain, Pery and Luffau (1976b). Rats were vaccinated orally with 2 mg ASE, 2 mg ESP or 100 dead worms. The protection induced against challenge was 46%, 75% and 39% respectively, thus showing that ESP antigens are capable of inducing the most effective protective responses.

The various reports discussed above are in general agreement with the results reported in the present experiment. Animals vaccinated with ASE showed only 17% protection but it should be noted that in this experiment only a single vaccinating dose of 1.7 mg ASE antigen/ rat was used. The other antigens used offered relatively good protection, e.g. 69% with ESP vaccination and about 99% when living stages of the parasite were used for vaccination.

However Murray et al (1979) demonstrated that, under certain conditions, ASE antigens could give good protection against infection. It was shown that protection increased by administering multiple doses of ASE antigens. Three successive intraperitoneal vaccinations with 5 mg ASE proteins + B. pertussis adjuvant induced 90% protection.

It was also reported by these workers that intraperitoneal vaccination was more effective than subcutaneous or oral vaccination.

In addition to investigating local antibody responses to different N. brasiliensis antigens, anti-parasite systemic antibody levels were also measured in each group of rats in the present experiment.

Serum haemagglutinating antibody responses to L4SE antigens both after vaccination and after challenge infection, were much stronger than anti-ESP antibody responses in all groups of rats. One surprising result was the relatively low anti-L4SE titre in the challenge control rats which had been infected with 5,000 L3 compared with the high anti-L4SE titre in Group I rats which had been vaccinated with 2,000 L3 larvae.

Serum IgA responses to L4SE,and ESP antigens in particular, were poor in all groups of rats and were much lower than intestinal and faecal IgA levels against these antigens in all groups of rats. Strong serum IgG responses were observed, especially to L4SE antigens. There was some evidence that living intestinal phases of N. brasiliensis produced slightly better serum IgG responses after vaccination compared to intragastric vaccination with ESP or ASE antigens. Strong serum IgM responses were recorded against L4SE in all groups, both after vaccination and after challenge. However IgM responses to ESP antigens were stronger in rats vaccinated with infective L3 larvae, L4 larvae and day 5 worms.

IgM anti-ESP responses in groups vaccinated with ESP or ASE antigens were very low. Nine days after challenge IgM levels in Groups I, II and III were lower than at the same time after vaccination. There was no correlation between serum antibody levels and protection.

Measurements of small intestine mucosal anti-L4SE immunoglobulins nine days after vaccination showed that living intestinal stages of N. brasiliensis stimulated higher IgA responses than intragastric vaccination with adult ESP or ASE antigens. Following vaccination, IgG responses were similar in all groups except IV. The latter group, vaccinated with ESP antigens, showed a significantly lower IgG response. Levels of IgM 9 days after vaccination were similar in all groups.

Small intestine mucosal anti-ESP IgA levels were similar in all groups except Group III. This group showed a lower IgA response. Levels of anti-ESP IgG showed little fluctuations between the groups. Group I anti-ESP IgM levels were the highest and Group IV the lowest 9 days after vaccination. The latter fact seems a little surprising since these rats (Group IV) were vaccinated with ESP antigens. After challenge anti-ESP IgG and IgM levels reached similar values for most groups. There was some evidence that rats vaccinated with L3 larvae subcutaneously or L4 larvae or D5 adult worms invoked higher immunoglobulin responses at mucosal surfaces, compared with intragastric vaccination with adult ESP or ASE antigens. There was no evidence of any correlation between protection and mucosal anti-parasite immunoglobulin responses.

Strong haemagglutinating antibody titres against L4SE antigens were detected in small intestine contents of all groups of rats 9 days after vaccination. Except in Group II, when IgA values showed a 50% fall, IgA levels 9 days after challenge infection were similar. The highest small intestine haemagglutinating titres against ESP

antigens were recorded in Groups I, II and III 9 days after vaccination. Groups II, III and IV showed very low anti-ESP titres 9 days after challenge. When compared with the relatively high anti-ESP titre in Group I, 9 days after infection, the titre in Group VI, the challenge control group, 9 days after challenge was very low despite being given a higher number of L3 larvae and this result can not readily be explained.

Haemagglutinating antibody titres against L4SE antigens were similar irrespective of the route of vaccination, although there is evidence that anti-ESP titres were higher in the rats vaccinated with living intragastric stages of the parasite (Groups I, II and III).

Small intestine IgA responses specific to L3SE and ASE antigens measured by immunoprecipitation tests were poor and there was no evidence of any correlation between the vaccination regime and the IgA responses. Similarly there was no obvious relationship between vaccination and small intestine IgA responses specific to L4SE antigens measured by the ELISA method. There is no logical explanation for the very low intestinal anti-L4SE IgA level observed in Group III. This group showed similar anti-L4SE haemagglutinating antibody titres in small intestine contents as those in other groups and also similar mucosal anti-L4SE IgA levels as the other groups.

When small intestine globulins IgG and IgM antibody responses to L4SE and adult ESP antigens were examined it was difficult to see any clear pattern emerging. The most striking feature was the very low anti-ESP IgM levels in small intestine globulins in Groups I and IV after vaccination and in Group II after challenge. Mucosal IgG

and IgM responses to L4SE antigen were, in general, higher than similar responses to ESP antigens. There appeared to be little similarity between antibody responses to L4SE and adult ESP antigens in small intestine mucosa compared with similar measurements on corresponding small intestine content samples. This was especially so with IgM antibody levels.

An examination of the results of both small intestine haemagglutinating antibody levels and anti-parasite immunoglobulin levels failed to show any correlation between these parameters and protection against infection in any of the vaccinated rats.

Anti-L4SE haemagglutinating antibody titres in faecal extracts of rats which had been vaccinated with living intestinal stages of N. brasiliensis L4 larvae, or day 5 adult worms, showed rapid rises on the days immediately after vaccination. The first sign of elevated titres in Group I was observed on day 5 after infection by which time adult worms are in the small intestine.

Antibody responses in rats vaccinated with adult nematode ESP or ASE antigens were slower to develop. The highest anti-L4SE antibody response was observed in the rats vaccinated with L4SE larvae. Poor anamnestic anti-L4SE antibody responses were observed in Groups I and V.

Anti-ESP haemagglutinating antibody responses in faecal extracts were similar in Groups I to IV following vaccination. Group V rats, vaccinated with ASE antigens showed low anti-ESP antibody titres following vaccination. Anamnestic anti-ESP titres were higher in the groups which had experienced living N. brasiliensis in the pre-challenge period.

There was a clear relationship between anti-L4SE and anti-ESP faecal haemagglutinating antibody titres and vaccination with living intestinal stages of N. brasiliensis. It was also observed that rats vaccinated with L4 larvae showed strong faecal antibody responses to L4SE antigen and rats vaccinated with ESP antigens intragastrically showed similar strong responses to ESP antigens.

Faecal IgA antibody levels against infective larvae (L3) measured by immunoprecipitation tests were poor and similar to intestinal anti-L3 IgA antibody levels. The levels of faecal IgA specific to ASE antibodies were higher than intestinal IgA specific to ASE in the same groups of rats at the same time after vaccination. These results are difficult to explain since results previously recorded in this study have generally shown a close correlation between coproantibody and intestinal antibody responses. However no correlation could be seen between the levels of faecal anti-ASE IgA response and protection in the present experiment. For example, Group V rats, vaccinated with ASE antigens, showed a 17.3% protection while the IgA response was of a similar order to those recorded for Groups II and III. The latter groups were vaccinated with L4 larvae and adult worms respectively and the degrees of protection were about 99%.

Considerable amounts of anti-L4SE and anti-ESP IgA antibodies were detected in faecal extracts. Group III rats, vaccinated with 1500 adult nematodes had the highest pre-challenge level of anti-ESP faecal IgA on day 12 after vaccination. The lowest level of anti-ESP faecal IgA on day 9 after vaccination was recorded in Group III. This group also showed the lowest level 9 days after vaccination.

Coproantibody monitoring showed a significant anamnestic response in faecal anti-L4SE IgA and anti-ESP IgA in rats vaccinated with ESP antigens (Group IV) 4 days after challenge. Such a result could possibly be due to antigenic similarities between developing larvae and adult nematode ESP. These results support the suggestion that immunological memory operates in the secretory IgA system (Andrew and Hall, 1982). These workers transferred thoracic duct lymphocytes to nonimmunised rats from donor rats which had been immunised by injecting killed Brucella abortus antigen into Peyer's patches. It was shown that the transferred TDL conferred on the recipient rats the ability to mount a substantial biliary IgA response to suboptimal doses of antigen. Transfer of serum from immune rats conferred no protection on the recipient rats and it was further shown that the ability of the TDL to transfer protection was destroyed by irradiating the TDL. These results suggest that there is a strong immunological memory in the IgA system and that circulating lymphocytes is the mediating factor.

It has been suggested that antigens present on parasite surfaces may have an important role in host parasite interactions. Using a technique for labelling surface antigens of N. brasiliensis larval and adult stages, Maizels et al (1983) showed that as the parasite develops through its cycle in the host, changes occur in the structure of the surface antigens. Furthermore these workers demonstrated that these different surface antigens are stage specific. Serum antibodies from single stage infections were shown to be reactive only with homologous antigen. The results obtained in this experiment also tend to suggest

antigen stage specificity. Prior to challenge infection serum from rats infected with L4 larvae, and with L3 larvae showed the highest binding of radiolabelled fourth-stage larvae surface antigens. However, in intestinal globulins, the highest reactivity was observed in Group III infected with 5 day old adult N. brasiliensis and, in contrast to serum, there was no anamnestic increase after challenge. A similar observation was made in rats vaccinated with ASE. Such a result seems to suggest that intestinal antibody responses to surface antigens are less stage specific than those observed in sera. The intestinal antibody isotypes reacting with surface antigens were not estimated. However analysis of faecal antibody levels to these antigens seems to suggest an IgA response.

The lack of stage specificity in local S-IgA responses, in contrast to the stage specificity observed in serum, may possibly be caused by differences in antigenic stimulation. It has been shown that serum and intestinal antibody responses can vary depending upon the nature of the antigen and the route of its administration. After enteric administration of cholera toxins it was found that the anti-toxin cell response in the small intestine lamina propria depended on the type of antigen administered (Pierce, 1978). Crude antigens were more effective than purified material. The IgA response of intestinal mucosa to Escherichia coli toxin was shown by Klipstein,Engert and Clements (1982), to be dependent on the antigenicity route and dosage of the immunogen. It was also shown by Ogilvie (1965a)that in N. brasiliensis infections protective antigens

released by the final larval moult and by adult stages are similar. The latter work also showed that immunity stimulated by adult worms acts on all intestinal stages of N. brasiliensis and that immunity stimulated by adult worms is not stage specific, but inhibits the development of larvae to maturity in a challenge infection.

The results of the present study failed to show a correlation between local intestinal responses to the various antigens used in the tests and the degrees of protection to challenge in the vaccinated rats. However there was a clear positive correlation between anti-ESP haemagglutinating antibody titres in faecal extracts, both before and after challenge, and the degrees of protection recorded.

An analysis of faecal extracts of rats following N. brasiliensis infection was carried out in Chapter 2 using gel filtration, immuno-electrophoresis and haemagglutinating antibody evaluation. The tests showed that the antibody activity against adult ESP antigens was related almost completely to the IgA fraction. Bearing the latter fact in mind, it is not unreasonable to relate the coproantibody activity against ESP antigens with faecal secretory IgA.

Immunoglobulin-A antibodies may also be stimulated by antigens irrelevant to parasite survival. Recent suggestions have been made that rather than having an important role in protective immunity, S-IgA antibodies may facilitate some form of symbiosis between the host and the parasite (Befus, 1982; Befus and Bienenstock, 1982). This possibility is considered in Chapter 6 and in the General Discussion.

CHAPTER 6

Some Observations on the Possible Role of Lung and Faecal IgA Antibodies in Immunity to *Nippostrongylus brasiliensis* in the Rat

Introduction

In the previous chapter local and systemic antibody responses to several parasite antigens were investigated in rats vaccinated with various developmental stages of N. brasiliensis and with ASE and adult ESP antigens. These results showed that local intestinal immunoglobulin responses did not correlate with the degrees of protection induced in the various groups of vaccinated rats. However a strong positive correlation was observed between anti-ESP faecal haemagglutinating antibody levels and protection in the vaccinated rats. It was shown in Chapter 2 that the most immunologically active immunoglobulin in faecal extracts of N. brasiliensis infected rats was IgA. This suggests a possible link between IgA activity in faeces and protection.

The experiments described in the previous four chapters showed that anti-nematode haemagglutinating antibodies could be detected in faecal and lung extracts from N. brasiliensis infected rats. Furthermore the experiments described in Chapter 4 showed that the appearance of lung antibodies was accompanied by an increase in total IgA concentration in the lungs of infected rats.

The two experiments described in this chapter were designed to investigate local responses to bronchial antigenic stimulation and to further investigate lung and faecal IgA responses to N. brasiliensis antigens using different levels and different sites of stimulation. The possible significance of bronchial and enteric IgA antibodies in conferring protection against challenge was also investigated.

Table 6.1

Experimental Plan

Group	Primary Stimulation With	Number of rats dissected at intervals after Vaccination			Challenge with 5000 normal larvae DAV *	Dissection of 5 rats on day after Challenge
		20 h	5 day	9 day		
A	2,000 normal larvae	5	5	5	14	9
B	2,000 larvae irradiated at 80 krad	5	-	5	14	9
C	2,000 larvae irradiated at 120 krad	5	-	5	14	9
D	2,000 larvae irradiated at 140 krad	5	5	5	14	9
E	2,000 larvae irradiated at 160 krad	5	5	5	14	9
F	2,000 larvae irradiated at 180 krad	5	5	5	14	9
G	1.7 mg of infective larval somatic proteins intravenously	-	-	2	14	9
H	1.7 mg of infective larval somatic proteins intragastrically	-	-	2	14	9
I	-	-	-	-	-	9
J	-	-	-	-	-	9

* Days after vaccination

Experiment I

Materials and Methods

Experimental Details

In this experiment 134 young female Hooded Lister rats were used. The animals were divided into 10 experimental groups. Six groups (A - F) were given immunising doses of 2,000 normal or irradiated (80 - 180 krad) N. brasiliensis larvae. Each rat in Group G was given 1.7 mg infective larvae somatic extract intravenously and Group H was given the same antigen by the intragastric route. On day 14 after infection or antigen inoculation the rats were challenged with 5,000 normal larvae, along with a group of 5 naive rats (Group I) which served as challenge controls. A further group of worm free rats (Group J) were included in the experiment. During the experiment, faecal samples for coproantibody evaluation were collected from the groups of rats every third day. Groups of 5 rats were killed and dissected at intervals, shown in Table 6.1. At necropsy the numbers of worms in the lungs and intestine were estimated. Samples of serum, lung and small intestinal mucosa were also collected.

This experiment was carried out concurrently with the experiment described in the previous chapter and Group A, rats infected with 2,000 infective larvae, GroupI, the challenge control group and Group J, the uninfected control rats were common to both experiments.

Parasitological Techniques

N. brasiliensis culture methods, infection, lung and intestinal worm recovery were as previously described.

Irradiation of Infective Larvae

Irradiation of third stage infective larvae was carried out as previously described.

Sampling Methods

Small intestine contents, small intestine mucosal extracts and lung extracts were prepared as previously described 9 days after vaccination and 9 days after challenge. Faeces for faecal extract preparation were collected every third day.

Parasite Antigens

Infective L3 larval and L4 larval somatic antigens and adult worm excretory-secretory products (ESP) were prepared as previously described. Larval (L4SE) somatic antigens and ESP antigens were used in serological tests.

Antibody Measurement

Passive haemagglutination tests were performed according to Herbert (1975) with modifications as previously described.

The microplate ELISA tests were accomplished using flat-bottomed microtitration plates (Dynatech-MICROELISA R) and 3,3', 5,5'-tetramethylbenzidine (TMB) as a chromogen. The optimal antigen concentrations determined in preliminary tests were 15 µg/ml of coating buffer for L4 somatic antigens and 20 µg/ml for adult excretory-secretory products. The plates were coated with 100 µl of antigen solution per well (18 hr, 4°C), washed three

Table 6.2

Number of larvae in the lungs (mean of 5 rats ± S.E.) of rats after vaccination and following challenge with 5 x 10³ N. brasiliensis

Group	Time after Vaccination			9 days After Challenge
	20 hrs	5 days	9 days	
A (2000 normal larvae)	1566 ± 29	23 ± 8	0	12 ± 7
B (2000 larvae irradiated at 80 krad)	943 ± 30	N.D.	12 ± 5	8 ± 3
C (2000 larvae irradiated at 120krad)	672 ± 23	N.D.	25 ± 7	12 ± 2
D (2000 larvae irradiated at 140krad)	627 ± 17	79 ± 8	49 ± 12	7 ± 3
E (2000 larvae irradiated at 160 krad)	402 ± 29	110 ± 20	103 ± 14	9 ± 2
F (2000 larvae irradiated at 180 krad)	257 ± 12	188 ± 19	122 ± 16	16 ± 8
G (1.7 mg of larval somatic proteins intravenously)	-	-	-	2 ± 2
H (1.7 mg of larval somatic proteins intragastrically)	-	-	-	N.D.
I (challenge control)	-	-	-	7 ± 5

N.D. = Not done

times with PBS-Triton X and incubated for 1.5 hr at room temperature with 100 µl of diluted sample per well (all samples were diluted to 500 µg protein/ml with PBS containing 0.1% bovine serum albumin). Following the incubation, the plates were processed as described in Chapter 5.

Haemagglutinating antibody levels against L4SE and ESP antigens were measured in serum, small intestine globulins and in faecal globulins.

Using the ELISA method anti-L4SE and anti-ESP IgA, IgG and IgM levels were measured in lung and small intestine mucosal extracts. Faecal IgA, IgG and IgM against ESP antigens were also measured.

Results

Lung Larval Burdens

The number of larvae in the lungs at various time intervals after infection is presented in Table 6.2. With increasing irradiation dose the number of larvae reaching the lungs decreased. However, on day 9 after vaccination with irradiated larvae a number of larvae were retained in the lungs (Groups B - F). These numbers increased with increasing doses of radiation. At the same time after vaccination with non-irradiated larvae (Group A) no larvae were detected in the lungs (Table 6.2). The numbers of larvae found in the lungs of vaccinated rats after challenge were not significantly different from those found in challenge control rats.

Intestinal Worm Burdens

In contrast to the larvae counts, the number of intestinal worms decreased with increasing doses of irradiation (Table 6.3). However, even in Group F infected with larvae irradiated at 180 krad, a few intestinal worms were found on day 5 after vaccination, although no worms were found four days later.

The number of worms detected after challenge with 5,000 non-irradiated larvae in Groups A - D was very low and did not exceed 1% of the challenge dose, whilst in Groups E and F, 4% and 27% respectively of the challenge infection became established. In Group G worm burdens were almost as high as in the challenge control rats. Intragastric administration of the antigens (Group H) marginally reduced establishment of the challenge infection, although the differences were not statistically significant.

Antibody Isotypes in Lung Extracts

Anti-L4SE Antibody Isotypes

The results of lung anti-L4SE antibody isotype measurements are shown in Figure 6.1. Results from individual animals are shown in Appendix F, Tables 1 - 3.

Lung IgA antibodies measured on day 9 after vaccination showed the highest level in the group vaccinated with 140 krad irradiated larvae (Group D). After challenge the only marked increase in IgA value was detected in the group vaccinated with 160 krad irradiated larvae (Group E).

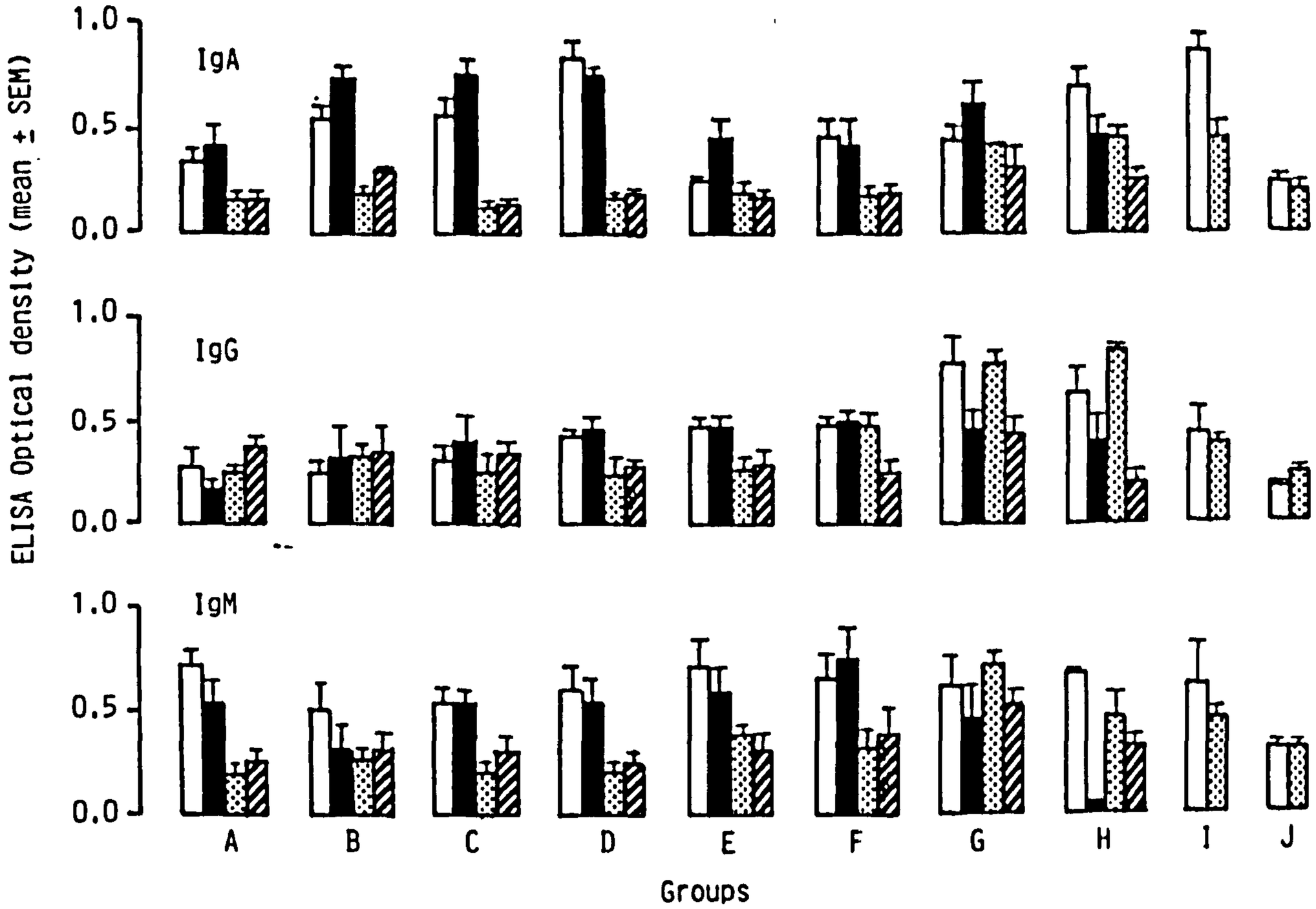

Fig. 6.1. Anti-parasite immunoglobulin levels in lung extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. The histograms represent the mean for 5 animals (± S.E.). (A = 0 krad, B = 80 krad, C = 120 krad, D = 140 krad, E = 160 krad, F = 180 krad, G = L3SE intravenous, H = L3SE intragastric, I = challenge controls, J = worm-free controls.)

□ = anti-L4SE 9 days after vaccination

■ = anti-L4SE 9 days after challenge

⊡ = anti-ESP 9 days after vaccination

▨ = anti-ESP 9 days after challenge

Levels of anti-L4SE IgG in lung extracts 9 days after vaccination were lower than corresponding IgA values, except in Groups G and H which were vaccinated with L3SE intravenously and intragastrically respectively. Immunoglobulin G levels in lung extracts of rats vaccinated with irradiated larvae increased with increasing irradiation dose. After challenge the latter groups showed small rises in IgG values, whereas significant IgG decreases were observed in the other groups.

Lung anti-L4SE IgM values 9 days after vaccination were highest in Groups A, E and F. In the groups vaccinated with irradiated larvae, IgM levels both after vaccination and following challenge tended to increase with higher radiation doses.

The results recorded showed no correlation between protection and anti-L4SE immunoglobulin levels in the lungs.

Lung Anti-ESP Antibody Isotypes

The values for lung anti-ESP antibody isotypes are shown in Fig. 6.1. Results from individual rats are shown in Appendix F, Tables 4 - 6.

In lung extracts of Groups A, C, D, E and F, IgA levels detected against ESP antigens were not significantly different from worm free controls (Group J). In Groups G and H, the IgA reactions were considerably higher, especially on day 9 after vaccination. However, after challenge only Group B showed higher IgA titres.

Anti-ESP IgG antibody levels in lung extracts of Groups A - F were significantly less than in Groups G and H 9 days after vaccination. After challenge, the level of IgG in all groups remained about or slightly lower than the level in the challenge control group.

Immunoglobulin M anti-ESP levels in all groups except G, H and I remained at or about the level recorded in the uninfected control groups both at day 9 after vaccination and after challenge. The highest lung IgM values were recorded in Group G before and after challenge.

There was no evidence of correlation between anti-ESP immunoglobulin levels in the lungs and protection.

Antibody Isotypes in Small Intestine Mucosal Extracts

Anti-L4SE Antibody Isotypes

Results of anti-L4SE antibody isotypes in small intestine mucosal extracts are shown in Figure 6.2. Results from individual rats are shown in Appendix F, Tables 7 - 9.

Immunoglobulin A responses to L4SE antigens in mucosal extracts showed a different pattern to that observed in lung extracts (Fig. 6.1). Nine days after vaccination the highest IgA concentration was observed in the group vaccinated with non-irradiated larvae (Group A). Among the groups vaccinated with irradiated larvae, C, E and F had levels of mucosal IgA above the levels in the uninfected control rats. In Groups G and H mucosal IgA levels were similar to those in the worm free controls.

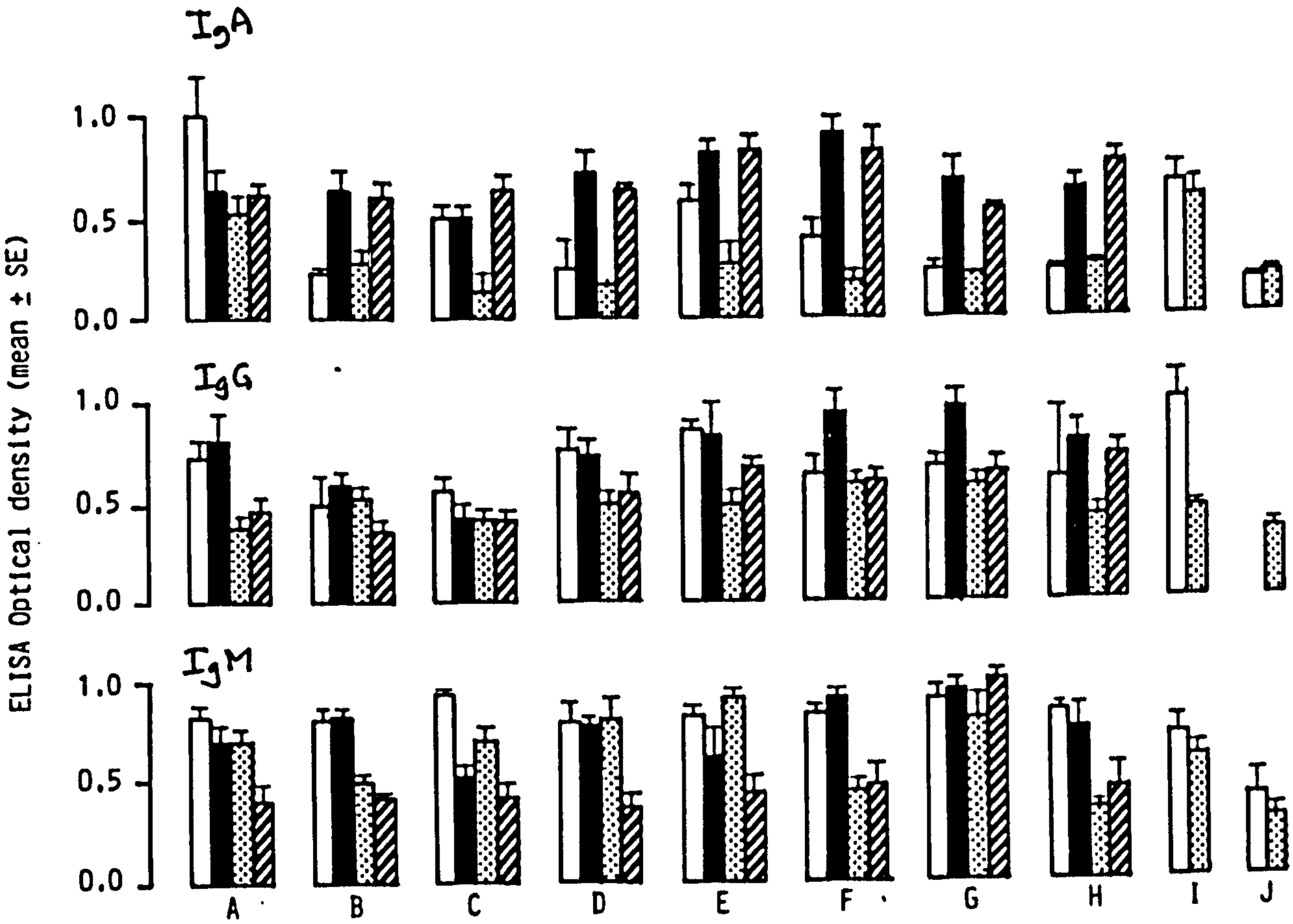

Fig. 6.2. Anti-parasite immunoglobulin levels in small intestine mucosal extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. The histograms represent the mean for 5 animals (± S.E.). (A = 0 krad, B = 80 krad, C = 120 krad, D = 140 krad, E = 160 krad, F = 180 krad, G = L3SE intravenous, H = L3SE intragastric, I = challenge controls, J = worm-free controls.)

□ = anti-L4SE 9 days after vaccination

■ = anti-L4SE 9 days after challenge

⊡ = anti-ESP 9 days after vaccination

▨ = anti-ESP 9 days after challenge

Intestinal mucosal anti-L4SE IgA levels measured in vaccinated animals after challenge were, in general, similar to those detected in challenge control rats (Group I).

In contrast to lung anti-L4SE IgA responses intestinal IgG responses to L4SE were at their highest in Groups A, D and E 9 days after vaccination. At this time mucosal IgG levels in the remaining groups were similar and at lower levels than in the former groups. After challenge IgG levels showed no clear trend, some groups recording values marginally higher or lower than pre-challenge values. Groups F, G and H showed substantial post-challenge IgG increases, approaching challenge control level in the former two groups.

Anti-L4SE intestinal IgM responses in the groups examined were more uniform than in corresponding lung IgM measurements nine days after vaccination.

After challenge anti-L4SE IgM values were generally unchanged except in Groups C and E which showed substantial falls.

Anti-ESP Antibody Isotypes

Figure 6.2 also shows the results of anti-ESP mucosal antibody isotype measurements. Results from individual rats are shown in Appendix F, Tables 10 - 12.

Small intestine mucosal anti-ESP specific IgA reached the highest level 9 days after vaccination in rats infected with normal larvae. After challenge infection the increase in IgA antibody levels in this group was proportionally much lower than in other experimental groups, when all levels recorded were similar to these in the challenge control group. Mucosal anti-ESP IgA levels were

comparatively much higher in Group A than in lung extracts and lower in Groups G and H. In the groups vaccinated with irradiated larvae, IgA levels were similar to those in worm-free controls (Group J). An almost identical pattern emerged in lung anti-ESP measurements in the latter groups. After challenge mucosal IgA levels in all groups rose to levels similar to, or higher than in challenge control rats (Group I).

Groups F and G showed the highest mucosal anti-ESP IgG levels 9 days after vaccination and Groups A, B and H the lowest, within a narrow range of O.D 0.4 to 0.7. After challenge the highest IgG levels were observed in the groups vaccinated with larvae irradiated with 160 and 180 krad and with L3SE given intravenously or intragastrically. The main difference between ESP specific mucosal IgG and lung IgG was that in lung measurements Groups G and H showed IgG levels about 2 fold greater than any other group on day 9 after vaccination.

Mucosal anti-ESP IgM levels showed the highest values 9 days after vaccination in Groups D, E and G. Anti-ESP immunoglobulin-M concentrations in Group H were similar to those in worm-free control rats. After challenge the IgM levels either fell or remained about their pre-challenge values. The exception was Group G which had been vaccinated intravenously with L3SE. This group showed a high post challenge IgM value.

Compared with worm free control animals, mucosal anti-ESP IgM values before and after challenge, showed greater increases than were observed in anti-ESP lung IgM levels.

There was no correlation between protection and anti-L4SE or anti-ESP immunoglobulin levels in small intestine mucosal extracts.

Haemagglutinating Antibody Levels in Small Intestine Contents

Results of small intestine haemagglutinating antibody measurements are shown in Figure 6.3. Results from individual rats are given in Appendix F, Tables 13 and 14.

Anti-L4SE Antibody Titres

Anti-L4SE antibody titres 9 days after vaccination were high and reached similar levels in all groups. After challenge anti-L4SE intestinal globulin titres remained high except in Group G. This low titre corresponded with the lowest protection, 3.9% 9 days after challenge. Otherwise there was no correlation between anti-L4SE intestinal antibody levels and protection.

Anti-ESP Antibody Titres

Anti-ESP small intestine globulin titres 9 days after vaccination were lower than anti-L4SE titres. Of the rats vaccinated with irradiated larvae, the lowest titre was observed in Group F, given 180 krad irradiated larvae. Similar titres were recorded for Group A, vaccinated with L3 larvae subcutaneously and Group G, given L3SE intragastrically. Anti-ESP titres on day 9 after challenge showed some correlation with protection.

Faecal Haemagglutinating Antibodies

Anti-L4SE Antibody Titres

The results are shown in Fig. 6.4.

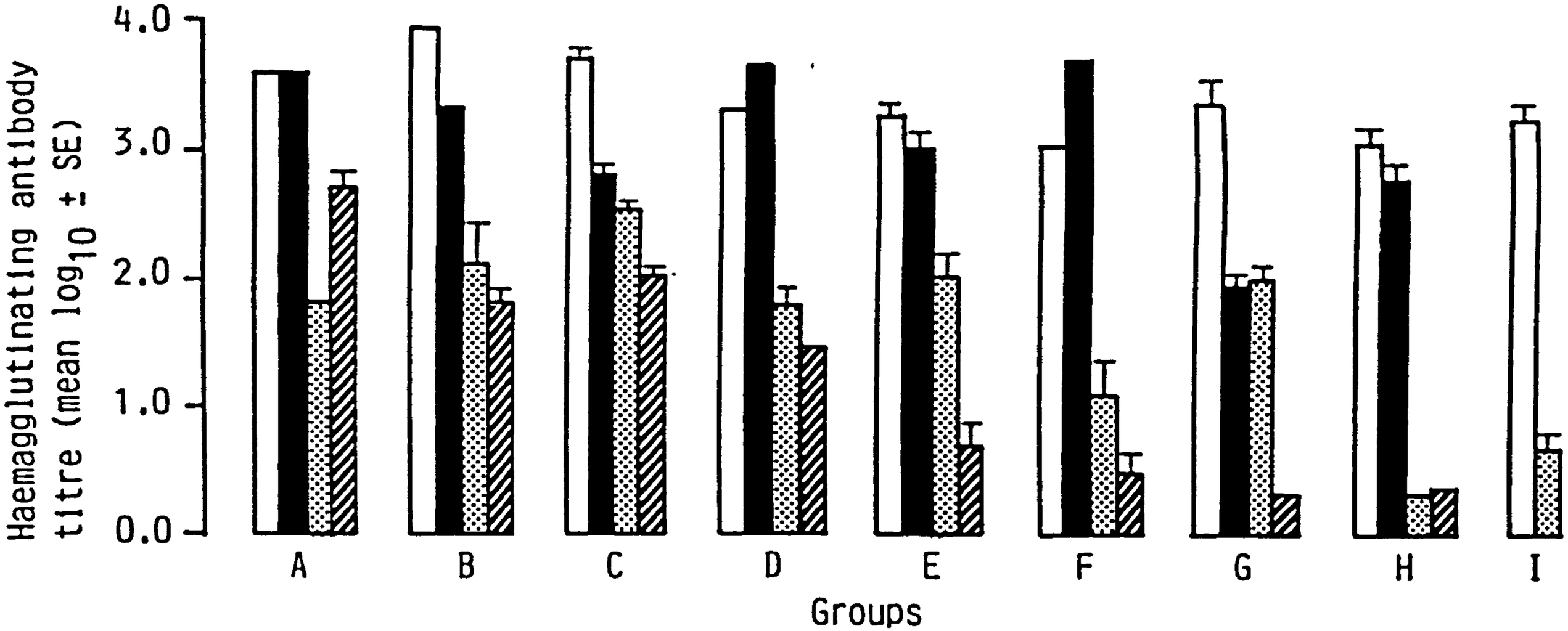

Fig. 6.3. Anti-parasite haemagglutinating antibody titres in small intestine contents of rats following vaccination and after challenge with 5 x 10^3 N. brasiliensis. The histograms represent the mean for 5 rats (log 10 ± S.E.). (A = 0 krad, B = 80 krad, C = 120 krad, D = 140 krad, E = 160 krad, F = 180 krad, G = L3SE intravenous, H = L3SE intragastric, I = challenge controls, J = worm-free controls.

□ = anti-L4SE 9 days after vaccination, ■ = anti-L4SE 9 days after challenge

▨ = anti-ESP 9 days after vaccination, ▨ = anti-ESP 9 days after challenge

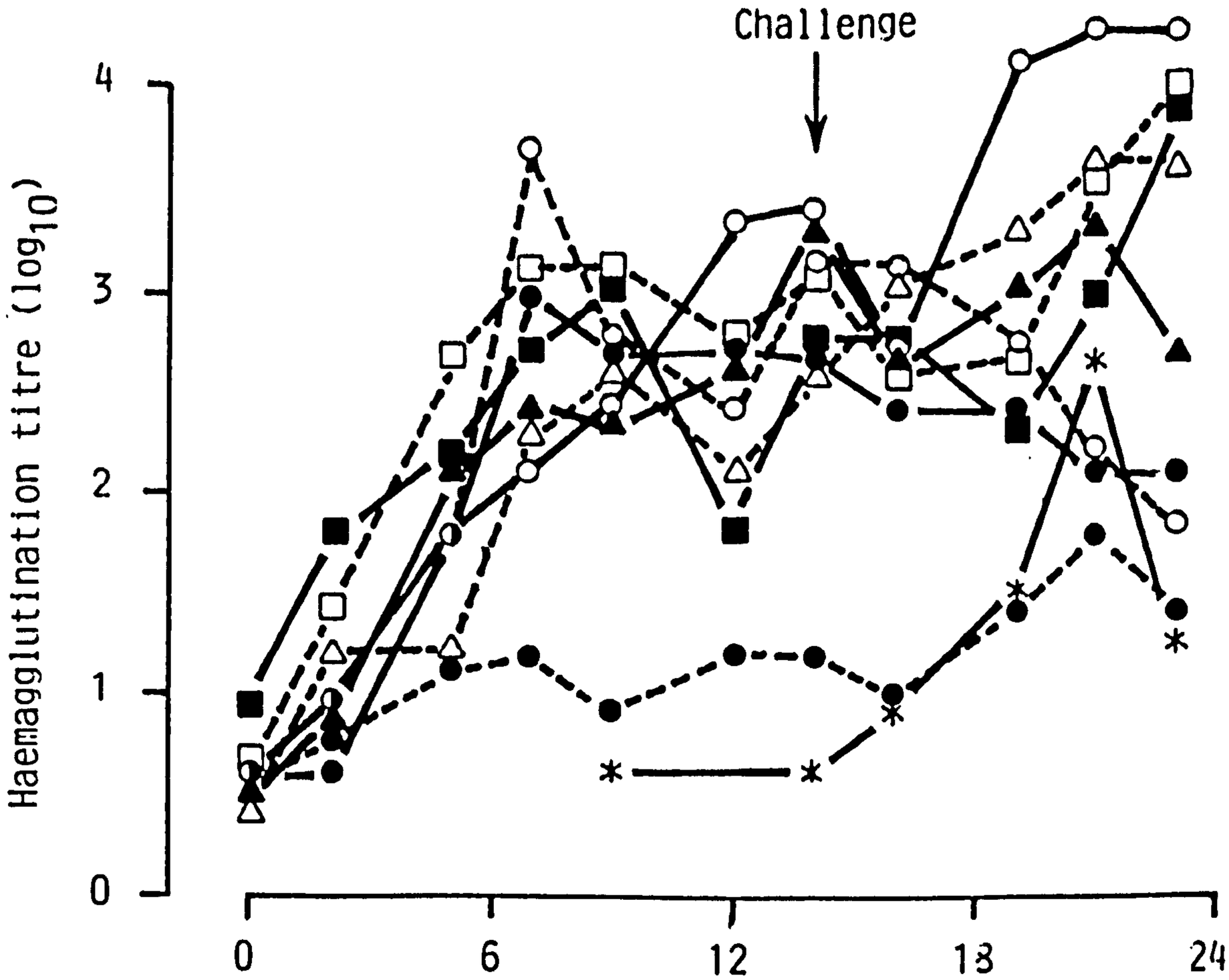

Fig. 6.4. Haemagglutinating antibody titres (log 10) against L4SE antigens in faecal extracts of rats following vaccination and after challenge with 5,000 N. brasiliensis and also in challenge control rats.

—●— 0 krad
—○— 80 krad
—▲— 120 krad
--△-- 140 krad
—■— 160 krad
--□-- 180 krad
--●-- L3SE iv
--○-- L3SE ig
—✱— Challenge controls

Table 6.3

Intestinal N. brasiliensis burdens (mean of 5 rats ± S.E.) in rats after vaccination and following challenge with 5 x 10^3 N. brasiliensis

Group	Days after Vaccination		9 Days After Challenge	Percentage of Protection
	5	9		
A (2000 normal larvae)	824 ± 6	519 ± 41	29 ± 16	98.7
B (2000 larvae irradiated at 80 krad)	N.D.	208 ± 12	9 ± 9	99.6
C (2000 larvae irradiated at 120 krad)	N.D.	26 ± 9	21 ± 20	99.3
D (2000 larvae irradiated at 140 krad)	103 ± 29	3 ± 2	43 ± 59	98.7
E (2000 larvae irradiated at 160 krad)	5 ± 3	0.4 ± 0.4	194 ± 62	93.0
F (2000 larvae irradiated at 180 krad)	2 ± 2	0	1334 ± 312	53.4
G (1.7 mg of L3SE intravenously)	-	-	2693 ± 262	3.9
H (1.7 mg of L3SE intragastrically)	-	-	2429 ± 348	13.3
I (challenge controls)	-	-	2801 ± 178	-

N.D. = not done

After primary infection the highest anti-L4SE antibody titres were observed in the rats vaccinated intragastrically with L3SE antigens. Except for Group G rats, vaccinated intravenously with L3SE antigens, all experimental groups showed good antibody responses to vaccination. There was, however, no relationship between the degrees of protection to reinfection and antibody titres, with the exception of Group G in which anti-L4SE antibody titres remained low after vaccination and the degree of protection to reinfection was only 3.9% (Table 6.3).

Following challenge all groups showed an anamnestic response except Group A rats, vaccinated with non-irradiated larvae and Group H rats, vaccinated intragastrically with L3SE antigens. Protection to reinfection was relatively low in Group H rats, 13.3%, but there was no correlation between antibody titres 9 days after challenge and protection.

Anti-ESP Antibody Titres

The results are shown in Fig. 6.5.

After primary infection the highest anti-ESP coproantibody titres were observed in the rats vaccinated with non-irradiated larvae (Group A). The haemagglutinating antibody levels in groups vaccinated with irradiated larvae were significantly lower and were proportional to the worm burdens 9 days after vaccination and inversely proportional to the irradiation dose. In Groups G and H, vaccinated with L3SE antigens intravenously and intragastrically respectively, coproantibodies to ESP antigens were lower than in the groups vaccinated with irradiated larvae.

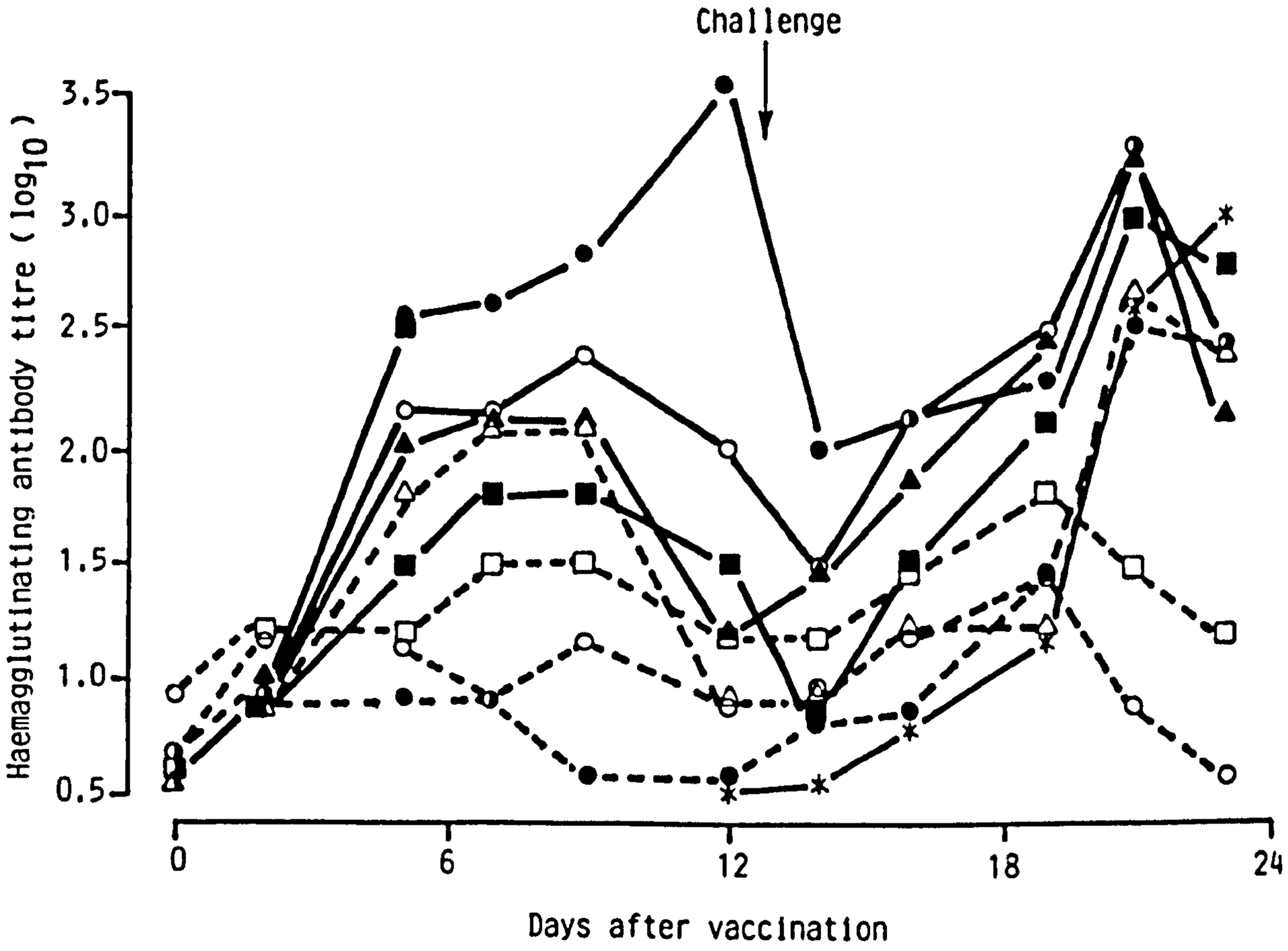

Fig. 6.5. Anti-ESP haemagglutinating antibody titres (log 10 of pooled sample from each group) in faecal extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis.

—●— 0 krad
—○— 80 krad
—▲— 120 krad
--△-- 140 krad
—■— 160 krad
--□-- 180 krad
--●-- L3SE iv
--○-- L3SE ig
—✱— Challenge controls

Table 6.4

Anti-ESP haemagglutinating antibody titres (mean log 10, days 7 - 12) in faecal extracts of rats after vaccination and percentage of protection induced

Group	Mean anti-ESP Haemagglutinating Antibody Titre (day 7 - 12)	Percentage of Protection
A (2,000 normal larvae)	3.010	98.7
B (2,000 larvae irradiated at 80 krad)	2.207	99.6
C (2,000 larvae irradiated at 120 krad)	1.806	99.3
D (2,000 larvae irradiated at 140 krad)	1.705	98.7
E (2,000 larvae irradiated at 160 krad)	1.705	93.0
F (2,000 larvae irradiated at 180 krad)	1.404	53.4
G (1.7 mg of L3SE intravenously)	0.802	3.9
H (1.7 mg lf L3SE intragastrically)	1.003	13.3

There was a significant positive correlation ($P < 0.001$) between anti-ESP faecal haemagglutinating antibody titres (day 7 - 12) and percentage protection.

A comparison of the mean anti-ESP faecal haemagglutinating antibody titres between days 7 and 12 after vaccination and the degrees of protection induced is shown in Table 6.4. This shows a significant positive correlation between protection and faecal IgA antibody levels, particularly in Groups G, H and F, where the mean antibody titres were 0.802, 1.003 and 1.404 respectively. The corresponding degrees of protection in these groups were 3.9%, 13.3% and 53.4%. Mean faecal IgA antibody levels were higher in the groups of rats which showed higher protection.

Following challenge infection a considerable increase in faecal antibody titres was observed. In Groups A - E the antibody levels increased to a similar degree by day 21 of the experiment (7 days after challenge) and then declined. In faeces of rats vaccinated with larvae irradiated at 180 krad the post-challenge increase was considerably lower. Similar results were obtained in Group H. Group G showed, after challenge, an increase in coproantibody titres until the end of the experiment in an identical manner to challenge control animals.

Faecal IgA Antibodies Against ESP Antigens

Levels of faecal IgA antibodies estimated by the ELISA tests are shown in Fig. 6.6. In Groups A and B the antibody levels increased until days 12 - 14 after vaccination and then gradually declined until the end of the experiment. In Groups C - F the maximal primary antibody response occurred at various times after infection. However, after challenge a rapid anamnestic increase

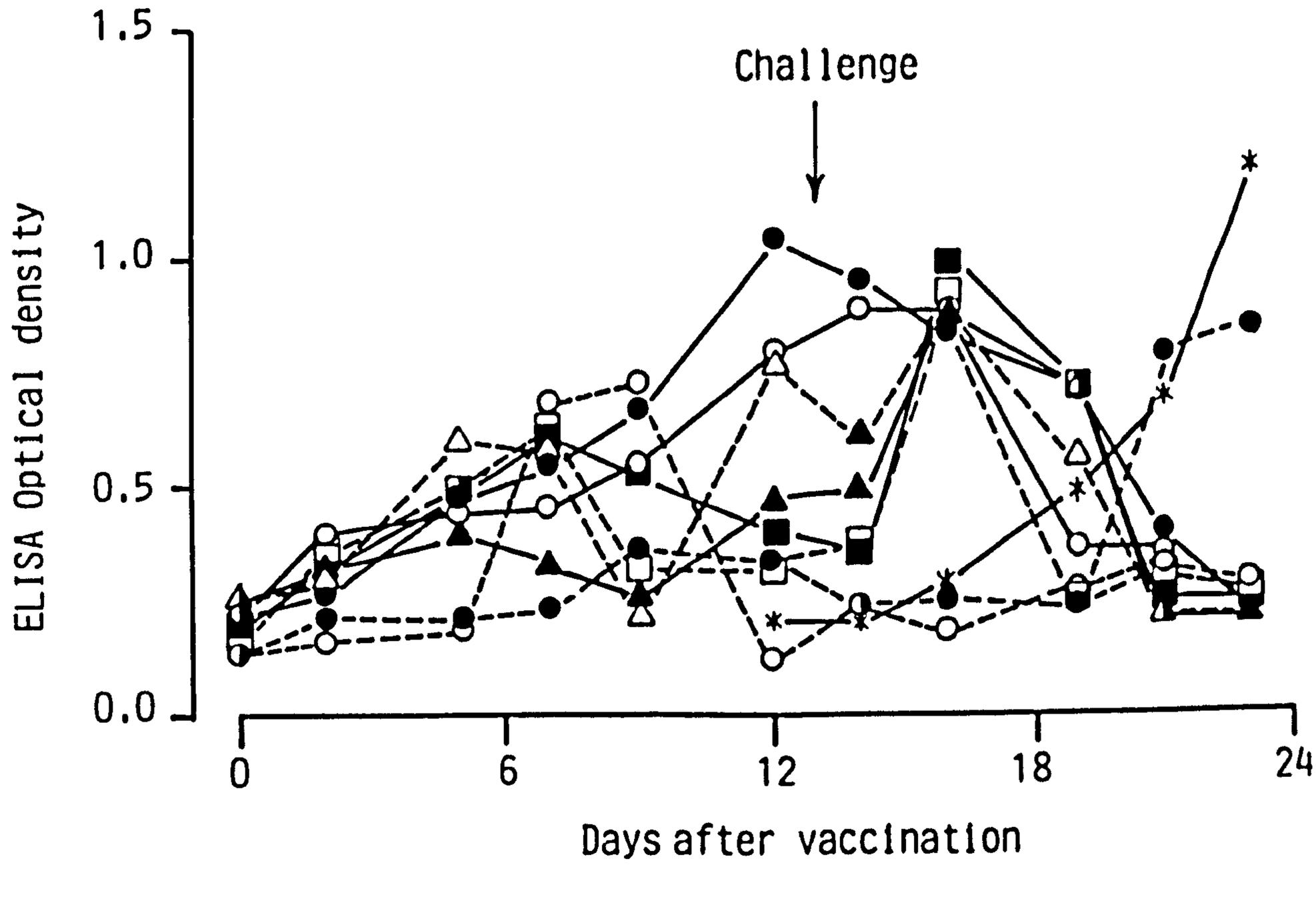

Figure 6.6. Anti-ESP IgA levels (pooled sample from each group) in faecal extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis

—●— 0 krad
—○— 80 krad
—▲— 120 krad
--△-- 140 krad
—■— 160 krad
--□-- 180 krad
--●-- L3SE iv
--○-- L3SE ig
—*— Challenge controls

Table 6.5

Anti-ESP IgA levels (mean ELISA O.D., days 7 - 12) in faecal extracts of rats after vaccination and percentages of protection induced

Group	Mean anti-ESP Faecal IgA Levels (Days 7 - 12)	Percentage of Protection
A (2,000 normal larvae)	0.761	98.7
B (2,000 larvae irradiated at 80 krad)	0.602	99.6
C (2,000 larvae irradiated at 120 krad)	0.344	99.3
D (2,000 larvae irradiated at 140 krad)	0.524	98.7
E (2,000 larvae irradiated at 160 krad)	0.510	93.0
F (2,000 larvae irradiated at 180 krad)	0.481	53.4
G (1.7 mg L3SE intravenously)	0.301	3.9
H (1.7 mg L3SE intragastrically)	0.546	13.3

to a similar level in all the groups was observed. Intragastric administration of L3 somatic antigens also induced a considerable primary response but there was no response to challenge. In rats vaccinated intravenously with L3 larvae (Group G), ELISA values after vaccination were slightly higher than uninfected controls only on two occasions (9 and 12 days post vaccination). After challenge, faecal IgA antibodies increased in this group in a similar manner to those observed in Group I (challenge control rats).

A comparison of the mean anti-ESP IgA levels between days 7 and 12 after vaccination and protection induced is shown in Table 6.5. The correlation between mean ELISA values and degrees of protection is less clear than that observed between faecal anti-ESP haemagglutinating antibody titres and protection. Nevertheless the highest ELISA values were observed in the rats infected with non-irradiated larvae and the lowest ELISA values in Group G, vaccinated intravenously with L3SE antigens. The latter group also showed the lowest protection against challenge, 3.9%.

Experiment 2

While caution should be observed in assuming that any conclusions drawn from *in vitro* experiments can be applied to *in vivo* situations, it is sometimes useful to conduct *in vitro* studies where corresponding *in vivo* tests may be difficult to apply. With these reservations in mind it was nevertheless thought useful to examine the effect of *in vitro* incubation of nematode specific IgA on adult *N. brasiliensis* isolated 5 days after infection.

Materials and Methods

Experimental Animals

Forty two rats were used. A group of 24 rats was infected with 5,000 L3 *N. brasiliensis* and killed on day 5 after infection when the intestinal worms were collected. The worms were divided into three groups and incubated with faecal IgA from infected rats, with faecal IgA from naive rats or with PBS. The worms were then transferred by stomach tube into groups of six naive rats. The faecal egg counts were monitored every day after worm transfer. On the sixth day after transfer, the rats were killed and the number of worms in the small intestine determined.

Isolation of IgA from Faeces

Faecal IgA was prepared by column chromatography on Sephadex G-200 as described in Chapter 2, using faecal extracts obtained from rats between 9 and 12 DAPI with 5,000 L3 *N. brasiliensis* larvae, and from naive control rats. The purity of the IgA fractions was confirmed by immunodiffusion against anti-rat IgA and against whole rat anti-serum (Miles Labs., U.S.A.). For worm incubations only fractions producing single precipitation arcs against both antisera were used. Infected rat IgA preparations showed 1:512 haemagglutination titres against adult nematode metabolites.

Worm Incubation and Transfer

Adult *N. brasiliensis* were collected from rats infected five days earlier. The nematodes were allowed to migrate from the open intestines into PBS at 37°C. They were then washed 10 times with warm PBS and counted. Aliquots of 5,000 worms were placed in the

Table 6.6

N. brasiliensis egg output (No. of eggs/g faeces) and worm burdens (mean of 6 rats ± S.E.) in rats infected intragastrically with nematodes preincubated with faecal IgA from infected rats, IgA from naive rats or PBS

Group	Eggs/g on days after worm transfer						No. of worms (± S.E.) Recovered 6 Days after Transfer
	1	2	3	4	5	6	
1 Worms incubated in faecal IgA from infected rats	950	950	7,200	2,950	6,000	1,150	109 ± 26
2 Worms incubated in faecal IgA from naive rats	1,000	1,350	2,400	2,875	4,150	5,200	39 ± 17
3 Worms incubated in PBS	1,050	5,400	1,350	2,950	5,000	1,650	100 ± 20

incubation media, consisting of 500 μg IgA from infected rats per 1.0 ml PBS (Group 1), 500 μg IgA from worm-free rats (Group 2) and in PBS alone (Group 3). The nematodes were incubated for four hours at 37°C and then doses of 700 worms were transferred into naive rats by gastric intubation (Spindler, 1936).

Results

Influence of Faecal IgA on Survival of Adult N. brasiliensis

There was no clear difference in nematode egg counts between rats infected with worms pre-incubated in PBS, faecal IgA from control or infected animals. Also the number of parasites recovered from rats given worms incubated in IgA from infected rats was similar to the PBS control group (Table 6.6). The mean worm burden in Group 2 rats, 6 days after transfer, where the worms were preincubated in faecal IgA from naive rats was significantly lower than in the other two groups.

Discussion

The results of the first experiment confirm the findings presented in Chapter 3. In the latter chapter it was observed that as the infective larvae were subjected to increasing irradiation doses, increasing numbers of parasites failed to proceed beyond the bronchial phase. This was accompanied by corresponding increases in anti-larval antibody levels in the lungs. In the results presented in this chapter this phenomenon was particularly pronounced in Groups B, C and D which had been vaccinated with larvae receiving irradiation

doses of 80, 120 and 140 krad respectively. In these groups lung anti-larval IgA levels were greatly elevated compared with rats vaccinated with normal larvae. Evidence of the involvement of lung immunoglobulins in lung humoral reactions to N. brasiliensis infections also corroborated earlier findings presented in Chapter 4. In Chapter 4 it was shown that elevated IgA, IgG and IgM concentrations could be detected in the lungs of rats 8 days after infection with N. brasiliensis.

The data presented in this chapter also highlights some new aspects by demonstrating the importance of antigen dose and the duration of antigenic stimulation in inducing lung IgA antibody responses to N. brasiliensis infections.

Although the total number of larvae passing through the lungs of rats vaccinated with non-irradiated larvae was more than double that of rats vaccinated with larvae irradiated at 120 and 140 krad the levels of anti-larval IgA antibodies found in the lungs of rats infected with irradiated larvae were much higher. Such a result may suggest that longer stimulation with smaller amounts of antigen(s) is more efficient for the induction of IgA antibody. However analysis of the anti-larval IgA results in groups vaccinated with highly irradiated larvae (Groups E and F) in which only 20% and 13% of infective dose reached the lungs, suggests that a threshold level of initial antigenic stimulation is necessary for an optimal lung IgA antibody response.

Levels of anti-larval IgA detected on day 9 after vaccination in lungs of Group F were slightly higher and, after challenge, equal

to those observed in Groups A and E. However, rats of Group F were only partly resistant to reinfection, while Groups A and E showed 98.7% and 93% protection against challenge respectively. These results seem to suggest that lung anti-larval IgA antibodies are not directly involved in specific host protective immunity. Nevertheless they may have an important role in host-parasite interactions (Befus and Bienenstock, 1982; Befus, 1982).

Rats vaccinated with L3SE antigens intravenously (Group G) showed IgA concentrations higher than Group E and similar to Group F yet protection in Group G was the lowest of all the groups, at 3.9%. This was further evidence of lack of correlation between lung IgA antibody levels and protection.

The high levels of anti-larval IgA found in lungs of rats vaccinated intragastrically (Group H) could be due to migration of IgA plasmocytes within the mucosal system (Montgomery et al, 1978; McDermott and Bienenstock, 1979; Tomasi, 1983). However, the possibility of local sensitisation after aspiration of the inoculant into the lungs during intragastric intubation cannot be excluded and further investigations are necessary to confirm this observation.

There was some evidence of a correlation between increasing anti-larval IgG levels in lung extracts both before and after challenge and the numbers of larvae trapped in the lungs on both day 5 and day 9 after vaccination (Groups B, C, D, E and F). Levels of anti-larval IgG in rats vaccinated with L3SE antigens intravenously (Group G) on day 9 after vaccination were relatively high. The explanation

for this may be that IgG is not a secretory immunoglobulin like IgA and is believed to be a serum transudate. The relatively high anti-L4SE IgG level in Group G on day 9 after vaccination could perhaps also be the result of some inoculant reaching the lungs. There is no obvious explanation for the high anti-adult nematode ESP IgG levels recorded for Groups G and H.

There was no clear relationship between levels of IgM anti-L4SE in lung extracts and either the route of antigen administration or the dose of antigen, with the possible exception of a suggestion of small increases in lung IgM levels with the increased numbers of larvae retained in the lungs (Groups B, C, D and E) on day 9 after vaccination. On the other hand the highest lung anti-L4SE IgM levels were recorded in Group A, where no larvae were found in the lungs 9 days after vaccination.

Concentrations of anti-parasite immunoglobulins recorded in the lungs of rats vaccinated with L3SE antigens intravenously or intragastrically were not translated into protection against challenge infection. These groups, G and H, showed the lowest protection.

Nine days after vaccination, the highest anti-L4SE small intestine mucosal IgA concentration occurred in Group A. This group, vaccinated subcutaneously with L3 larvae had the highest intestinal worm burdens at this time.

No clear relationship between mucosal IgA levels and vaccinating regimes emerged in the other groups. Intestinal mucosal IgA responses to L4SE antigens were low in groups vaccinated with L3 larvae, Group G and H. The latter values were similar to those in worm-free controls.

The absence of living stages of N. brasiliensis in the intestine resulted in a poor IgA response. After challenge anti-L4SE levels of mucosal IgM were similar to levels in challenge controls except in groups D, E and F. In the latter groups, vaccinated with 140 krad, 160 krad and 180 krad irradiated larvae, post-challenge mucosal IgM levels showed significant increases. These values correlated with increasing numbers of worms found in the intestine at this time but correlated negatively with protection. These results provide further evidence of the lack of a direct relationship between intestinal IgA antibodies and protection. Except for Group A, small intestine mucosal anti-ESP IgA levels on day 9 after vaccination were at worm free control level. After challenge, intestinal mucosal anti-ESP IgA levels were similar to challenge control values, except in Groups E and F. The higher post-challenge anti-ESP IgA values in these groups again correlated with the intestinal worm burden and were negatively correlated with protection.

There was no clear relationship between intestinal worm burdens and intestinal mucosal anti-parasite IgG levels on day 9 after vaccination or after challenge. There was also no evidence of a relationship between anti-parasite intestinal IgG levels and protection.

Intestinal mucosa anti-L4SE IgM values were similar in all groups on day 9 after vaccination. After challenge anti-L4SE IgM levels in Groups C, E and F were lower but there was no obvious significance in these results.

Anti-ESP intestinal mucosa IgM concentrations 9 days after vaccination with irradiated larvae showed increases from Group B to Group E. However Group F concentration was similar to Group B. There was no indication that anti-parasite intestinal mucosa IgM values correlated with either worm burdens or protection.

Haemagglutinating anti-L4SE antibody levels in intestinal contents showed only minor differences 9 days after vaccination in all groups. After challenge Group G (vaccinated intravenously with L3 larvae) concentration was significantly lower than the challenge control group or Group A after vaccination. There is no apparent explanation for the latter result. Anti-ESP antibody levels in small intestine contents showed no correlation with either worm burdens on day 9 after vaccination or after challenge. The relatively high intestine contents anti-ESP antibody titre in Group G 9 days after intravenous vaccination with L3SE antigen correlated well with anti-parasite IgG and IgM concentrations in intestinal mucosa samples of this group at the same time. The very low small intestine contents anti-ESP antibody titre in Group H, vaccinated with L3SE intragastrically, also correlated with Group H anti-ESP IgA, IgG and IgM in small intestine mucosal extracts 9 days after vaccination.

A close negative correlation was observed between the titres of faecal haemagglutinating anti-ESP antibodies and numbers of worms recovered from the intestines 9 days after vaccination. Antibody titres showed a strong positive correlation with protection. These findings were similar to those observed in Chapter 5. Following challenge, increases in coproantibody levels and worm burdens in

rats immunised intravenously with larval somatic antigens (Group B) were similar to the values observed in challenge control rats.

The changes observed, following vaccination, in parasite-specific faecal IgA levels measured by the ELISA method correlated less closely than anti-ESP faecal haemagglutinating titres with protection. However there was some suggestion of a link between anti-ESP IgA levels and protection. Anti-parasite faecal IgA responses to challenge in the immunised rats reached a maximum earlier than the haemagglutinating antibody levels. Immunochemical analysis of faecal antibody in primary infected rats (Chapter 2) suggested that the antibody activity was related to the IgA fraction of the faecal globulin. The lack of a close correlation between haemagglutination titres and ELISA values in the present experiment is probably due to differences in availability of the antigen determinants in haemagglutination and ELISA tests (Catty et al, 1981).

It is possible that the IgA antibodies present in the faecal extract of infected animals are induced by antigens not directly associated with protective immunity and the results of the second experiment tend to support this suggestion since the number of worms established in naive rats after incubation with faecal IgA (collected from primary infected rats on days 9 - 12 after infection, i.e. just before adult worm expulsion) were almost identical with the number of worms recovered where the worms had been pre-incubated with PBS. Similarly Gerber et al (1976) failed to detect any differences in the mortality of adult N. brasiliensis cultured in media containing serum and intestinal IgA from hyperimmune rats.

The reduction of worm establishment following incubation with faecal IgA from uninfected rats is difficult to explain. It may be due to cross-reactivity of nematode antigens with antibodies produced against bacteria or other intestinal antigens since investigations on conventional and axenic mice have shown an occurrence of secretory IgA antibodies against both normal flora of alimentary tract and enteric pathogens (Benveniste, Lespinats and Salmon, 1971; Ebersole and Molinari, 1977). It was also shown that human gastrointestinal secretions were capable of agglutinating enteric microorganisms (McClelland, Samson, Parkin and Shearman,1972).

Although surface antigens of adult N. brasiliensis have been studied recently (Maizels et al, 1983) the antigenic component crucial for nematode survival remains undetermined. On the other hand, Pery, Petit, Poulain and Luffau (1974)and Pery (1977) reported the occurrence in N. brasiliensis antigenic preparations of components common or closely related to bacterial and other intestinal antigens. Thus it is possible that faecal IgA from uninfected rats reacted with such antigens whilst such cross-reacting antibodies have been absorbed by the parasites, in the case of faecal IgA, from infected rats. It is also noteworthy that Jacqueline, Crinquette, Bout, Barrois and Vernes (1981) reported an inhibiting effect of non-specific IgA from rat bile on the fecundity of female Trichinella spiralis.

In conclusion an analysis of the results produced in this chapter have shown that strong local antibody responses, particularly anti-larval IgA responses, were elicited in the lungs of rats

vaccinated with irradiated larvae. It was also observed that this response was more dependent on the duration of antigenic stimulation than on the antigenic dose. However it appeared that a threshold quantity of antigen was essential to induce anti-larval IgA lung responses. Nematode specific immunoglobulins resulting from intravenous stimulation with infective larvae somatic antigen did not appear to have protective properties. Comparison of lung anti-larval IgA antibody in rats resistant or susceptible to challenge suggested that IgA lung antibodies are not directly involved in specific host protective immunity.

Levels of faecal haemagglutinating antibodies against adult nematode ESP antigens showed a significant positive correlation with the numbers of worms recovered from the intestines following vaccination and also with the degrees of resistance to reinfection. Anti-ESP specific IgA levels in faecal extracts correlated less well with worm numbers and protection to challenge. However preincubation of adult N. brasiliensis in media containing faecal IgA from primary infected rats had no adverse effect on the ability of these worms to establish and survive in naive rats. Mucosal IgA antibody levels specific to L4SE or adult nematode ESP antigens did not show any correlation with protection against reinfection. While results of in vitro experiments should be interpreted with caution, the direct involvement of secretory IgA in protection against N. brasiliensis in the rat is still open to speculation. One possibility is that faecal IgA antibodies are derived from stimulation by antigens not directly involved in protective immunity. It has

been suggested that an important role for dimeric IgA may be to provide a means of removing dimeric IgA-immune complexes from the circulation via the parenchymal cells in the liver and bile secretion (see review by Bienenstock and Befus, 1980).

It has been postulated that rather than play an essential role in host resistance oligomeric IgA secreted locally may facilitate symbiosis. It has been suggested that host-parasite symbiosis is enhanced by mechanisms at mucosal surfaces which suppress IgG antibody production and enhance IgA responses. Local IgA antibody is thought to minimise the uptake of antigen at mucosal surfaces and limit the formation of potentially damaging antigen-antibody complexes in the blood and the transport of oligomeric IgA via the bile is thought to facilitate the removal from the circulation of any such complexes (see reviews by Befus, 1982; Befus and Bienenstock, 1982; Bienenstock and Befus, 1983).

While the role of secretory IgA at mucosal surfaces is still uncertain it is most improbable that this highly specialised immunoglobulin does not have a significant part to play in the relationship between the host and intestinal parasites. This is an obvious area for further research and it would be useful to determine which parasite antigens, surface, secretory or somatic, are most involved in eliciting secretory IgA responses.

GENERAL DISCUSSION

The experiments described in Section I of this study have provided a considerable amount of information on local immune responses to both larval stages of N. brasiliensis in the lungs and to adult stages of the nematode in the small intestine.

The results showed that one consequence of the presence of adult N. brasiliensis in the small intestine was a hypertrophy and proliferation of Peyer's patches. These findings were in line with earlier reports that Peyer's patches responded to the presence of antigenic material in the intestine. It was shown by Pollard and Sharon (1970) that Peyer's patches in mice responded to bacterial and viral antigens in the intestine. A role for Peyer's patches in the processing of antigenic material in the lumen of the intestine was suggested by Bockman and Cooper (1973) who demonstrated the pinocytosis of ferritin particles from the surface of Peyer's patch epithelium. Results obtained in the present study suggest that Peyer's patches may also play an important role in the sampling of N. brasiliensis antigens and in facilitating the stimulation of B lymphoblasts prior to their migrating to immunoglobulin secreting sites in the small intestine lamina propria.

Results of experiments conducted in Chapter 1 showed that there was a hyperplasia of IgA containing cells in the small intestine coinciding with the presence of adult N. brasiliensis. Many workers have demonstrated a causal relationship between the presence of micro-organisms in the lumen of the intestine and the development

of secretory immunoglobulin cells in the small intestine. The general introduction contains a review of some of the relevant literature.

Immunological evaluation of small intestine mucosal extracts and faecal extracts following primary infection showed that total IgA and IgG levels in these preparations were greatly elevated. Analysis of faecal extracts of rats following N. brasiliensis infection showed that while IgG was the most abundant immunoglobulin present, IgA was many times more immunologically active than IgG or IgM.

Changes in mucosal and faecal immunoglobulin concentrations following a primary N. brasiliensis infection closely matched each other and were similar to the findings of Poulain et al (1976a) who observed marked increases in IgA levels in small intestine fluid, coinciding with the presence of adult N. brasiliensis in the gut. Sinski and Holmes (1977 and 1978) also demonstrated strong local intestinal anti-parasite IgA, IgG and haemagglutinating antibody responses to N. brasiliensis.

It was additionally noted in this study that there was a second faecal IgA peak between 18 and 24 DAI. It was suggested that transport of serum derived IgA via bile secreted into the intestine represented a significant proportion of intestinal IgA (Jackson et al, 1978; Orlans et al, 1978). Lemaitre-Coelho et al (1978) suggested that 90% of specific IgA antibodies in the intestine of the rat originate from bile. More recently Brown, Poulain and Pery (1981) showed that levels of biliary IgA antibody

specific to N. brasiliensis ESP increase until 28 DAI. It is therefore possible that, in the late stages of N. brasiliensis infection, i.e. by 24 DAI, the higher amounts of IgA detected in the faeces may be largely a result of biliary secretion.

The results of the experiments carried out in Chapter 2 showed that mucosal and faecal haemagglutinating antibody titres against adult N. brasiliensis excretory-secretory and somatic antigens and against larval somatic antigens increased sharply following N. brasiliensis infection and, in general, mucosal and faecal extract titres closely mirrored each other. In Chapter 3 anti-ESP coproantibody levels were closely correlated with protection against challenge and in Chapter 4, coproantibody levels correlated with antibody levels in both small intestine mucosal extracts and small intestine contents.

An examination of the accumulated data suggests that immunological evaluation of faecal extracts can provide information on local intestinal immune responses to intestinal parasites thus allowing sequential changes in immune responses to be monitored during the course of an infection. However it is also important to attempt to evaluate the significance of local immune responses, particularly the role of S-IgA in protective immunity. The significance of local immunity in protection against intestinal parasites has always been speculative.

The direct involvement of secretory-IgA in protective immunity in intestinal parasitic infections was reported by Lloyd

and Soulsby (1978). These workers showed that the passive transfer of S-IgA obtained from T. taeniaeformis infected mice to the intestines of 4 week old recipients, transferred protection against infection. Most of the investigations of local immunity in intestinal parasitic infections have shown a temporal relationship between local immune responses and the presence or termination of a parasite population, suggesting a speculative link between the two phenomena.

Some of the experiments described in Section I were designed to examine the possible relationship between local immune responses to N. brasiliensis antigens and protective immunity. Several workers have suggested that excretory-secretory products (ESP antigens) are important in the induction of protective antibody responses. Excretory-secretory antigens are excreted or secreted at anal or oral orifices respectively and these antigens include cuticular material and enzymes such as acetylcholinesterases. Evidence has been produced to support the view that N. brasiliensis ESP antigens are capable of stimulating protective responses to infection (Denham, 1969; Poulain et al, 1977b; Day, Howard, Prowse, Chapman and Mitchell, 1979; Bolla and Weinstein, 1980). It is noteworthy that coproantibodies specific to adult ESP antigens (Chapter 2) showed the highest levels on 10 - 12 DAI, immediately prior to and during the period of maximum worm expulsion. The results presented in Chapter 3 showed a close correlation between coproantibodies to ESP antigens and worm numbers in the intestines at 10 DAI. These findings suggest a possible link between ESP antigens and the stimulation of protective responses.

Results produced in Chapter 5 showed that although intragastric vaccination with adult ESP antigens was not as effective as vaccination with living intestinal parasites, i.e. 69% protection compared to 99%, adult ESP antigens were more capable of stimulating protective responses than adult somatic (ASE) antigens, i.e. 69% compared to 17%. An investigation of larval antigens (Katiyar, Govil and Sen, 1972) also showed that larval somatic antigens offered little protection against infection. However it has been demonstrated that ASE antigens can give good protection against infectionbut only if the rats are given multiple inoculations with relatively high quantities of antigen (Murray et al, 1979).

Most of the evidence suggests that in N. brasiliensis infections protective responses are induced by the excretory-secretory products of living intestinal nematodes.

Anti-parasite local intestinal secretory antibody responses to intragastric vaccination with living stages of N. brasiliensis and with adult worm ESP and ASE antigens were investigated in Chapter 5. The results appeared to indicate that the major antigens stimulating intestinal antibody responses were common for all intestinal stages of N. brasiliensis. Ogilvie (1965a)similarly suggested that parasite antigens released by L4 larvae or adult worms were similar in nature.

Secretory IgA, IgG and IgM responses to the various antigens investigated did not correlate with the degrees of protection induced by the antigens. However there was a good correlation between anti-ESP haemagglutinating antibody titres in faecal extracts and

protection. It had previously been shown that although IgG was the predominant immunoglobulin in faecal extracts of N. brasiliensis infected rats, the IgA fraction of faecal extracts had the highest anti-parasite immunological activity. A possible link can therefore been seen between faecal IgA and protection against infection.

The mode of action of IgA secreted locally in the intestine is a matter for much speculation. It may be that these antibodies are stimulated by antigens not directly associated with protective immunity. The results of the experiment in which adult worms were pre-incubated with S-IgA from infected rats and with PBS prior to their transfer to naive rats may support this suggestion. The number of worms established in naive rats after incubation with faecal IgA from infected rats was almost identical with the number established after incubation with PBS.

The suggestion has recently been made that the ability of secretory IgA to remove from the circulation antigenic materials which have crossed the various mucosal surfaces and the ability to form antigen-antibody complexes which have a blocking action at mucosal surfaces may be an important function of this immunoglobulin,(see reviews by Bienenstock and Befus, 1980; Befus, 1982; Befus and Bienenstock, 1982 and Bienenstock and Befus, 1983).

It was shown by Andre, Lambert, Bazin and Heremans (1974) that local immunisation of rat intestine with human serum albumin led to a marked decrease in the capacity of the intestinal epithelium to absorb human serum albumin administered intragastrically two weeks

later. It was suggested that this was due to binding of the challenge dose of human serum albumin with secretory antibody which was thought to be S-IgA.

The work of Peppard, Orlans, Payne and Andrew (1981) illustrated clearly the role of secretory IgA in the transport and removal of macromolecules from the circulation. These workers injected dimeric human IgA into rats and recovered 40% of the injected IgA from bile in the six hours after injection. In addition they found that the dimeric human IgA was able to carry with it macromolecular material in the form of a complex which included rat secretory component. Monomeric IgA similarly injected was not transported into bile. When dimeric rabbit IgA was injected intravenously into rats, 18% was recovered in six hours in the bile. Monomeric rabbit IgA similarly administered was not detected in bile. The process was believed to be mediated by hepatocytes which synthesise SC. The SC is thought to act as a specific receptor on the cell for polymeric IgA. Peppard et al (1981) suggested that important functions of secretory IgA transport were the removal of potentially hazardous antigens, the removal of immune complexes thus preventing systemic responses and the recycling of IgA.

Further evidence of biliary removal of immune complexes was reported by Russell, Brown and Mestecky (1981). These workers injected intravenously a ^{14}C-labelled hapten-human serum albumin complex into mice. Large quantities of the radiolabelled immune complex were recovered in the bile between 1 and 3 hours after injection. Secretory component was shown to be a glycoprotein synthesised by

hepatocytes (Socken, Simms, Nagy, Fisher and Underdown, 1981). The latter workers also showed that, in the rat, a hepatic IgA transport system functioned as an in vivo clearing mechanism for antigen-antibody complexes.

Reviews of investigations into the function of IgA secreted locally at mucosal surfaces led to the suggestion that S-IgA at mucosal surfaces may not be an obstacle to parasite survival (Befus, 1982). Rather than embarrass the parasite, S-IgA may facilitate a form of symbiosis by the processes of minimising the uptake of antigens and the formation of potentially damaging antigen-antibody complexes.

While not excluding an anti-parasite role for intestinal S-IgA, Befus and Bienenstock (1982) in their review concluded that ultimately non-specific inflammatory events in the intestine may be responsible for expulsion of a parasite population and that these events were either the result of antigen specific action or were amplified by such action.

There is much evidence that intestinal mucus may play a significant role in the process of exclusion or expulsion of an intestinal parasite population (see review by Miller, 1983). A protective role for mucus in N. brasiliensis infections was suggested by Miller, Huntley and Wallace (1981). These workers showed that when 4 day old N. brasiliensis were transferred into the duodenum of rats which had been primed by infection 18 days previously, the transferred worms did not penetrate between the villi. It was also observed that those worms which were adjacent to the villi became enveloped in mucus and within

4 hours of intraduodenal challenge about 85% of the worms were expelled. When the intestines of the challenged rats were perfused with saline, 28% - 36% of the parasites were adherent to the mucosa and 3% - 23% were trapped in mucus in the immune rats. However in previously uninfected control rats 70% - 80% of the worms were adherent to the mucosa. It was also observed by Miller et al (1981) that while exclusion of the worms from the mucus was evident 30 minutes after challenge, significant mucus trapping did not occur for a further 90 minutes.

Further evidence of the involvement of superficial mucus in the immune exclusion or entrapment of N. brasiliensis comes from the work of Miller and Huntley (1982a) who treated immune rats with a mucolytic or mustard oil intraduodenally to disperse or deplete small intestine mucus. It was then shown that when these treated immune rats were challenged with pre-adult N. brasiliensis parasite expulsion did not occur, although it was not established that mucus trapping or blocking was affected. However, Miller and Huntley (1982b) showed that immune rats pretreated 48 hrs or 24 hrs before challenge with corticosteroids were highly susceptible to infection with N. brasiliensis and the worms were not trapped or excluded from the mucosa by mucus. The effect that corticosteroid has on intestinal mucus is not understood but it has been suggested (Menguy and Masters, 1963) that it depletes the mucosa of mucin glycoprotein.

There have been reports of an interrelationship between mucin glycoproteins and parasite-specific antibodies. Dobson (1966 and 1967) found high levels of parasite-specific haemagglutinating antibodies in the superficial mucus from the intestines of sheep infected with

Oesophagostomum columbianum. Because of the viscosity and lack of solubility it is difficult to analyse the intestinal mucous layer for immunoglobulin levels (Allen, 1981; Forstner, Wesley and Forstner, 1982). The latter workers showed that gel filtration chromatography did not permit the separation of immunoglobulins from mucous glycoproteins. In tests with Salmonella typhimurium bacteria it was demonstrated that when the bacteria was sensitised with S-IgA their affinity for the mucus belt of the intestine of rats was increased (Magnusson and Stjernstrom, 1982). These workers also showed in an in vitro test that sensitisation of the bacteria with S - IgA increased their affinity for hog mucin. Therefore small amounts of specific antibody in conjunction with mucin glycoproteins may have substantial agglutinating properties. Similar findings were reported by Lee and Ogilvie (1982) who noted that in vitro trapping of T. spiralis larvae in extracts of mucus was promoted by pre-incubation of the larvae in either immune or normal rat serum. The latter workers suggested that a specific component, presumably antibody, and a non-specific component, possibly complement, were involved in the retention of larvae in the mucous layer. Since the cuticles of nematodes are antigenic (Phillipp, Parkhouse and Ogilvie, 1980) and also capable of activating complement (Mackenzie, Jungery, Taylor and Ogilvie, 1980) the suggestion has been made (see review by Miller, 1983) that the presence of either specific antibodies or of complement in the superficial mucus may promote the interaction of mucus with the cuticular surface of nematodes. It was also suggested that such a mechanism

may account for the trapping of N. brasiliensis in mucus. Thus a role has been proposed for both mucus and locally secreted intestinal antibodies in the immune expulsion of N. brasiliensis. Miller (1983) expressed the view that it was unlikely that antibodies or lymphoid cells were solely responsible for the immune expulsion of parasitic nematodes but that their primary role was perhaps the induction of pathophysiological changes in the mucosal epithelium. The expulsion of the parasites may be promoted by these non-specific changes in cooperation with anaphylactic antibodies and local parasite-specific immunoglobulins. When the processes involved in the immune termination of a N. brasiliensis population are finally explained it is likely that antibody secreted locally in the intestine will be shown to play a significant part.

N. brasiliensis also undergoes a pre-intestinal parasitic stage in the lungs of the host animal. Local immune responses in bronchus associated lymphoid tissue (BALT) are therefore of interest in the study of local immune responses to N. brasiliensis. Although most of the studies of local immunity in the respiratory tract have been carried out in the last 20 years, as early as 1929 Bull and McKee showed that rabbits could be specifically immunised against pneumococci by nasal vaccination and that this immunity occurred in the absence of a serum antibody response. Askonas and Humphrey (1958) showed that intravenous vaccination with pneumococcal organisms led to significant antibody levels in the lungs. More recently immunity in the respiratory tract has been the subject of a great deal of investigation (see review by McDermott, Befus and Bienenstock, 1982).

Although immunity to N. brasiliensis in the respiratory tract was not the main topic of this study, local antibody levels in lung extracts of rats infected with irradiated and non-irradiated larvae were measured. The first experiment (Chapter 3) showed that anti-parasite antibodies could be detected in the lungs of rats following vaccination with larvae attenuated by 40, 80 or 120 krad gamma irradiation. After challenge anti-parasite antibodies were detected in both the rats vaccinated with irradiated and non-irradiated larvae.

The results discussed in Chapter 4 indicated that the depression of local IgA and IgG responses in the lungs may lead to an increase in the numbers of parasites establishing in the intestine following N. brasiliensis infection. In the concluding chapter of Section I, it was shown that strong anti-larval IgA responses were elicited in the lungs of rats vaccinated with irradiated larvae. It was noted that while a threshold quantity of larval antigen was essential to induce an IgA response in the lungs, the duration of the antigenic stimulation was more important than the quantity of antigenic material present. There was no indication from any of the experiments that lung anti-larvae IgA antibody was directly involved in specific host protective immunity.

It is important to note that the results discussed in Chapter 4 showed that coproantibody levels correlated closely with levels of antibody in both small intestine contents and in small intestine mucosa. These findings indicate that local antibody responses to gastrointestinal parasites can be monitored by coproantibody measurement. The

advantages of studying immune responses by this method are obvious. Measurements can be made at intervals, daily or weekly, during an experiment without resort to post-mortem material. Coproantibody measurements have recently been employed to assess the influence of diet on local immune responses of sheep vaccinated against Haemonchus contortus (Wedrychowicz, Abbott and Holmes, 1984) and also in the study of local antibody responses in the bile and faeces of sheep infected with Fasciola hepatica (Wedrychowicz, Turner, Pfister, Holmes and Armour, 1984).

In a review of the impact of subclinical parasitism in ruminants Coop (1982) suggested that the impact of low-level parasitism on individuals or herd performance may be greater than previously considered. The further suggestion was made that specific diagnostic indicators to assist in the early detection of low level infections would be a useful aid. It is anticipated that, in the future, copro-antibody detection and measurement may provide a useful technique for the study of local antibody responses to parasites in the intestinal tract and that such techniques might also prove to be useful in the development of new in vitro diagnostic tests for intestinal parasites in domestic animals and man.

SECTION II

Pathogenesis and Local Immunity in the Mongolian Gerbil (*Meriones unguiculatus*) Infected with *Trichostrongylus colubriformis*

INTRODUCTION

Trichostrongylus colubriformis is an important pathogenic nematode of the Trichostrongylidae family and commonly found in the small intestine of sheep and goats. One of the earliest records of the naturally occurring disease is that of Waterman (1918) who reported an outbreak in goats.

The pathogenic effects of T. colubriformis in sheep have been well documented by many workers. The symptoms produced in all species of host are similar. These symptoms are anorexia, abdominal pain, progressive weakness with or without diarrhoea and reduction in body weight gain or weight loss.

When lambs were given trickle infections over periods of two to eight months, diarrhoea commenced two weeks after the first infection and continued until death. These animals also were anorexic, depressed and showed signs of abdominal pain (Andrews, 1939). Weight gains in T. colubriformis infected lambs were shown to be inversely correlated with the infective larval dose (Andrews, Kauffman and Davis, 1944). Sub-clinical T. colubriformis infections in sheep did not lead to diarrhoea but live-weight gain was reduced (Franklin, Gordon and Macgregor, 1946). Low level T. colubriformis infections were shown to reduce wool production by 40% (Carter, Franklin and Gordon, 1946). Goats given experimental infections of more than 200,000 larvae died by 28 days after infection of acute disease showing symptoms of anorexia and progressive weakness (Fitzsimmons, 1966). The experiments of Fitzsimmons (1966) also showed that

infective doses of 60,000 larvae produced a chronic form of the disease in goats with symptoms of progressive loss of condition leading to emaciation before death.

Roseby and Leng (1974) studied rates of production, secretion and recycling of urea in T. colubriformis infected lambs. These workers showed that between 15 and 35 DAI, parasitised lambs had higher plasma urea levels than their pair-fed controls. These elevated plasma urea levels were associated with an increase in the rate of excretion of urinary urea. The suggestion was made by Roseby and Leng (1974) that the urea lost by the parasitised animals was produced from ammonia released from amino acids in the tissues and in the gastrointestinal tract. It was shown by Sykes and Coop (1976) that T. colubriformis infections in growing lambs reduced food intake by 9% and reduced weight gain in infected animals by 50% compared with pair-fed controls.

It has been shown by many workers that a consequence of gastrointestinal parasitic infections in sheep is often an increased loss of plasma proteins across the intestinal epithelium. This phenomenon was demonstrated in sheep infected with Ostertagia circumcincta (Holmes and Maclean, 1971), and in sheep infected with Haemonchus contortus (Dargie, 1975).

In a study of the pathogenesis of T. colubriformis in lambs Barker (1973a) showed that following infection with 85,000 or 100,000 T. colubriformis larvae there was an increase in plasma protein loss into the gut and that this plasma loss coincided with other signs of

trichostrongylosis namely inappetence, weight loss and hypoproteinaemia. Increased gastrointestinal leak of plasma proteins in T. colubriformis infected lambs has also been demonstrated by Steel, Symons and Jones (1980) and by Poppi, MacRae and Corrigal (1981).

The interrelationships between levels of T. colubriformis infections and changes in body weight, wool production, enteric plasma loss, hypoalbuminaemia and in other metabolic parameters were assessed by Steel et al (1980). These workers studied groups of lambs which were dosed weekly with either 300, 950, 3,000, 9,500 or 30,000 T. colubriformis larvae for 24 weeks. Liveweight gains over the 24 week study were reduced in the lambs receiving 3,000, 9,500 or 30,000 larvae to 67%, 61% and 49% respectively compared to weight gains in the worm-free controls. Wool growth in the latter group of lambs was also reduced to 79%, 47% and 44% of that recorded in the control animals between weeks 8 and 12 of the experiment and at the same time food intake was 71%, 61% and 44% of that in the uninfected lambs.

The anorexia, reduction in weight gain and loss of wool production recorded in the infected lambs by Steel et al (1980) showed a close temporal relationship with disturbances in nitrogen metabolism, hypoalbuminaemia and intestinal plasma loss. The lambs subjected to weekly dosing with 30,000 larvae showed a significant negative nitrogen balance on weeks 4, 8 and 12 after the commencement of infection. Intestinal plasma loss and reduced plasma albumin levels were recorded during the twelfth week of infection in all groups of lambs receiving 950 or more larvae per week.

Throughout the world domestic livestock represent an important source of food and other products of major economic importance such as wool. Production losses due to parasitic diseases are of major significance and it has been estimated that wastage of livestock products as a result of parasitism totalled $6 billion (Kelly and Hall, 1979).

Strenuous efforts have been made in the last few decades to control animal helminthiasis by the use of anthelmintic drugs. Control has been achieved to a considerable degree following the introduction of thiabenzole and several other benzimidazole derivatives. Unfortunately the use of benzimidazole broad-spectrum anthelmintics has led to the increasing occurrence of drug-resistant strains of important parasites. Such a phenomenon is a source of major concern and the study of drug resistance in animal helminths has become an important area of parasitological research (see review by Kelly and Hall, 1979). In a review of anthelmintic resistance in nematodes (Prichard, Hall, Kelly, Martin and Donald, 1980) described several field reports and laboratory studies of drug-resistant strains of T. colubriformis in sheep.

In order to achieve a fuller understanding of drug resistance further laboratory investigations will be necessary. However, the use of substantial numbers of large animals in laboratory studies of drug resistance can be very costly and in many laboratories this factor has a limiting effect on research into diseases of animals such as sheep and cattle. There are obvious important advantages in identifying small animal hosts for such research, although great

care must be taken in selecting such a small laboratory animal. The parasite or organism should develop in the small animal host in a similar manner to that in the natural host and the pathogenic effects of infection should also be similar.

The suitability of several such hosts for T. colubriformis has been evaluated by Williams and Palmer (1964). Guinea pigs were found to be suitable hosts for T. colubriformis and the parasite was serially passaged through more than fifty generations with success. Rabbits were shown to be suitable laboratory hosts for the maintenance of T. colubriformis, but from an economic point of view, this animal is not ideally suited for large numbers of tests. The suitability of mice and rats as hosts for T. colubriformis has also been evaluated. Young male rats were found to be most susceptible but the infection terminated after 3 or 4 weeks and the optimum worm burdens were recovered 14 days after infection (DAI).

Guinea pigs were used to assay resistance to anthelmintics in ovine isolates of T. colubriformis (Kelly, Sangster, Porter, Martin and Gunawan, 1981). In the latter study several anthelmintics were tested and the guinea pig was considered to be a useful model for such a study. The economic advantages were obvious but there was a major drawback. The experiments had to be terminated at 10 DAI since expulsion of T. colubriformis in the particular breed of guinea pig used commenced at 14 DAI and all tests had to be completed prior to this time. There are therefore obvious disadvantages in studying T. colubriformis infections in the guinea pig.

Following their evaluation of the suitability of small animal hosts for T. colubriformis, Williams and Palmer (1964) concluded that the Libyan gerbil (Meriones libycus) was a suitable host for both Trichostrongylus colubriformis and Trichostrongylus axei. There have also been a number of reports that the Mongolian gerbil (Meriones unguiculatus) can be successfully infected with T. colubriformis and with T. axei. Leyland (1963) investigated the suitability of the Mongolian gerbil as a host for T. axei. The average prepatent period was found to be 21.8 days and satisfactory levels of fecundity rates were recorded. Worm recoveries ranged from 16% to 100% of the administered dose and in several cases 60% of the administered dose was recovered at 60 and 76 DAI. Kates and Thompson (1967) showed that gerbils could act as hosts for mixed infections of T. axei and T. colubriformis simultaneously administered. The latter workers (Kates and Thompson, 1968) also showed that, under similar conditions, two strains of young white rats were almost completely resistant to a mixed culture of T. colubriformis and T. axei, whereas the Mongolian gerbil could be readily infected.

The gerbil was used by Ostlind and Cifelli (1981) to test the efficacy of anthelmintics against T. colubriformis infections. The latter experiments were terminated at 9 DAI. Mongolian gerbils were also successfully used as hosts for T. colubriformis in a test on the efficacy of anthelmintics at 30 DAI (Panitz and Shum, 1981a). The latter workers (Panitz and Shum, 1981b) found that adult T. colubriformis in the gerbil 28 DAI were located exclusively in the small intestine.

Unfortunately the infections in the above reports have usually been terminated soon after patency and there are no reports of the study of T. colubriformis infections in small animal hosts over a long period. These aspects are important since small animal hosts for T. colubriformis in current common usage, such as the guinea pig or immunosuppressed rat, show relatively transient infections which normally self-cure soon after patency is achieved, e.g. 12 - 20 days post-infection (Herlich, 1958; Connan, 1966 and Rothwell and Griffiths, 1975), and therefore their value as models in anthelmintic screening and immunological investigations is limited. Furthermore, in the guinea pig considerable variability in susceptibility between individual animals has been reported (Williams and Palmer, 1964

The current interest in the development and identification of drug resistant strains of T. colubriformis in the field, and also the need for improved models of gastrointestinal helminth parasitism in which to study pathogenic effects and immunological responses, justified a more detailed evaluation of the infection pattern and pathogenic effects of T. colubriformis in the Mongolian gerbil. In Section II of this study the establishment, fecundity and survival of a T. colubriformis population in young Mongolian gerbils was followed for 60 days following infection with a strain of T. colub - riformis susceptible to the benzimidazole group of anthelmintics.

Clinical signs of the disease in the gerbils were monitored throughout the period of study. A comparison of the pathogenesis of benzimidazole-susceptible and benzimidazole-resistant strains of T. colubriformis in the gerbil was also conducted.

In the final chapter of this thesis, parasite-specific antibody levels were measured in gerbils following vaccination with T. colubriformis larvae attenuated with varying doses of gamma radiation and in gerbils following vaccination with non-irradiated larvae. Local intestinal and coproantibody levels were reassessed following challenge with non-irradiated T. colubriformis larvae and the relationship between protection against reinfection and local immune responses was examined.

CHAPTER 1

Studies on the Pathogenesis of *Trichostrongylus colubriformis* in the Mongolian Gerbil (*Meriones unguiculatus*)

Table 1.1

Experimental Design

No. of Gerbils	Infective Dose of *T. colubriformis* Larvae	Gerbil Weights	Faecal Egg Counts (eggs/g)	Necropsy* of 5 gerbils on D.A.I.
45	1,500	Weekly	Three times per week	10, 15, 20, 25, 30, 40, 50, 60
15	Control Group	Weekly	Three times per week	18, 45

* Worm burdens, packed cell volumes, serum protein and serum albumin concentrations were determined.

Introduction

Although a number of workers have used the Mongolian gerbil as an experimental host for T. colubriformis infections, particularly in the study of the efficacy of anthelmintics, the the pattern of infection and pathogenic effects of the parasite have not been studied. The experiment described in this chapter allowed such an evaluation to be made over the period 0 - 60 DAI.

Experiment 1

In this experiment, the numbers of T. colubriformis eggs recovered in faeces of gerbils following infection with T. colubriformis and the worm burdens were studied at intervals from infection until 60 DAI. Body weight changes, plasma protein concentrations and packed cell volumes were also measured.

Experimental Design

The experimental plan is shown in Table 1.1. Nine groups of 5 gerbils were infected with 1,500 infective third stage T. colubriformis larvae. The gerbils were weighed every 7 days from 11 DAI until 60 DAI. Faeces was examined daily for parasite eggs from 10 DAI until patency at 17 DAI, then three times weekly for the duration of the experiment. A group of 5 gerbils was necropsied on the days shown in Table 1.1. Worm burdens were ascertained and blood collected for packed cell volume determination and for the preparation of serum. Total serum protein and serum albumin concentrations in the serum samples were estimated.

Experimental Animals

Sixty gerbils, 52 female (cages A, B, D and E) and 8 male (cage C), approximately two months old, were used. Three weeks before infection the animals were treated for one week with an anthelmintic in the feed (220 parts per million morantel tartrate Suiminth, Pfizer, Sandwich, Kent, England) in Spratts No. 2 powdered rodent diet in order to eliminate any possible existing gastrointestinal nematode infections.

Throughout the subsequent study the gerbils were kept in groups of 5 animals in plastic cages with grid floors and provided with a commercial pelleted rodent diet (No. 41, Angus Milling Co., Perth) and fresh water. At weekly intervals all the animals were weighed.

Gerbil Body Weights

The body weights of all infected and control gerbils were determined weekly commencing at 11 DAI. In addition, body weights of gerbils were recorded immediately prior to necropsy (see experimental plan).

Parasitological Techniques

T. colubriformis Larvae

A benzimidazole-susceptible strain of the parasite was originally obtained from the Central Veterinary Laboratories, Weybridge, England and had been maintained by passage through parasite-free lambs at Pfizer Central Research Laboratories, Sandwich, Kent, England, for at least 10 years.

Infection of Gerbils

This was carried out as described in the General Materials and Methods.

T. colubriformis Egg Counts and Determination of Worm Burdens

These were carried out as described in the General Materials and Methods.

Packed Cell Volume Measurement

Immediately prior to necropsy blood was withdrawn by cardiac puncture under deep anaesthesia induced with trichloroethylene. Immediately after collection two heparinised capillary tubes were filled for determination of the packed cell volume (PCV) by the microhaematocrit method.

Serum Preparation

The remainder of the blood collected prior to necropsy was placed in a clean glass centrifuge tube. After 3 hours at room temperature the serum was removed and stored at -20°C until required.

Serum Total Protein Estimations

Serum total protein estimations were carried out as described in the General Materials and Methods.

Serum Albumin Estimations

Serum albumin concentrations were estimated by continuous flow analysis, (Standard Technicon Auto-Analyser II), based on the bromo-cresol green method (Rodkey, F.L., 1965).

Results

Survival of Gerbils Following Infection with 1,500 T. colubriformis

Eight of the infected gerbils, 15% of the total, died between 10 and 20 DAI. Prior to death, these animals showed signs of inappetance, poor condition and most were diarrhoeic. The surviving infected gerbils, while showing some loss of condition were generally not diarrhoeic. Since most of the deaths in the eight fatal cases occurred overnight, it was not possible to ascertain body weights or worm burdens in these animals.

Changes in Gerbil Body Weights Following Infection with 1,500 T. colubriformis

Changes in the body weight of gerbils following infection with T. colubriformis and of the corresponding group of worm-free control animals are shown in Fig. 1.1.

Loss of weight and/or reduction in weight gain was observed in the infected gerbils compared with the non-infected controls. The weight of the infected gerbils was significantly lower than the controls on days 18, 46, 53 and 60 after infection (Appendix G, Table 1). Over the 60 day experiment the infected animals showed a net weight loss of 3 g whilst the control animals had a mean gain of 8 g. Towards the end of the experiment there was evidence of an increase in the difference between the weights of infected and control gerbils.

Weights of individual gerbils recorded immediately prior to necropsy are shown in Appendix G, Table 2.

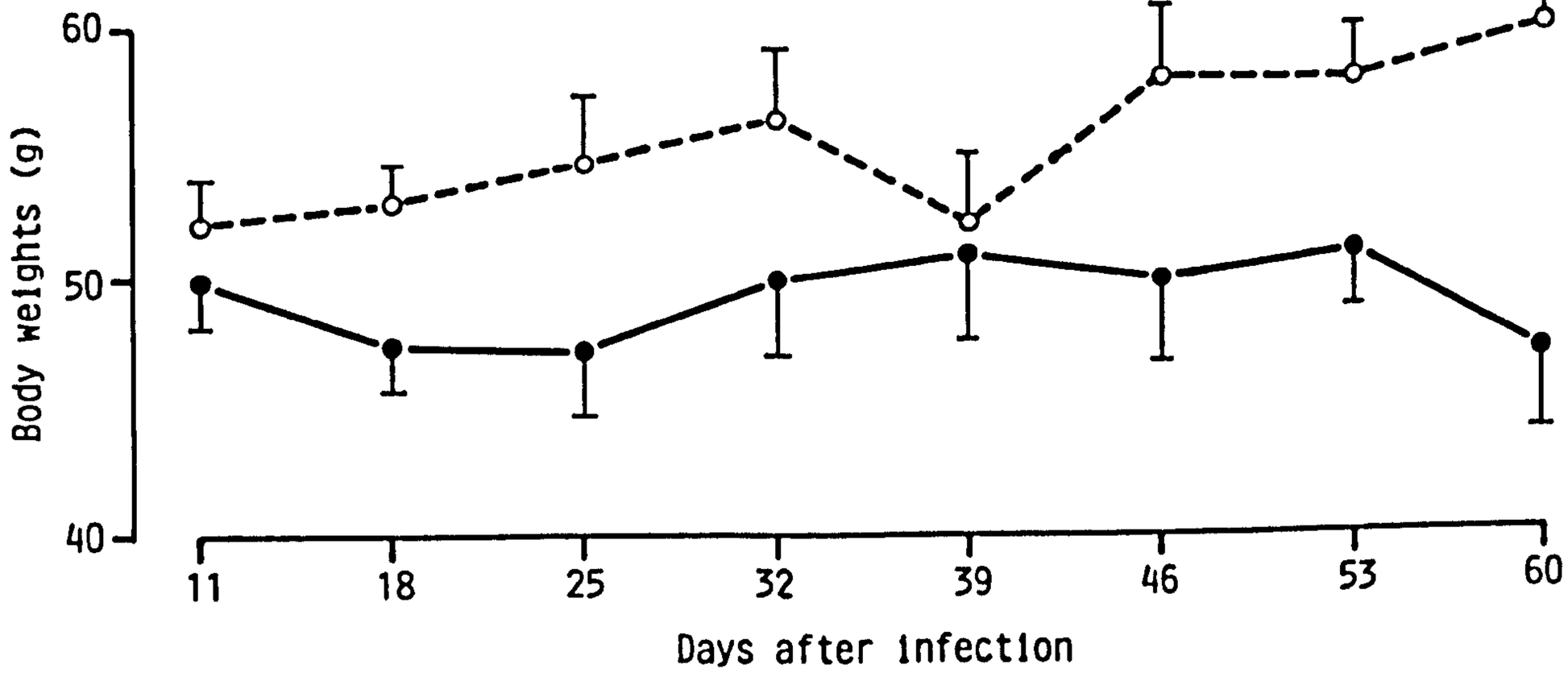

Fig. 1.1. Body weights of gerbils (mean ± S.E.) following infection with 1,500 T. colubriformis and in worm-free controls.

———— infected, ---------- control.

Trichostrongylus colubriformis Egg Counts

The numbers of T. colubriformis eggs recovered in faeces of infected gerbils are shown in Fig. 1.2.

The infections became patent on 17 DAI and faecal egg counts remained high for an extended period. Results from individual cages are shown in Appendix G, Table 3.

Worm Burdens

T. colubriformis worm burdens in the infected gerbils are shown in Fig. 1.3.

Worm burdens in the groups of animals dissected at various times after infection showed a similar pattern to the faecal egg output. High worm burdens were detectable between 20 and 40 DAI, during which time approximately 20% of the infective larval dose was recoverable. Results from individual gerbils are shown in Appendix G, Table 2.

Comparison of T. colubriformis Infections in Male and Female Gerbils

There was no evidence of any significant differences in gerbil body weights, worm burdens, serum protein levels or packed cell volumes in the male gerbils compared with the female gerbils, (see Appendix G, Tables 2 and 3). However only one group of male animals was included in the study and these observations should be viewed with this point in mind.

It was additionally noted that after 40 DAI there was a significant negative correlation between worm burdens and gerbil body weights at necropsy (Fig. 1.4).

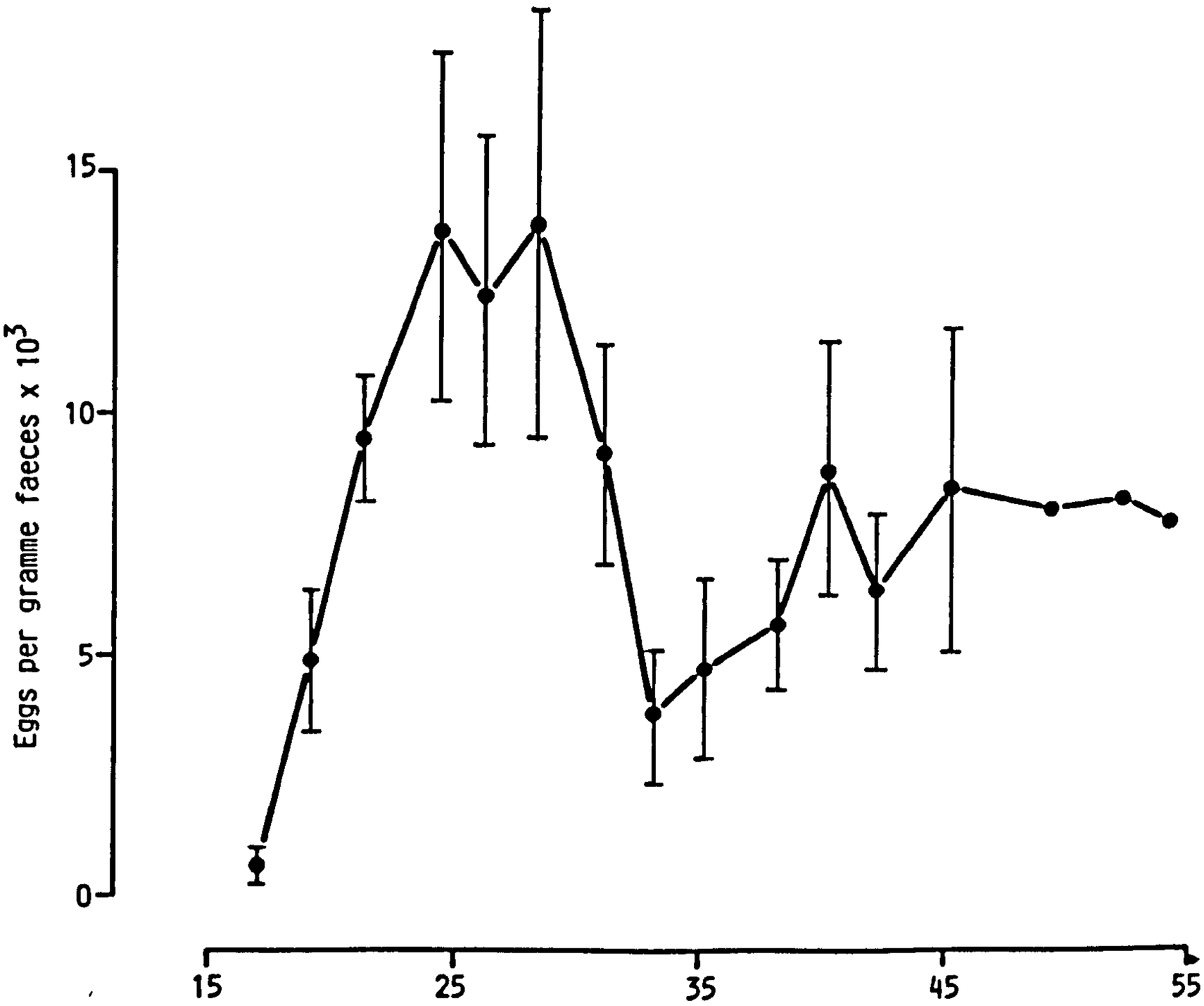

Fig. 1.2. T. colubriformis egg counts in faeces of gerbils (eggs/g x 10^3) following infection with 1,500 larvae.

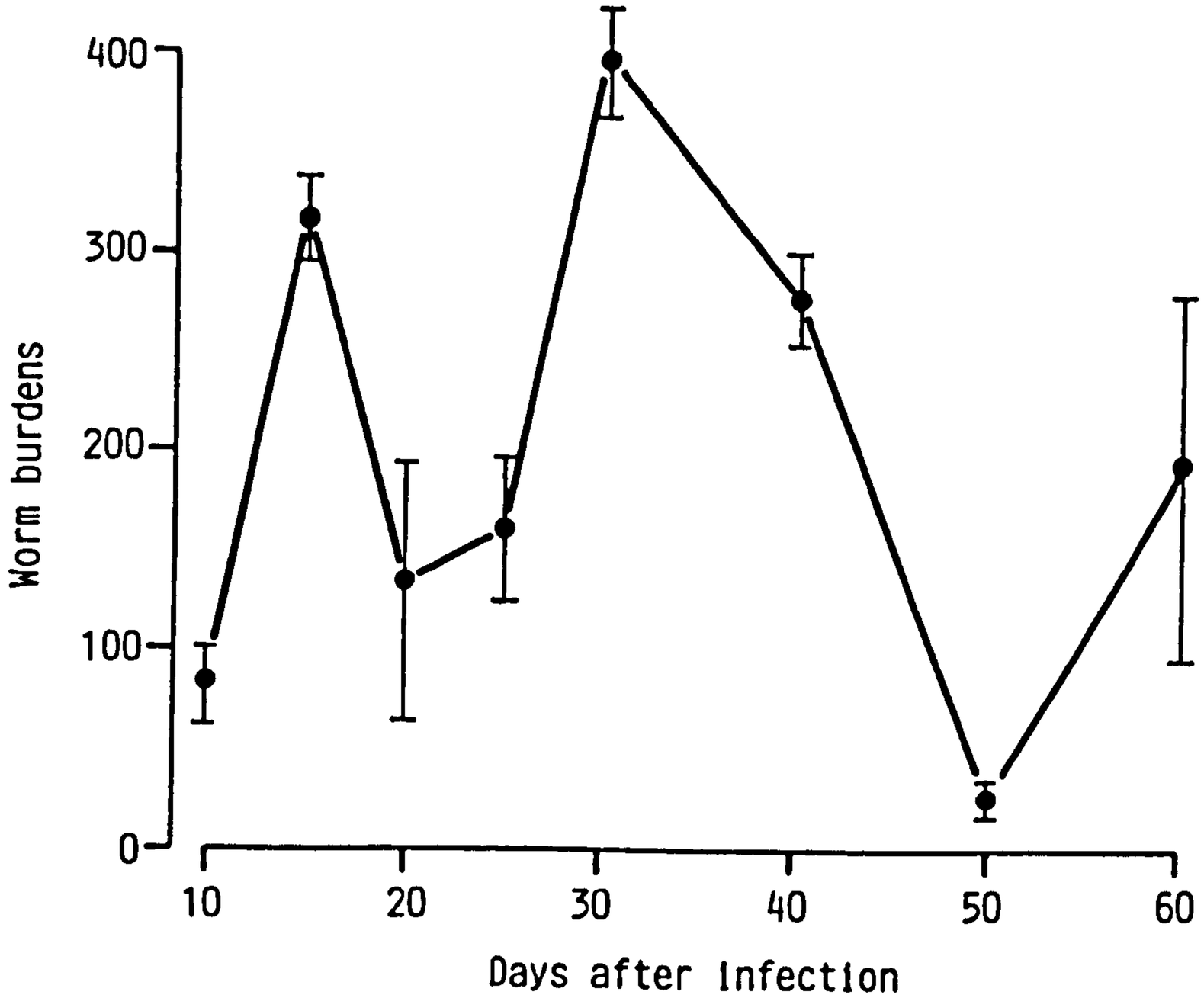

Fig. 1.3. T. colubriformis worm burdens in gerbils following infection with 1,500 larvae.

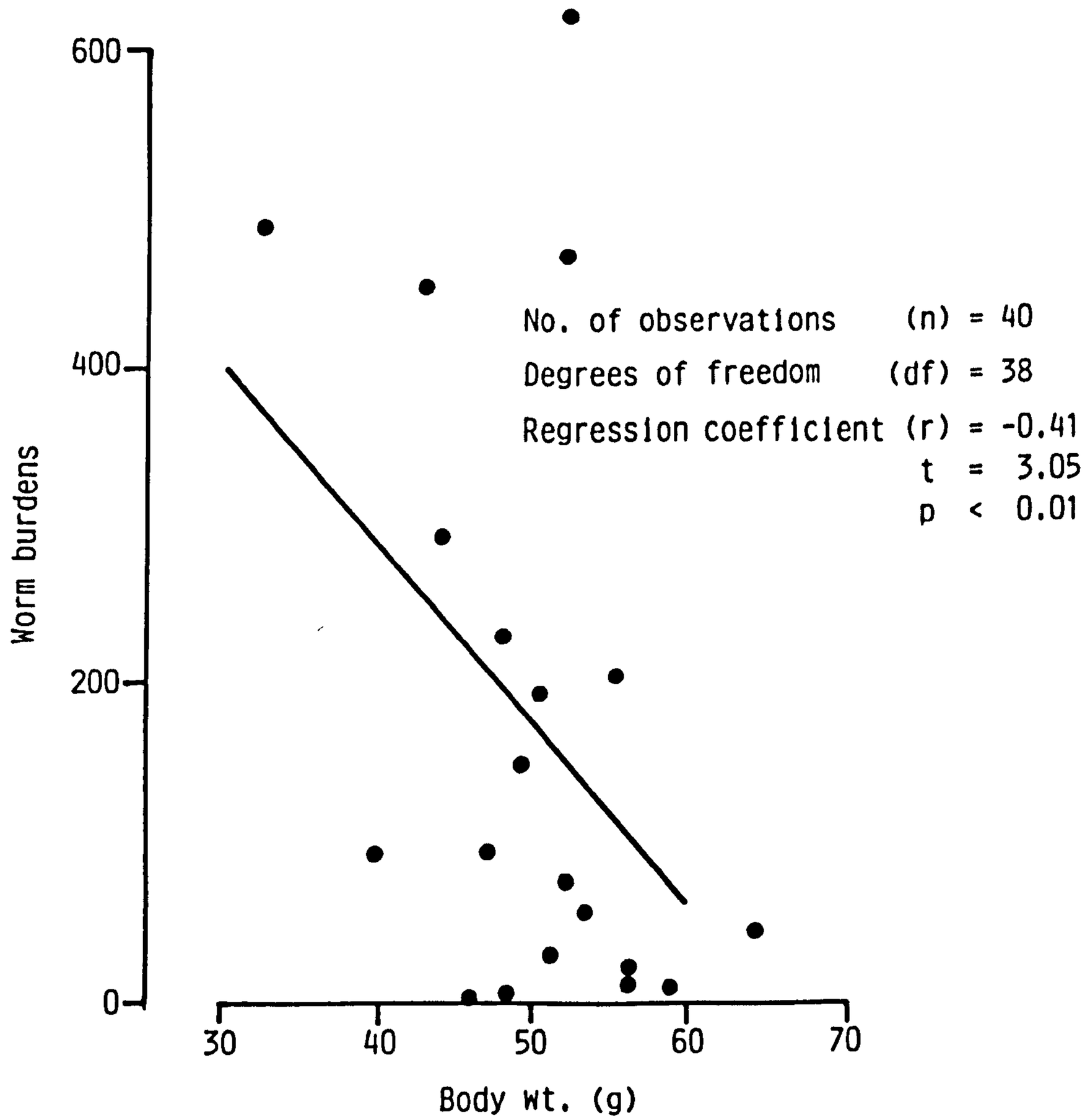

Fig. 1.4. Correlation between worm burdens and gerbil weights between days 40 - 60 after infection with 1,500 T. colubriformis.

Serum Protein Concentrations in Gerbils Following Infection with T. colubriformis

Serum total protein levels remained fairly constant in the infected gerbils over the period of the experiment. However a modest hypoalbuminaemia developed in the infected gerbils with a fall in the mean albumin level from 2.9 g/100 ml at 10 DAI to 1.6 g/100 ml at 60 DAI. No significant alterations were detected in the control animals. Serum globulin levels appeared to increase as a result of infection.

Results from individual infected animals are shown in Appendix G, Table 3 and results from the control animals in Appendix G, Table 4.

Changes in Packed Cell Volumes of Gerbils Following Infection with T. colubriformis

There were no significant changes in packed red cell volumes in gerbils following infection with T. colubriformis.

Discussion

In this experiment and in experiments to be described later, worms were recovered from the contents of the small intestine and the mucosal wall of the small intestine. No attempt was made to collect the nematodes from other parts of the gastrointestinal tract since it was shown by Panitz and Shum (1981b) that in T. colubriformis infections in the gerbil 99% of the adult worms could be recovered from the small intestine.

Following a single infection with 1,500 T. colubriformis larvae, approximately 20% of the infective dose became established and worm burdens generally remained high over a prolonged period. Though there were some fluctuations in the numbers of parasites recovered, these may be attributable to the small numbers of gerbils in each group and to their random selection. Similarly a 17% worm establishment was reported by Panitz and Shum (1981b) in gerbils 28 days after infection with 1,000 T. colubriformis of bovine origin.

The infections were patent 17 days after infection. These findings were similar to previous reports that the prepatent period for T. colubriformis in the gerbil was 16 days (Herlich, 1958; Gordon, Mulligan and Reinecke, 1960; and Sturrock, 1963). Gerbil parasite burdens were associated with high faecal egg counts. However it is important to appreciate that inappetance can have an effect on the numbers of eggs per gramme of faeces. It was shown by Abbott (1982) that inappetence in Haemonchus contortus infected lambs led to greatly reduced faecal mass. The inappetant lambs had very high faecal egg counts compared to lambs which had received the same larval dose but showed no signs of loss of appetite or reduced faecal output, though the total numbers of eggs excreted per day was similar in both groups. A similar situation could have arisen in some of the T. colubriformis infected gerbils, leading to the assessment of egg output in terms of eggs/g faeces giving higher values than otherwise might have been obtained. It therefore might have been more satisfactory if the total egg output per animal per day had been measured, though this was prevented by practical considerations.

The infected gerbils showed either reduced weight gains or weight losses compared to the non-infected control animals. However, the premature removal by death of the 8 most adversely affected gerbils from the experiment by 20 DAI distorted to some extent, changes recorded in body weights later in the experiment. Since the animals that died were in every case showing signs of emaciation prior to death the assumption can be made that the difference between body weights between infected and control animals was in reality greater than that recorded by comparing surviving animals with the controls. T. colubriformis egg output may also have been higher in the animals that died but this suggestion is speculative.

The negative correlation between worm burden and body weight in the surviving gerbils indicated that gerbils carrying the highest parasite burdens tended to suffer the greatest weight losses. Andrews et al, (1944) reported similar findings in lambs infected with T. colubriformis. It is also noteworthy that T. colubriformis in sheep has been shown to produce anorexia, emaciation, weakness, weight loss and sometimes diarrhoea (see Review by Fitzsimmons, 1969) and closely similar findings were observed in the infected gerbils in the present experiment.

There appeared to be no difference in susceptibility to T. colubriformis infections between male and female gerbils. Similar findings were previously reported by Kates and Thompson (1968).

There was no evidence of anaemia, as detected by packed cell volume measurements in any of the infected gerbils. Other

workers (Andrews, 1939; Kates and Turner, 1953; and Fitzsimmons, 1966) also showed that anaemia was not a feature of T. colubriformis infections in sheep, lambs or goats. However, hypoalbuminaemia is a feature of T. colubriformis infection of sheep (Steel et al, 1980) and in the present experiment plasma total protein and albumin levels in infected animals were slightly lower than in the non-infected control animals, though the differences were not statistically significant.

The results showed that the pathogenic effects of T. colubriformis infections in the gerbil were essentially similar to those observed in sheep and thereby suggested that the gerbil is a suitable laboratory host for the sheep nematode T. colubriformis. Furthermore, they showed that, because of the high level of establishment and extended duration of infection, the gerbil is superior to the other small animal models previously described.

In the following chapter, experiments are described in which the Mongolian gerbil is used in studies of the comparative pathogenicity of drug-susceptible and drug-resistant strains of T. colubriformis.

CHAPTER 2

A Study of the Pathogenesis of Benzimidazole-resistant and Benzimidazole-susceptible Strains of *Trichostrongylus colubriformis* in the Mongolian Gerbil (*Meriones unguiculatus*)

Introduction

The results discussed in Chapter 1 clearly demonstrated that in terms of establishment, duration of infection and pathophysiology, T. colubriformis infections in the gerbil were closely similar to those in sheep. The gerbil therefore appears to be superior to other small animal hosts and promises to be a useful model in studies of both helminth immunity and in the evaluation of drug resistant strains of T. colubriformis. With regard to the latter aspect, there have been reports that the pathogenesis of drug resistant strains of H. contortus may be different from the pathogenesis of drug sensitive strains of parasites. It was reported by Drudge, Leyland and Wyant (1957) that a strain of H. contortus resistant to the anthelmintic phenothiazine had a higher infectivity for sheep, 52%, compared with an infectivity of 33% for a non-resistant strain of parasite. A similar phenomenon was observed by Kelly, Whitlock and Thompson (1978) who showed that 55% of a benzimidazole-resistant strain of H. contortus established in worm-free sheep compared with 40% of a drug-susceptible strain. The latter workers also showed that the drug-resistant strain of H. contortus had a higher egg output and caused a more severe anaemia than the drug-susceptible strain.

However the basis of the altered pathogenesis has not been adequately investigated. The experiments to be described in this chapter were designed to investigate the comparative pathogenesis of benzimidazole-susceptible and benzimidazole-resistant strains of T. colubriformis in the Mongolian gerbil.

Table 2.1

Susceptible Strain Infections (BS)			Resistant Strain Infections (BR)			Necropsy DAI
Cage	No. of Gerbils Necropsied	Sex	Cage	No. of Gerbils Necropsied	Sex	
N	4	♀	D	4	♀	10
N	5	♀	D	5	♀	20
K	5	♀	F	5	♀	30
O *	5	♂	I *	5	♂	40
O *	5	♂	I *	5	♂	50
J *	5	♀	G *	5	♂	
L *	5	♂	H *	5	♂	
M *	5	♂	E *	4	♀	

* Gerbils in these cages were used for monitoring T. colubriformis egg output.

27 worm-free control gerbils were also included in the experiment.

In Experiment 1, changes in gerbil body weights, mortality rates, T. colubriformis egg output and worm burdens were recorded following infection with either a drug-susceptible or a drug-resistant strain of the parasite. In addition comparisons were made of plasma protein concentrations in both groups of infected gerbils.

It seems probable that the intestines of T. colubriformis infected animals undergo considerable changes due to the presence of the parasites. To investigate this aspect of T. colubriformis infections, mucosal tissue was taken from gerbils 20 days after infection with either drug-susceptible or drug-resistant strains of the parasite and from control animals. These tissues were examined by electron microscope scanning.

In Experiment 2 plasma protein loss into the gastrointestinal tract of gerbils infected with drug-susceptible or drug-resistant strains of T. colubriformis was measured using ^{51}Cr-chromic chloride labelled plasma proteins.

Experiment 1

Thirty-nine gerbils were infected with a benzimidazole-susceptible (BS) strain of T. colubriformis and 38 gerbils were infected with a drug-resistant (BR) strain of the parasite. These animals were monitored for 50 days following infection. At regular intervals groups of infected animals were necropsied (Table 2.1) for serum, tissue samples and the determination of worm burdens. A group of 27 gerbils served as worm-free controls.

Materials and Methods

Experimental Animals

Young gerbils, approximately 2 months old were used. Three weeks before the study commenced they were treated with a broad spectrum anthelmintic, as described in Chapter 1, to eliminate any existing nematode infections.

Parasitological Techniques

T. colubriformis Larvae

Two strains of T. colubriformis were used, a benzimidazole-susceptible (BS) strain and a benzimidazole-resistant, VRSG,* (BR) strain. Both strains had been maintained by passage through parasite-free lambs at Pfizer Central Research Laboratories, Sandwich, Kent, England.

Infection of Gerbils with T. colubriformis

Infection of gerbils with 1,500 T. colubriformis larvae of either the BS strain or the BR strain was carried out as previously described.

Egg Counts in Faeces of T. colubriformis Infected Gerbils

T. colubriformis egg counts in faeces of gerbils were carried out as previously described. From the BS strain of infections, cages J.L.M. and O were used for thrice weekly counts. Gerbils in cages J and M were transferred to clean cages each week to minimise

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* Veterinary Research Station, Glenfield, Australia.

the risk of reinfection from faecal material adhering to the cage grids. From the BR strain infections, cages E, G, H and I were used for egg counts thrice weekly and animals in cages E and H were transferred to clean cages every week.

Preparation of Serum and Serum Protein Analysis

Blood was collected at necropsy and serum prepared for total protein and albumin estimation as previously described.

Examination of Small Intestines of Gerbils Following Infection with T. colubriformis and of Non-infected Gerbils

Small intestines were removed from gerbils infected with either BS or BR strain of the parasite 30DAI and from non-infected gerbils. The gross appearance of the intestines of the infected animals was compared with intestines removed from control animals.

Preparation of Tissue for Scanning Electron Microscope Examination

Small pieces of tissue were removed from the intestines of both groups of infected gerbils and from a non-infected animal on day 20 after infection. The tissues were taken 16 cm from the pyloric end of the small intestine and were fixed in dilute Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M buffer) for a minimum of 24 hours. The tissues were then washed in buffer, dehydrated in a series of acetones and critical point dried in a Polarton Critical Point drier. The specimens were attached to aluminium stubs with silver paint and sputter coated with gold/palladium. Tissues were viewed and photographed in a Philips 501 B scanning electron microscope at 30 Kv.

Results

Survival of Gerbils Following Infection with 1,500 Drug-Susceptible or Drug-Resistant T. colubriformis

By 27 DAI, 18 gerbils from the groups infected with the BS strain of T. colubriformis had died, 46% of the population. Only 2 of these deaths occurred after the 20th day of infection. In contrast, in the gerbils infected with the BR strain of the parasite there were only 4 deaths by 20 DAI and then another 7 deaths by 27 DAI, 29% of the population. The infected gerbils showed signs of oedema in the later stages of the experiment.

As a consequence of the high death rate among both experimental groups, the experiment had to be terminated at 50 DAI, rather than at 70 DAI which was the original intention.

T. colubriformis Egg Counts in Faeces of Gerbils Infected with Drug-Susceptible or Drug Resistant Strains of the Parasite

The infections became patent on 17 DAI. The rate of increase in faecal egg output was greater in the gerbils infected with the BS strain of the parasite, reaching a level of 1.8×10^4 eggs/g at 24 DAI. At the same time the level of egg output in the animals infected with the BR strain of the parasite was 1.3×10^4 eggs/g (Fig. 2.1).

There was no evidence of any significant differences between egg output in the groups of gerbils remaining in the same cages throughout the experiment, cages L and O compared with J and M and cages E and H compared with G and I, for the BS and BR strain

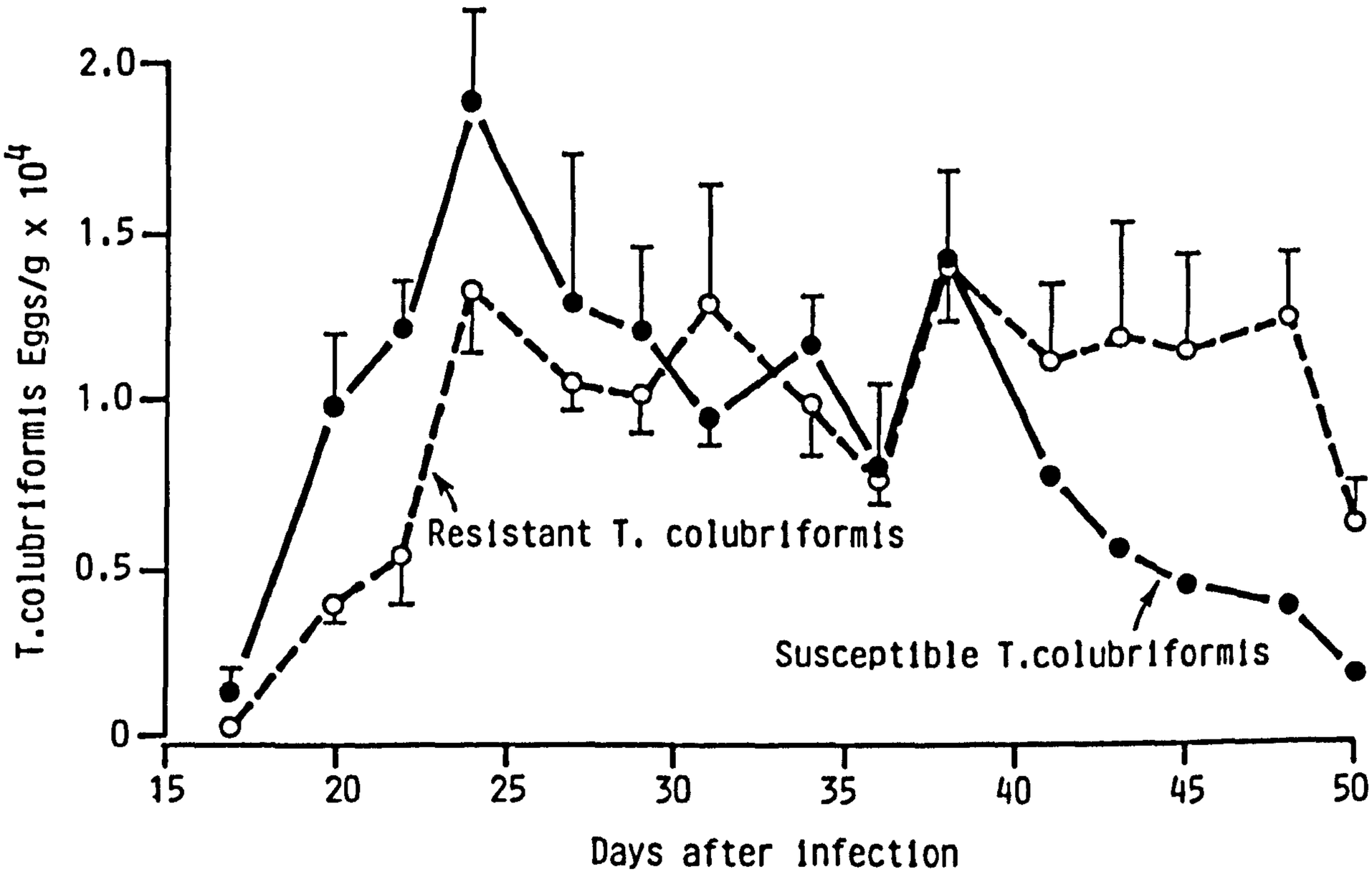

Fig. 2.1. T. colubriformis egg counts in faeces of gerbils infected with drug-susceptible or drug-resistant strains of the parasite (results represent mean count for 2, 3 or 4 cages ± S.E.)

infections respectively. Faeces from gerbils in cages A, B and C which housed worm-free controls were examined for T. colubriformis eggs at intervals throughout the experiment and all tests were negative.

Results for individual cages are shown in Appendix H, Table 1.

Worm Burdens in Gerbils Infected with Drug-Susceptible or Drug-Resistant Strains of T. colubriformis

Worms recovered from the small intestines of gerbils between 10 and 50 DAI are shown in Fig. 2.2. Greater numbers of worms were found in the BS strain infections. Between 10 and 40 DAI the ratio of worms recovered in the BS compared with the BR strain infections varied from 1.8:1 on 20 DAI to 1.2:1 on 40 DAI. Worm burdens in the BS strain infections were significantly higher on 20 DAI and on 30 DAI than in the BR strain infections.

Results for individual gerbils are shown in Appendix H, Table 2.

Changes in Weights of Gerbils Following Infection with Drug-Susceptible or Drug-Resistant Strains of T. colubriformis

(1) The weights of groups of five gerbils necropsied on 10, 20, 30, 40 and 50 DAI were recorded immediately prior to necropsy. The results are shown in Fig. 2.3.

There were no significant differences between the weights of the gerbils infected with BS or BR strains of T. colubriformis. Both groups of gerbils showed modest weight gains between 10 and 50 DAI. Results for individual gerbils are shown in Appendix H, Table 2.

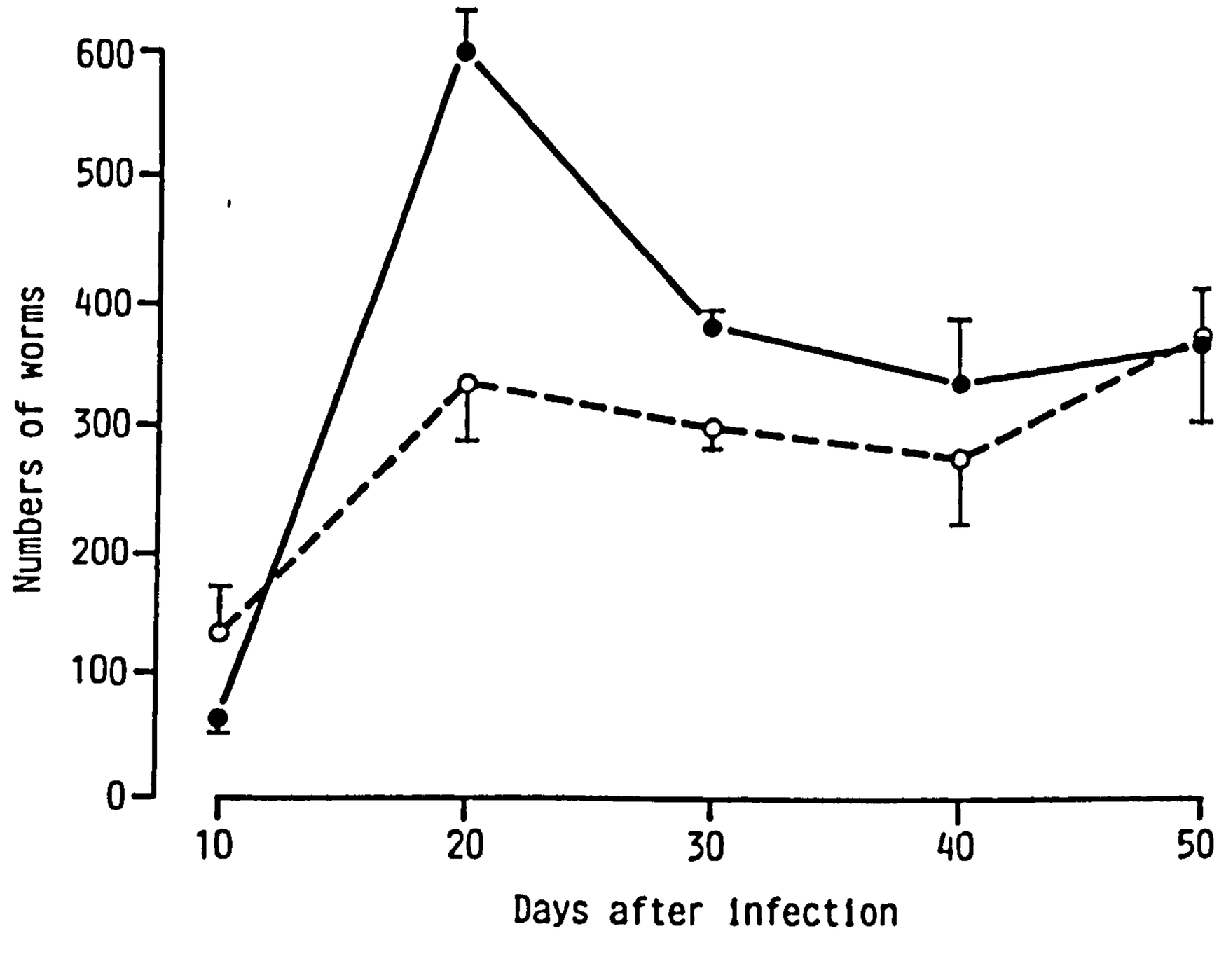

Fig. 2.2. Numbers of worms (mean ± S.E.) recovered from gerbils following infection with 1,500 drug-susceptible or drug-resistant strain T. colubriformis

——●—— drug susceptible T. colubriformis

----o---- drug resistant T. colubriformis

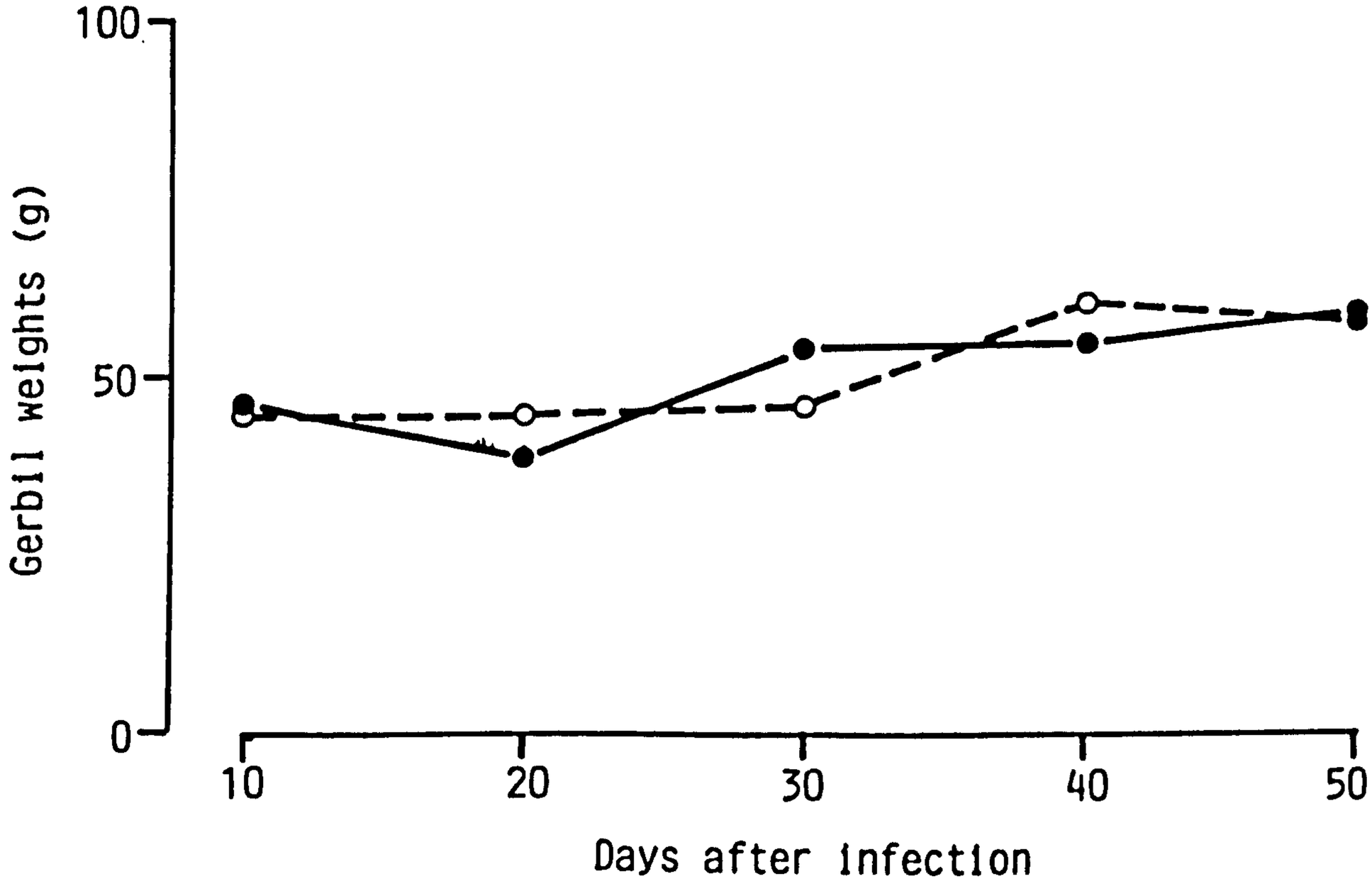

Fig. 2.3. Weights of gerbils (mean of 5 ± S.E.) following infection with drug-susceptible or drug-resistant strain of T. colubriformis.

——●—— drug-susceptible T. colubriformis

----o---- drug-resistant T. colubriformis

(2) The weights of all gerbils were recorded weekly beginning at 14 DAI until 42 DAI. The results are shown in Fig. 2.4.

By 14 DAI the mean weight of gerbils infected with the BS strain of T. colubriformis was 50.4 g while the mean weight of the gerbils infected with the BR strain of the parasite was 50.0 g. These weights compared with a mean value of 58.1 g in the worm-free control gerbils, (see Appendix H, Table 3 for the numbers of animals in each group).

From 21 DAI the surviving gerbils in both infected groups showed steady gains in weight, reaching control values by 42 DAI in the case of the gerbils infected with the BS strain of T. colubriformis and by 49 DAI in the other infected group. The mean weights of the control gerbils on days 14, 21, 28, 35 and 42 of the experiment were 58.1 g, 56.6 g, 59.2 g, 58.5 g and 56.8 g respectively.

Examination of Small Intestines Removed from Infected and Control Gerbils at 30 DAI

Small intestines removed from infected gerbils on day 30 after infection were distended both laterally and longitudonally compared with intestines removed from worm-free gerbils.

Serum Total Protein and Serum Albumin Levels in Gerbils Following Infection with Drug-Susceptible or Drug-Resistant Strains of T. colubriformis

In both groups of infected animals there was a moderate hypoproteinaemia compared with the worm-free controls. There was evidence of hypoalbuminaemia, particularly in the drug-susceptible strain infections between 30 and 50 DAI (Fig. 2.5).

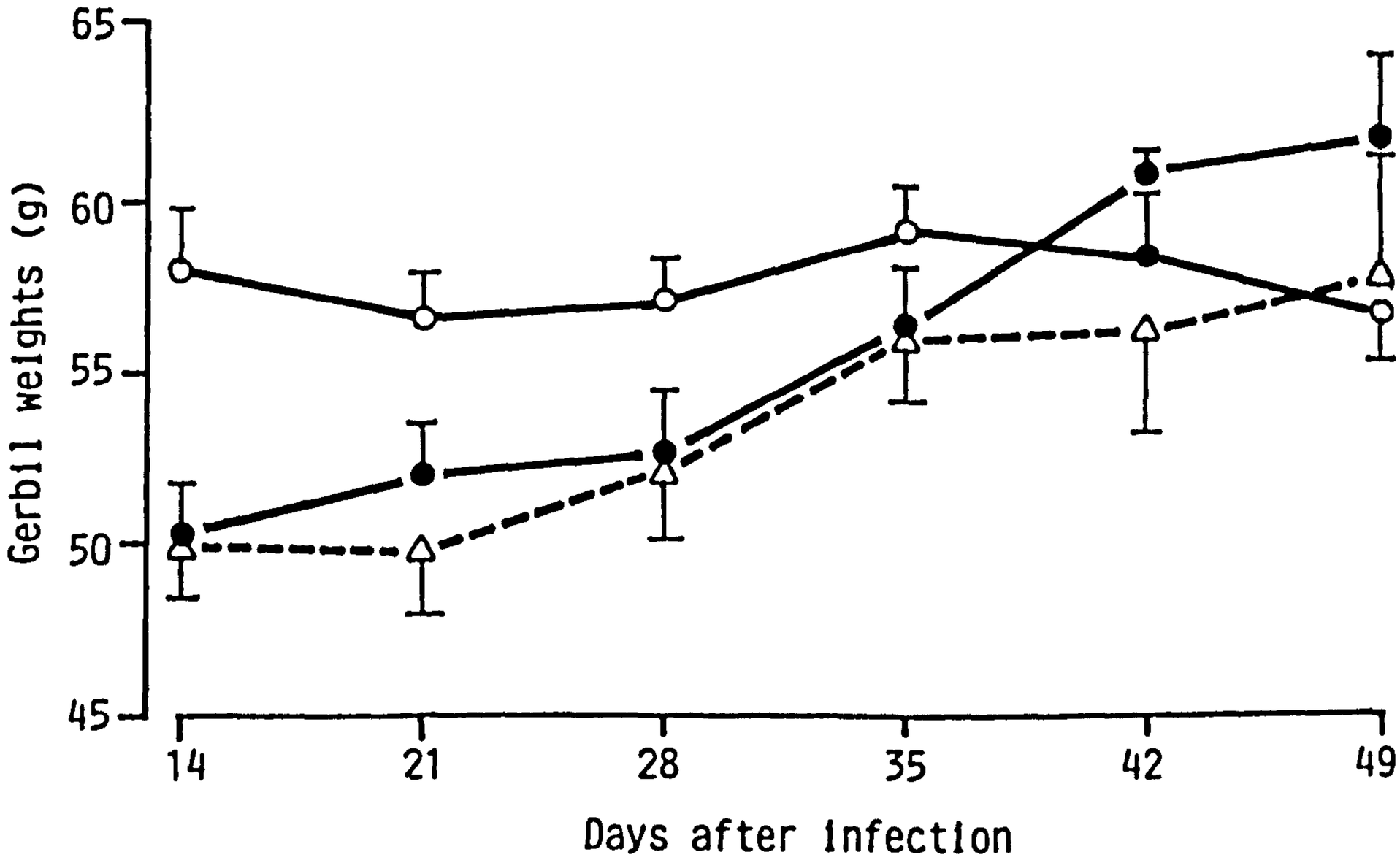

Fig. 2.4. Weights of gerbils following infection with drug-susceptible or drug-resistant strain of T. colubriformis and weights of control gerbils.

—●— drug-susceptible strain T. colubriformis

----△---- drug-resistant strain T. colubriformis

—○— worm-free controls

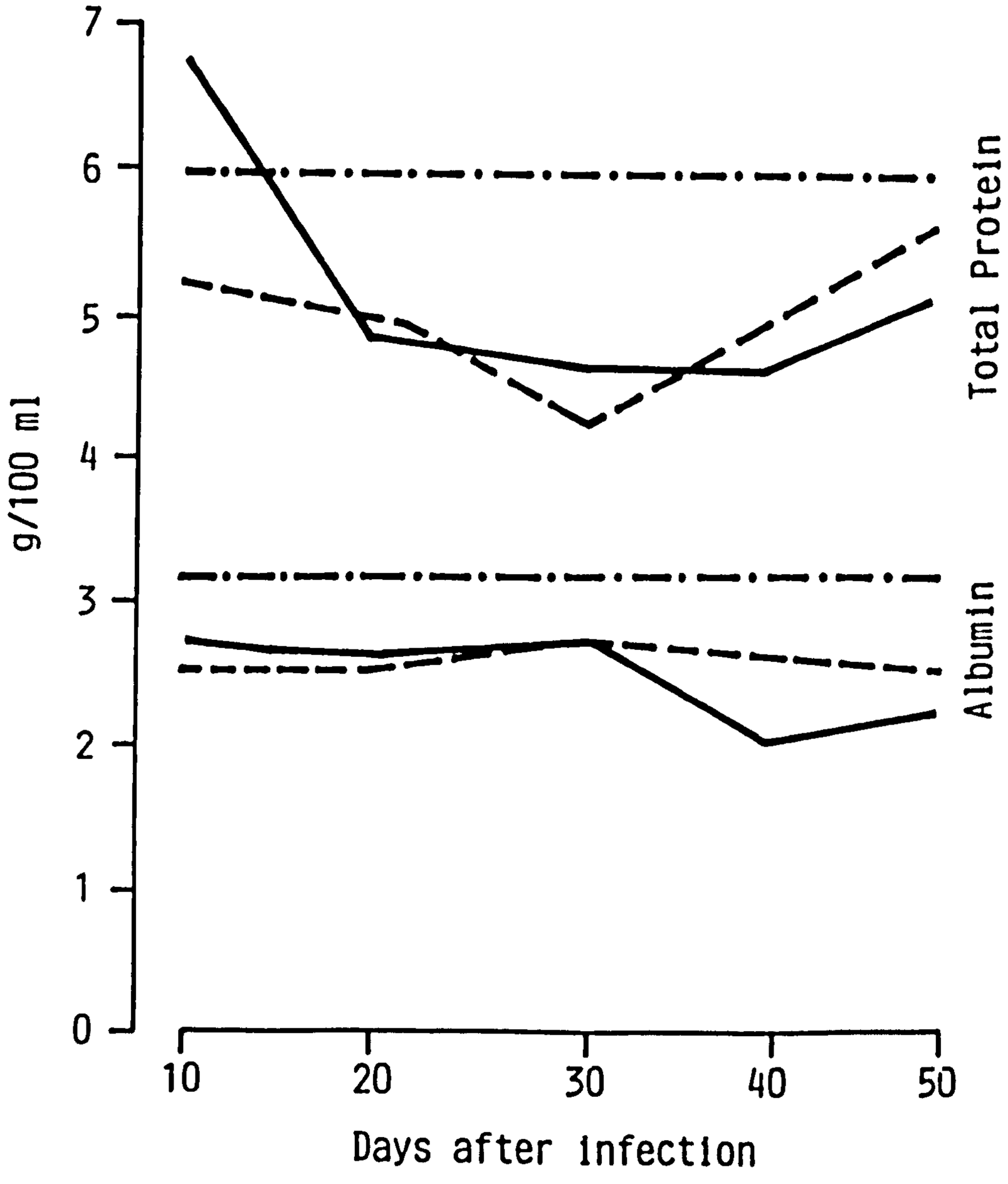

Fig. 2.5. Serum total protein and serum albumin concentrations in gerbils following infection with drug-susceptible or drug-resistant strains of T. colubriformis and in worm-free control animals.

——— drug-susceptible strain of T. colubriformis

- - - - - - - - drug-resistant strain of T. colubriformis

•—•—• worm-free controls

Results from individual gerbils are shown in Appendix H, Tables 5 and 6.

Examination of Scanning Electron Micrographs

Figure 2.6 shows villi from the small intestine of a worm-free control gerbil at magnification 1 x 320. The villi appeared in the regular spatulate formation normally seen in the small intestine.

Figure 2.7 shows intestinal villi from a gerbil infected with the BS strain of T. colubriformis. The magnification was 1 x 320. The villi showed signs of severe atrophy and lacked the regular spatulate formation observed in the mucosa of the uninfected gerbil. There were also signs of extensive flattening of the microvilli.

Figure 2.8 shows intestinal villi from a gerbil infected with the BR strain of T. colubriformis at magnification 1 x 320. The villi in this tissue also showed evidence of atrophy and of irregular column formation when compared with the villi of the control gerbil. However the villi in the BR strain infections were less adversely affected than those in the BS strain infections, e.g. there was less evidence of flattening of the microvilli.

Figure 2.9 shows a 1 x 160 magnification of the drug-resistant strain worm embedded in the intestinal mucosa.

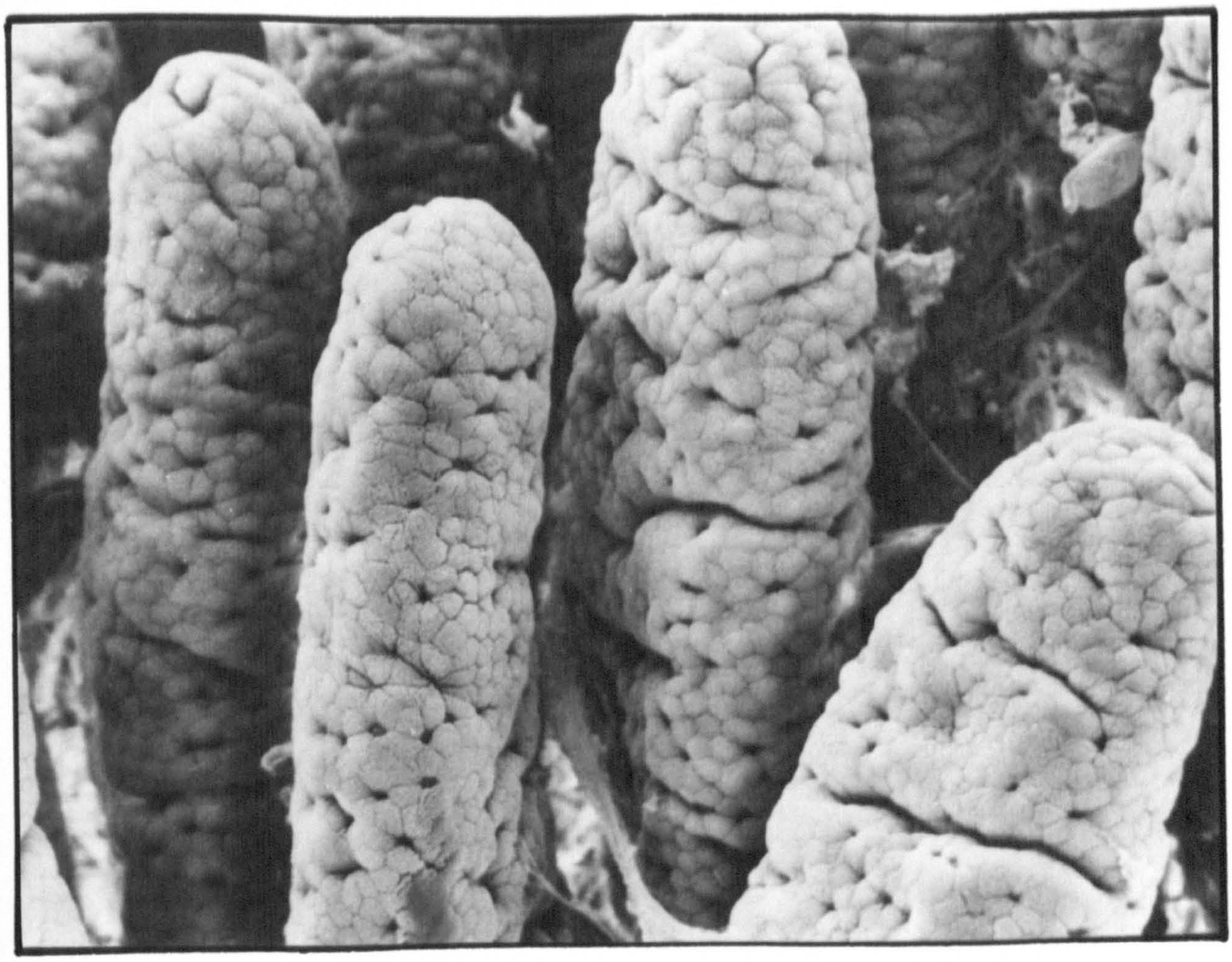

Fig. 2.6. Villi from the small intestine of a worm-free control gerbil at magnification 1 x 320.

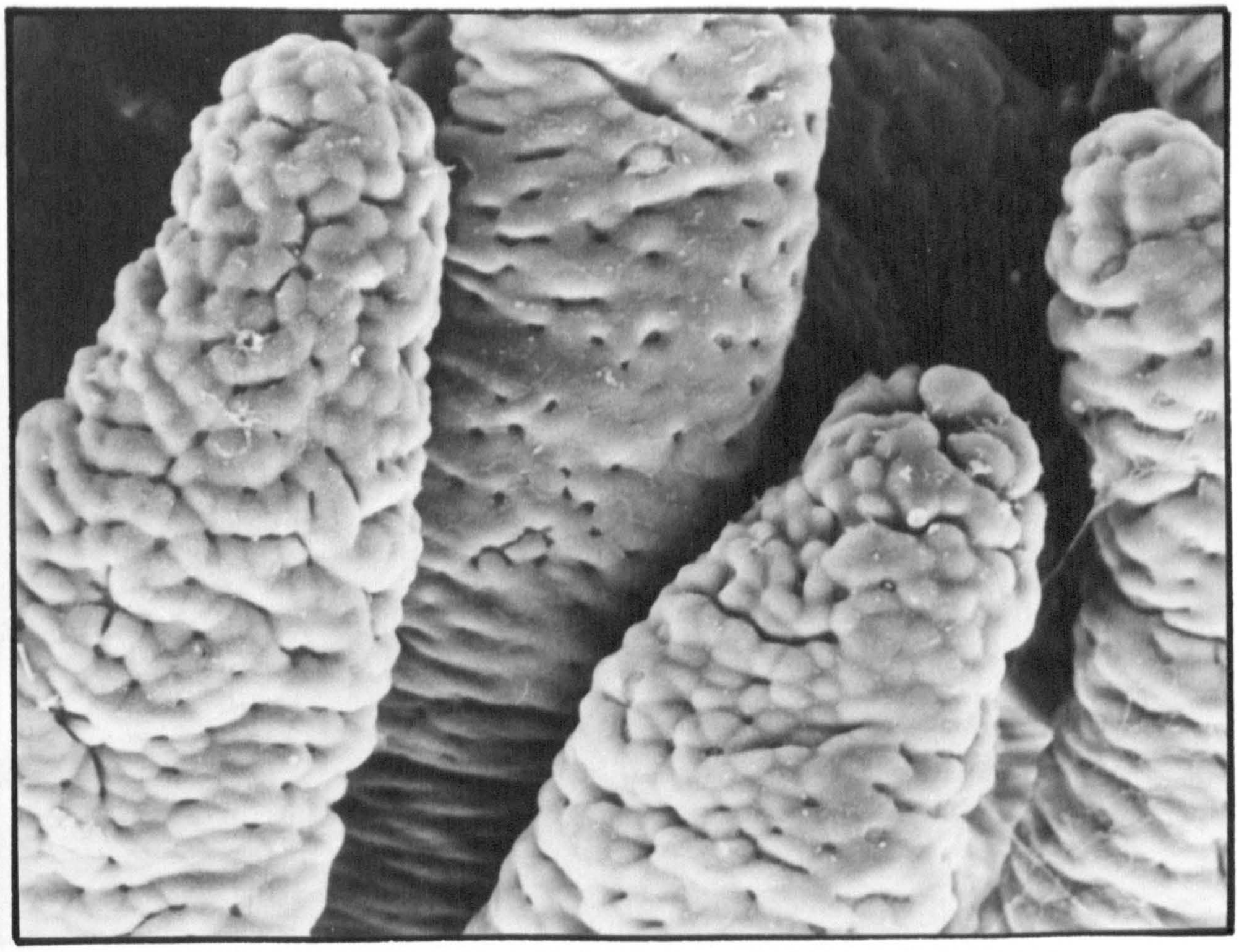

Fig. 2.7. Villi from the small intestine of a gerbil 20 days after infection with 1,500 larvae of a drug-susceptible strain of T. colubriformis. (Magnification 1 x 320)

Fig. 2.8. Villi from the small intestine of a gerbil 20 days after infection with 1,500 larvae of a drug-resistant strain of T. colubriformis. (Magnification 1 x 320)

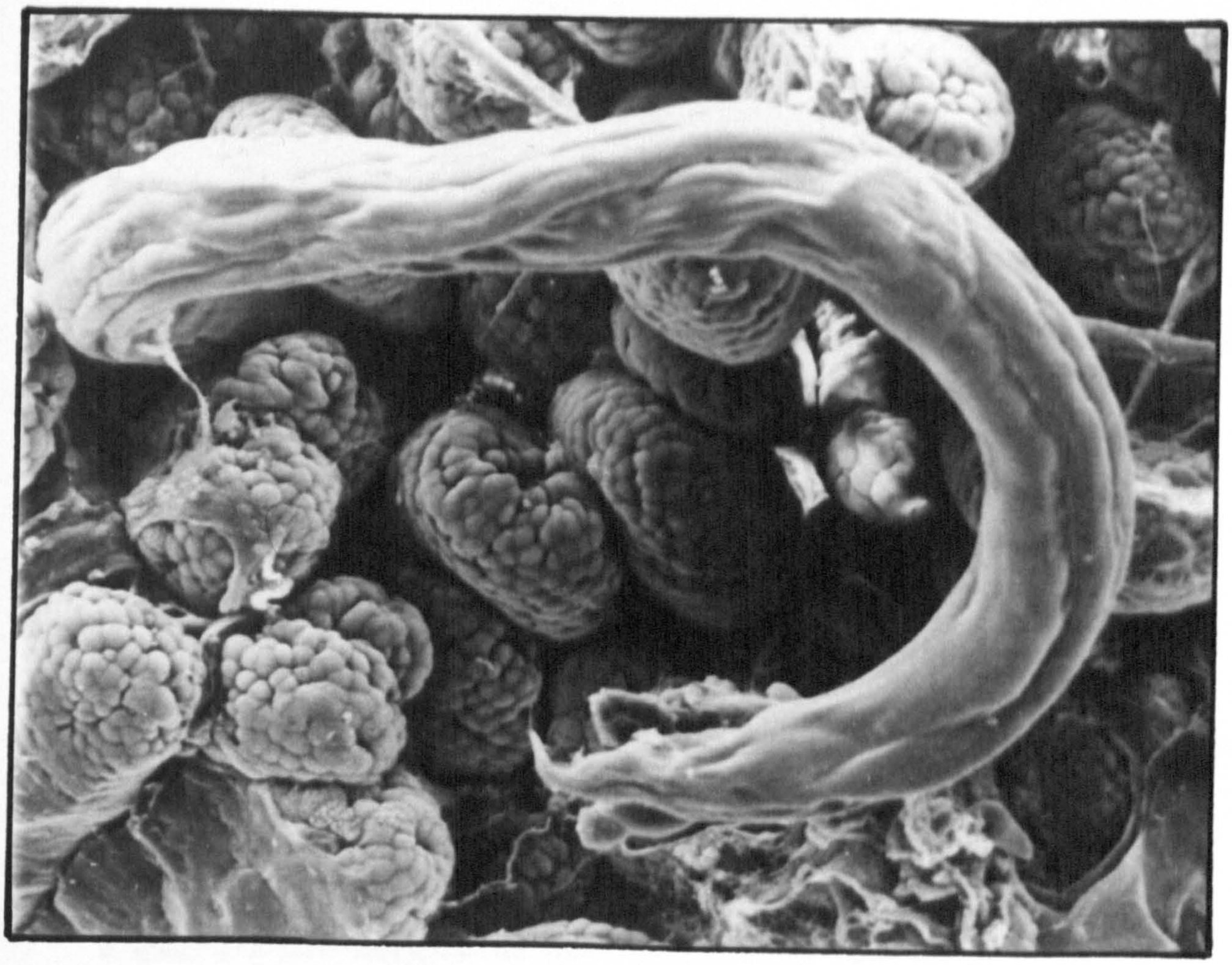

Fig. 2.9. T. colubriformis worm (drug-resistant strain) embedded in the small intestine mucosa of a gerbil 20 days after infection. (Magnification 1 x 160)

Experiment 2

Measurement of Gastrointestinal Plasma Loss in Gerbils Infected with the Drug-Susceptible or Drug-Resistant Strains of T. colubriformis

Materials and Methods

The method used to measure the loss of plasma proteins into the lumen of the intestine utilises the *in vivo* labelling of plasma proteins with ^{51}Cr-chromic chloride and the detection of the ^{51}Cr label in the faeces of the animal (Walker-Smith, Skyring and Mistilis, 1967). Chromium is not reabsorbed from the intestine and the amount of ^{51}Cr appearing in the faeces gives a quantitative measure of the ^{51}Cr-labelled plasma leaking into the intestine. An evaluation of the various methods available for quantifying gastrointestinal protein loss (Maclean, 1974) showed that the method of *in vivo* labelling described by Walker-Smith *et al*,(1967) was superior to other methods in normal circumstances. Several workers have shown good correlations between increased enteric plasma loss and increased catabolic rates of albumin in many gastrointestinal disorders.

Experimental Animals

On three successive weeks of the experiment, two gerbils were randomly selected from the group of gerbils infected with the BS strain of *T. colubriformis*, two from the gerbils infected with the BR strain and two from the worm-free controls.

Injection of ^{51}Cr-Chromic Chloride

Each gerbil was injected intraperitoneally with 50 µci of ^{51}Cr-chromic chloride solution (Amersham International, Amersham, England). At the same time 50 µci of the ^{51}Cr-chromic chloride was diluted to 100 ml in a volumetric flask. This solution, or ^{51}Cr standard, was used to ascertain the total ^{51}Cr radioactivity counts injected into the gerbils.

Housing of Gerbils

The gerbils were placed in metabolism cages which allowed urine and faeces to be separated (Fig. 2.10). Each metabolism cage contained two gerbils from each of the three groups of animals.

Calculation of Plasma Loss into the Intestine

The total weight of faeces voided by each pair of gerbils each day was recorded. Any faecal pellets which had become contaminated with (radioactive) urine were then discarded. The urine-free faeces was then reweighed and transferred to a vial for ^{51}Cr radioactivity assay in a gamma spectrometer (Packard Instruments, Ltd.). The radioactivity of each sample in counts per minute was then adjusted to give the radioactivity of the total weight of faeces produced by the gerbils in the 24 hour collection period.

By counting aliquots of the ^{51}Cr standard the total injected radioactivity was calculated. The total radioactivity (^{51}Cr counts/minute) in the daily faecal output of each pair of gerbils was expressed as a percentage of the total injected radioactivity. The cumulative radioactivity appearing in the 2nd, 3rd

Fig. 2.10. Gerbil metabolism cages.

and 4th days after injection was ascertained. These values were directly proportional to the quantity of plasma protein leaking into the gut during the time of collection. Gastrointestinal protein leak was measured in pairs of gerbils from the three groups of animals in the following periods after injection, 29 - 31 days, 36 - 38 days and 43 - 45 days.

Results

Gastrointestinal Plasma Loss in Gerbils Following Infection with Drug-Susceptible or Drug-Resistant Strains of T. colubriformis

The cumulative ^{51}Cr radioactivity expressed as a percentage of the injected ^{51}Cr between 29 and 31 DAI, 36 and 38 DAI and 43 and 45 DAI in both groups of infected gerbils and in pairs of control gerbils is shown in Fig. 2.11.

There was a significant leakage of plasma into the intestinal tract of the infected animals compared with the worm-free control animals. In the period 29 - 31 DAI, the loss of plasma into the gut in the gerbils of both infected groups was approximately three times greater than in the control animals. From 36 - 38 DAI the gerbils from the group infected with the BR strain of T. colubriformis showed a gastrointestinal plasma leak of almost three times that observed in the controls, while the gerbils infected with the BS strain of T. colubriformis showed a loss of four times the control value. In the remaining period studied, 43 - 45 DAI, the gastrointestinal plasma loss in the gerbils infected with the BR strain

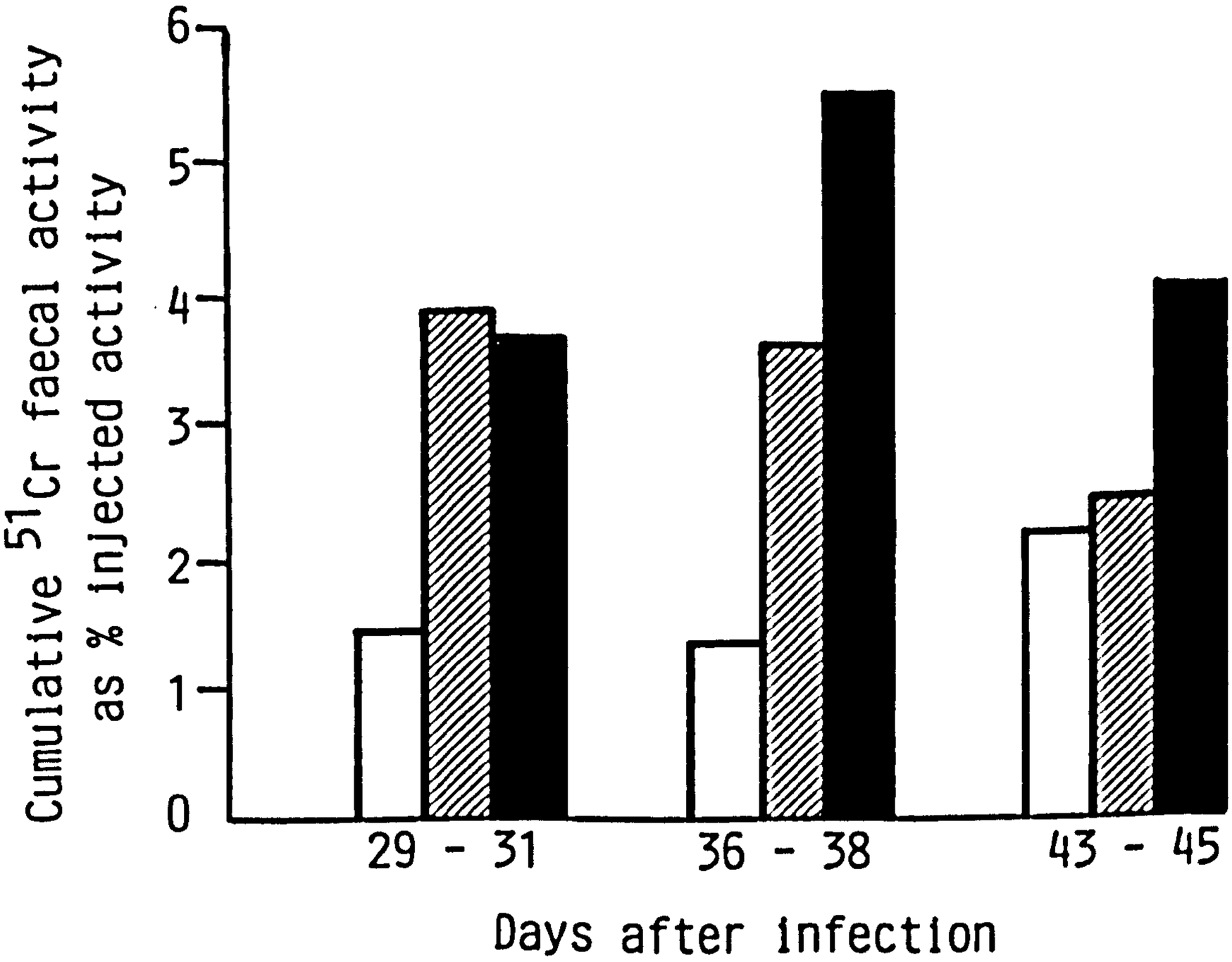

Fig. 2.11. Cumulative faecal ^{51}Cr radioactivity (% injected radioactivity) in gerbils following infection with 1,500 drug-susceptible or drug-resistant strain of T. colubriformis and in worm-free controls.

□ = control gerbils
▨ = BR strain infected
■ = BS strain infected

of T. colubriformis was only slightly greater than in the control animals whereas the loss in the animals infected with the BS strain of parasite was almost two times that of the control animals.

Discussion

The results presented in this chapter suggest that the benzimidazole-susceptible strain of T. colubriformis was more pathogenic than the benzimidazole-resistant strain.

By 27 DAI 46% of the gerbils infected with the BS strain of the parasite had died compared with 29% in the animals infected with the BR strain of T. colubriformis. It is noteworthy that Prichard, Kelly and Thompson (1978) found that 32% of sheep infected with a mixed culture of benzimidazole-susceptible H. contortus and T. colubriformis died within 6 weeks of infection, whereas no deaths occurred in a group of sheep given the same number of drug-resistant larvae, 5,000 H. contortus and 30,000 T. colubriformis, nor did the sheep in the latter group show clinical signs of disease, while surviving sheep in the former group were clearly showing signs of disease.

Compared with the weights of uninfected control animals, the weights of gerbils in both infected groups were depressed. This was so between days 14 and 42 after infection in the gerbils infected with drug-susceptible parasites, and between 14 and 49 days after infection in the gerbils infected with the drug-resistant T. colubriformis.

Worm burdens in the gerbils infected with the BS strain of T. colubriformis were much higher than in the BR strain of the parasite on day 20 after infection (662 ± 34 compared to 339 ± 47). Worm burdens recorded in the former group of gerbils were also higher than in the group infected with drug-resistant T. colubriformis on days 30 and 40 after infection but at 50 DAI the worm numbers were approximately equal. At 20 DAI and 30 DAI the differences in worm burdens between the two groups of gerbils was statistically significant. These results showed a higher establishment of the parasites in the drug-susceptible strain.

The higher worm burdens in the gerbils infected with the BS strain of T. colubriformis was reflected in higher faecal egg output than in the infections with the BR strain of T. colubriformis, from patency until 30 DAI. However between 38 DAI and 50 DAI parasite egg output fell sharply in the former group of gerbils, while remaining relatively high in the animals infected with the BR strain of T. colubriformis. Higher parasite egg output in the 7 weeks following infection was another indication of the greater pathogenicity of the BS strain of T. colubriformis.

A moderate plasma hypoproteinaemia and hypoalbuminaemia was observed in both groups of infected animals. Compared with the control gerbils, elevated plasma leak into the intestinal tract was observed during three experimental periods between 29 DAI and 45 DAI. It is highly probably that the loss of plasma into the gut as a result of the presence of the parasites in the intestine is responsible for the hypoproteinaemia observed. There was further

evidence that the BS strain of T. colubriformis was more pathogenic than the BR strain in that the gastrointestinal plasma leak recorded in the former group of gerbils between 36 DAI and 43 DAI was greater than in the gerbils infected with drug-resistant parasites.

Barker (1973a) showed that in T. colubriformis infected sheep, there was a gastrointestinal plasma loss which coincided with a plasma hypoproteinaemia. Elevated gastrointestinal loss in T. colubriformis infected sheep was also shown by Steel et al (1980) and by Poppi et al (1981).

Some of the changes occurring in the intestinal mucosa of the gerbils as a result of the presence of the parasites were illustrated in the scanning electron micrographs of sections of intestinal mucosa. Villus atrophy and flattening of the mucosa were observed in tissues taken from gerbils infected with both strains of T. colubriformis. Similar findings were reported by Barker (1973b) who examined, by scanning electron microscope, the duodenal mucosa of lambs following infection with 85,000 to 140,000 T. colubriformis. The heavily infected areas of the intestines of the lambs showed abnormalities ranging from shortening of the villi to total villus atrophy and the mucosa showed irregular masses and ridges. Barker (1973b) also observed that the nematodes usually lay in tunnels across the endothelium with mid-body or anterior or posterior ends protruding into the lumen. A similar picture was observed in the present study in which a worm was clearly seen protruding from a tunnel between the villi. In considering the

possible causes of villus atrophy in T. colubriformis infections Barker (1973b) suggested that the location and degree of gastro-intestinal plasma loss may be related to the extent of villus atrophy.

Coop and Angus (1975) have also reported extensive flattening of the mucosa and villus atrophy in sheep infected with T. colubriformis. These workers noted that the altered morphology of the intestine in T. colubriformis infections was similar to that observed by other workers in coeliac disease in man and in N. brasiliensis infections in the rat. The pathological effects of T. colubriformis infection on the small intestine mucosa of young calves has also been investigated. Shayo and Benz (1979) showed that villus atrophy and necrosis and erosions of the surface of the epithelial cells in the lamina propria were a consequence of T. colubriformis infection in the calves and also that the changes in the mucosa were related to the severity of infection rather than the duration. Results of electron microscope scanning of intestines of rats infected with N. brasiliensis also showed atrophy of the villi of the small intestinal epithelium (Martin 1980).

In conclusion the results presented in this chapter showed that the drug-susceptible strain of T. colubriformis used in the infections was more pathogenic than the drug-resistant strain and these findings were similar to observations previously reported by other workers investigating drug-susceptible and drug-resistant strains of mixed T. colubriformis and H. contortus in sheep (Prichard, et al, 1978). The findings of this study however are in contrast to investigations of comparative infections and pathogenicity of

H. contortus in sheep where the drug-resistant strains of parasite were found to be more pathogenic (Drudge et al, 1957; Kelly et al, 1978).

Finally the results of the experiments conducted in Chapter 2 showed that the pathology of T. colubriformis infections in the gerbil was similar, in many aspects, to T. colubriformis infections in sheep. The latter findings support those of Chapter 1, which indicated that the Mongolian gerbil is a suitable laboratory small animal host for the study of T. colubriformis infections. In particular T. colubriformis infections in the gerbil should provide a suitable model for the study of anthelminthic resistance.

CHAPTER 3

Local and Systemic Antibody Responses in Gerbils Following Vaccination with Irradiated or Non-irradiated *Trichostrongylus colubriformis* Larvae and after Challenge with Non-irradiated Larvae

Introduction

The experiments described in Section I of this study showed that nematode-specific coproantibodies could be detected in faecal extracts of rats following infection with N. brasiliensis and that changes in coproantibody levels during an infection reflected changes in antibody levels in both small and large intestinal contents and in small intestine mucosal extracts. In particular, it was shown that levels of faecal haemagglutinating antibodies against N. brasiliensis ESP antigens showed a positive correlation with worm burdens following vaccination and also with the degrees of resistance to reinfection.

The findings of Chapters 1 and 2 (Section II) demonstrated that the Mongolian gerbil was a suitable host for the study of T. colubriformis infections. Some of the techniques used to study local immune responses in Section I were employed in the experiment to be described in this chapter to study local immunity in gerbils infected with T. colubriformis. The experiment was designed essentially to examine local and systemic antibody responses in gerbils following both vaccination with irradiated T. colubriformis larvae and after challenge with non-irradiated larvae.

Materials and Methods

Experimental Design

Ten groups of worm-free gerbils designated as groups A, B, C, D, E, F, G and H were used. The first 6 groups (A - F) containing 10 female gerbils were vaccinated with 1,500 T. colubriformis third

Table 3.1

Experimental Design

Group	Krad	Day 0 Vaccination	25 DAV *	26 DAV Challenge (normal larvae)	24 DAC †
A (10)	0	1500 L_3	Kill (5)	1500 L_3	Kill (5)
B (10)	20	1500 L_3	Kill (5)	1500 L_3	Kill (5)
C (10)	40	1500 L_3	Kill (5)	1500 L_3	Kill (5)
D (10)	60	1500 L_3	Kill (5)	1500 L_3	Kill (5)
E (10)	80	1500 L_3	Kill (5)	1500 L_3	Kill (5)
F (10)	120	1500 L_3	Kill (5)	1500 L_3	Kill (5)
G (5)	-	-	-	1500 L_3	Kill (5)
H (5)	-	-	-	1500 L_3	Kill (5)
I (5)	-	-	-	-	Kill (5)
J (5)	-	-	-	-	Kill (5)

*DAV - Days after vaccination

†DAC - Days after challenge

Faecal samples were collected three times per week for the first 42 days after vaccination and then daily till the end of the experiment.

stage larvae irradiated at 0, 20, 40, 60. 80 and 120 krad respectively. On day 25 after vaccination five gerbils from each group were randomly selected and necropsied. Worm burdens were estimated, serum and mucosal samples and large and small intestinal fluids collected and processed as previously described. On day 26 after vaccination the remaining vaccinated animals, along with a challenge control group (Group G) were challenged with 1,500 third stage non-irradiated infective T. colubriformis larvae. Fifty days after vaccination the remaining gerbils were killed and samples prepared. The remaining group, Group H, acted as normal non-vaccinated non-challenged controls.

The weights of the animals were measured at weekly intervals. Faecal samples for T. colubriformis egg counts and coproantibody analysis were collected thrice weekly (see Table 3.1).

Experimental Animals

Eighty 6 - 8 week old female gerbils were housed in wire-floored cages. Faecal samples from each group were collected and examined for any prior helminth infection. Following the finding of oxyurid eggs in some faecal samples, all the animals were treated orally with 3 mg/kg body weight of Fenbendazole suspension (Panacur, Hoechst AG, Germany). On the day of treatment all the animals were transferred to new cages to avoid further infection. This treatment was repeated five days later at which time they were again moved to new cages as a precautionary measure and left for one week prior to vaccination. At the time of vaccination, faecal samples

collected from all groups were negative for any helminth eggs or larvae. Before vaccination the gerbils were divided into ten groups and weighed.

Infective Materials

Normal third stage infective larvae of T. colubriformis used in this experiment were maintained by regular passage through parasite-free lambs and were kindly provided by Dr. R.L. Coop of the Moredun Institute, Edinburgh, and stored in the laboratory at 4°C for two days before use. The parasites were morphologically normal and actively moving.

Irradiation

Six perspex test tubes were each filled with 10 ml water-parasite suspension (about 20,000 T. colubriformis larvae in each tube) and irradiated at 0, 20, 40, 60, 80 or 120 krad as previously described.

Infection

Larvae were counted and adjusted to 1500 larvae/0.5 ml of suspension before infection. Six groups of gerbils (10 animals per group) designated A, B, C, D, E and F were dosed orally with 1,500 T. colubriformis larvae as described in the General Materials and Methods. The larval suspension was mixed well before each administration. Ten gerbils (Group G) were used as challenge controls and Group H acted as worm-free controls.

Parasitological Techniques

T. colubriformis Faecal Egg Counts

The number of T. colubriformis eggs per gramme faeces was counted as previously described.

Trichostrongylus colubriformis Worm Counts

The following procedure was adopted:

Five animals from each group were killed at 25 days after vaccination and a further five animals 24 days after challenge. After the collection of blood, as previously described, the abdominal cavity was opened and the large and small intestines were carefully separated and placed in different labelled beakers.

The content of the small intestine of each animal was flushed into a beaker and the lumen was rinsed with PBS pH 6.0 containing 0.02% sodium azide (Sigma Chemical Company). This was then centrifuged for 10 minutes at 3,000 g. The supernatant was collected and subjected to protein extraction as described in the General Materials and Methods. The sediment was transferred to a tea strainer placed over a beaker filled with PBS pH 6.0 and immersed in a warm water bath at 37°C. After 5 hours the worms which had collected at the bottom of the beakers were carefully counted under a dissecting microscope and the number recorded as the number of worms recovered from the small intestinal contents.

The opened intestine was cut lengthwise into two equal parts on a glass plate using a sharp scalpel. One half was put in a sieve placed over a beaker containing PBS pH 6.0 and was left to stand in a water bath at 37°C for at least 5 hours. The number

of worms recovered was then multiplied by 2 to give the number of worms that lodged in the mucosa and/or on the wall of the small intestine. The remaining half of the small intestine was gently scraped with a scalpel and the tissue put in a pre-weighed universal bottle. The weight of the scraping was then ascertained and stored at -20°C until required.

The sum of the worm counts from the small intestinal fluid and the mucosa was considered as the total number of worms recovered from the small intestine.

Small Intestine and Large Intestine Contents, Small Intestine Mucosal Extracts and Faecal Extracts

The treatment of the contents of the small and large intestines and the preparation of extracts of the small intestine mucosa and of faeces was as described in the General Materials and Methods.

Preparation of Parasite Antigens

The following antigens were prepared as described in the General Materials and Methods:

(i) Trichostrongylus colubriformis larval somatic extracts (L3SE) were prepared from infective (L3) larvae. For serological tests extracts containing 2.9 mg protein per ml were used.

(ii) Adult worm somatic extract (ASE)

Preparations containing 1.6 mg protein per ml were used in serological tests.

(iii) Adult worm excretory-secretory products (ESP)

Extracts containing 2 mg of protein per ml were used in serological tests.

Table 3.2.

T. colubriformis faecal egg counts (eggs/g faeces) in gerbils following vaccination with irradiated and non-irradiated larvae and after collection

Group	DAV				DAC												
	17	20	22	25	1	3	6	8	10	13	15	17	19	21	22	23	24
A (0 krad)	4733	13800	6100	13900	10800	9200	12250	7350	6500	6500	8200	8550	16700	7700	7050	3400	850
B(20 krad)	0	900	860	13500	2350	1450	2600	1750	1700	1400	1250	2150	4200	5550	5800	3500	1500
C(40 krad)	0	0	0	50	0	0	0	0	0	0	50	500	2200	1450	1050	900	1500
D(60 krad)	0	0	266	0	0	0	0	0	0	0	0	100	1950	1500	450	650	900
E(80 krad)	0	0	0	50	0	0	0	0	0	0	0	50	1050	250	250	500	900
F(120 krad)	0	0	0	0	0	0	0	0	0	0	0	950	3150	1650	3400	2150	2400
G (CC)	0	0	0	0	0	0	0	0	0	0	0	650	7300	5450	4300	4650	3500

CC = Challenge Controls

Immunological Methods

Gel Double Diffusion and Passive Haemagglutination tests were performed as previously described.

Results

T. colubriformis Egg Counts per Gramme Faeces (epg)

The results are shown in Table 3.2.

Eggs were first detected in Groups A (4733 epg) and B (900 epg) animals on day 17 and day 20 after vaccination respectively. In Group A the faecal egg output remained generally high until day 46, i.e. 20 days after challenge. The maximum number of eggs excreted in this group before challenge was 13,900 per gramme.

Although a lower number of eggs were found in faecal samples of Group B animals, the pattern of faecal egg output was very similar to that in gerbils immunised with non-irradiated larvae (Group A). Except for the transient appearance of eggs in the faeces on day 22 after vaccination in Group D and on day 25 after vaccination in Groups C and E (50 - 266 epg), eggs were not observed in Groups C, D, E or F until about 15 - 17 days after challenge. Egg output then reached a peak (1,050 - 16,700 epg) in all groups 20 days after challenge. At the end of the experiment, faecal samples from all the groups were positive for T. colubriformis eggs, the highest count being recorded in the challenge controls and gerbils vaccinated with larvae attenuated at 120 krad (about 2,400 - 3,500 epg).

Table 3.3

Recovery of T. colubriformis worms (mean for 5 gerbils ± S.E.) from gerbils following vaccination with 1500 irradiated larvae and after challenge with 1500 non-irradiated larvae

Group	Radiation Dose krad	Vaccination Phase No. of worms recovered 25 DAV				Challenge Phase No. of worms recovered 24 DAC			
		Mucosal $\bar{x}$	S.I.C.* $\bar{x}$	Total $\bar{x}$ ± S.E.	% Establishment	Mucosal $\bar{x}$	S.I.C.* $\bar{x}$	Total $\bar{x}$ ± S.E.	Estimated % Establishment from Challenge infection
A	0	319.0	20.0	339±35	22.6	497	4	501±154	10.8
B	20	266.0	16.0	282±33	18.8	356	36	392±128	7.4
C	40	30.0	1.5	33±14	2.1	123	10	133±20	6.8
D	60	87.0	2.0	89± 5	5.9	70	6	76±25	-
E	80	12.5	0.5	13± 7	0.9	100	5	105±35	6.1
F	120	2.5	0.5	3± 1	0.2	136	4	142±37	9.4
G	-					277	6	233±22	15.5

* SIC = small intestine contents

DAV = Days after vaccination

DAC = Days after challenge

In the challenge control animals (Group G) eggs were first observed after the same period of exposure as in the gerbils vaccinated with non-irradiated larvae (Group A), i.e. 17 days post-infection but the egg counts did not reach as high a level as in Group A before challenge.

Worm Burdens

The total number of worms recovered 25 days after vaccination and 24 days post-challenge are shown in Table 3.3. Worm establishment during the immunisation phase was inversely proportional to the radiation dose applied to attenuate the larvae, with the exception of Group D, in which the vaccinating larvae were irradiated at 60 krad ($r = -0.843$, $P < 0.001$). Thus 22.6% of the vaccine worms were established in Group A (vaccinated with non-irradiated larvae) whilst the lowest number (0.2%) of parasites was recovered from Group F (vaccinated with larvae irradiated at 120 krad). The worm counts after challenge infection also showed that gerbils in Group A harboured the highest number of worms.

The number of worms due to the challenge infection in each group 24 days after challenge was estimated by subtracting the mean of the number of worms found in the five gerbils necropsied 25 days after vaccination from the mean number of worms recovered in the five gerbils necropsied, in corresponding groups, 24 days after challenge. It is appreciated that it was impossible to obtain a direct count of the worms originating from the challenge infection and the number which remained from the vaccinating infection.

Worm counts 24 days after challenge showed that gerbils in Group A harboured the highest estimated number of challenge infection worms(10.8% of challenge dose). No clear pattern of protection against challenge could be ascertained from the worm counts 24 days after challenge since it was not possible to count the worms remaining from the vaccinating dose. Groups D and E appeared to have the highest levels of protection and this correlated with the relatively low counts of T. colubriformis eggs in the faeces of Groups D and E at the same time.

As shown in Table 3.3 a greater number (88 - 99%) of worms were recovered from the mucosa than from the small intestinal contents on both necropsy days.

The mean percentage worm establishment in the challenge control gerbils was about 30% lower than that found in Group A gerbils infected with the same number of non-irradiated larvae and necropsied after the same period of expsosure following a primary infection.

Gerbil Body Weight Changes

Figure 3.1 shows the changes in the body weights of the gerbils recorded during the course of the study. Initially, the mean body weights of all vaccinated groups were similar and remained statistically unchanged until day 8 after vaccination. There was then a loss of body weight in Group A and B and this loss was statistically significant compared to Groups C - F on day 15 after vaccination and this difference increased by day 25 after vaccination.

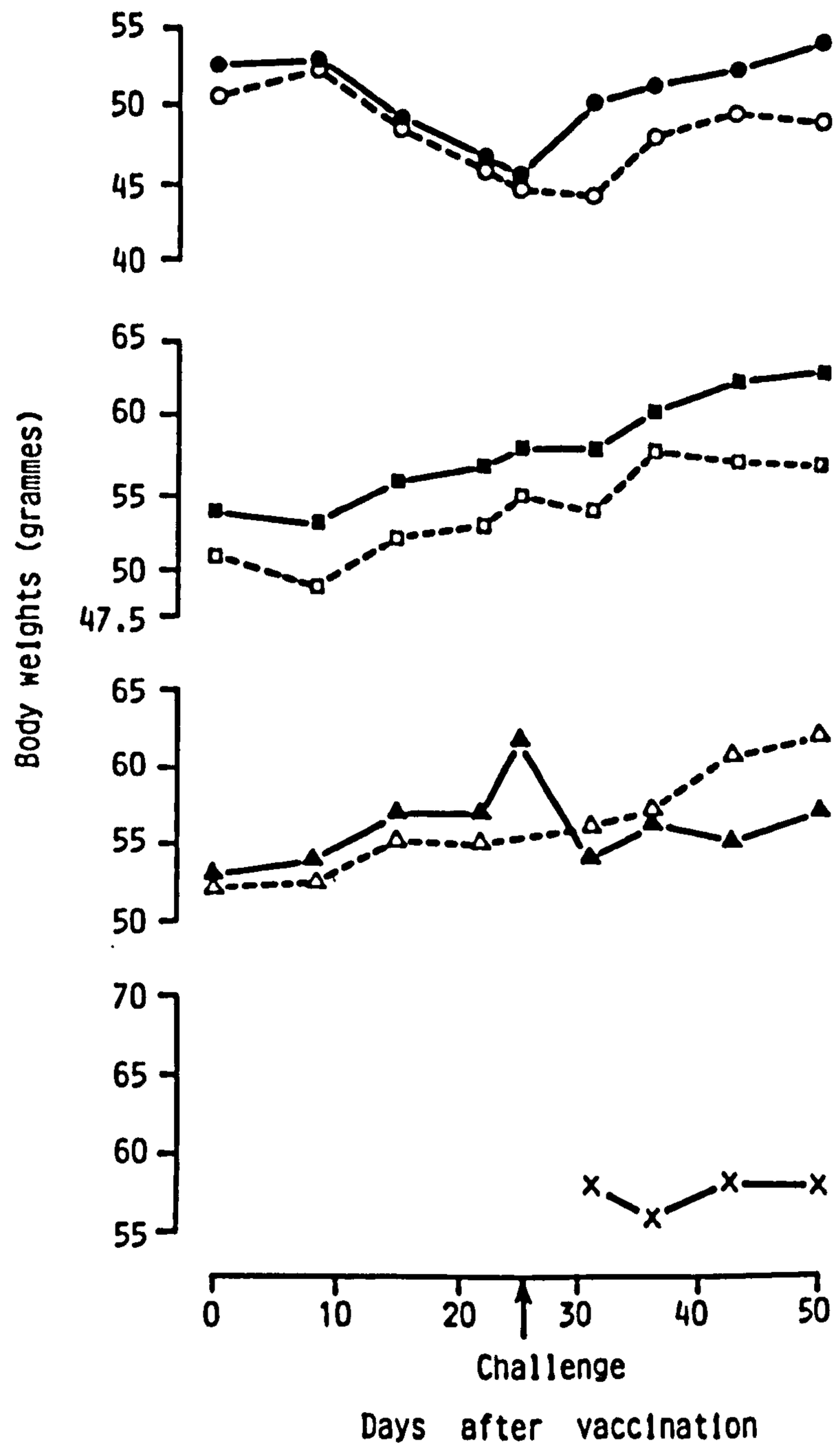

Fig. 3.1. Gerbil body weights (mean of 5) following vaccination with irradiated or non-irradiated larvae and after challenge with 1,500 T. colubriformis larvae.

--○--A (0 krad) —●— B (20 krad) --□- C (40 krad)
—■— D (60 krad) —▲— E (80 krad) --△- F (120 krad)
—X— G (Challenge controls)

Following challenge, gerbils in Groups A and B showed weight gains, the latter group reaching pre-vaccination weights by 24 days after challenge. After slight weight reductions following vaccination Groups C and D showed weight gains throughout the experiment and, except for a reduction in weights in Group E following challenge, a similar pattern emerged in Groups E and F. Following infection, the challenge control gerbils showed small weight losses.

Double Diffusion Tests

Gel immunodiffusion tests showed no precipitating antibodies in small or large intestinal contents in mucosal or faecal extracts or in serum from any of the experimental animals against any of the worm antigen preparations, L3SE, ASE or ESP antigens.

Haemagglutinating Antibody Titres in Serum of Gerbils Following Vaccination and Challenge

The results are shown in Fig. 3.2.

Anti-L3SE Antibody Titres

Levels of antibodies reacting specifically with L3SE antigens were low in all groups at 25 days after vaccination and were not detectable in Group C (vaccinated with larvae at 40 krad). Anti-L3SE titres showed a significant positive correlation with numbers of worms established following vaccination ($r = 0.750$, $P < 0.01$). Anti-L3SE antibodies showed the highest levels in Group C 24 days after challenge and similar levels were recorded in Group F (vaccinated with larvae at 120 krad) after challenge.

Results for individual gerbils are shown in Appendix I, Table 1.

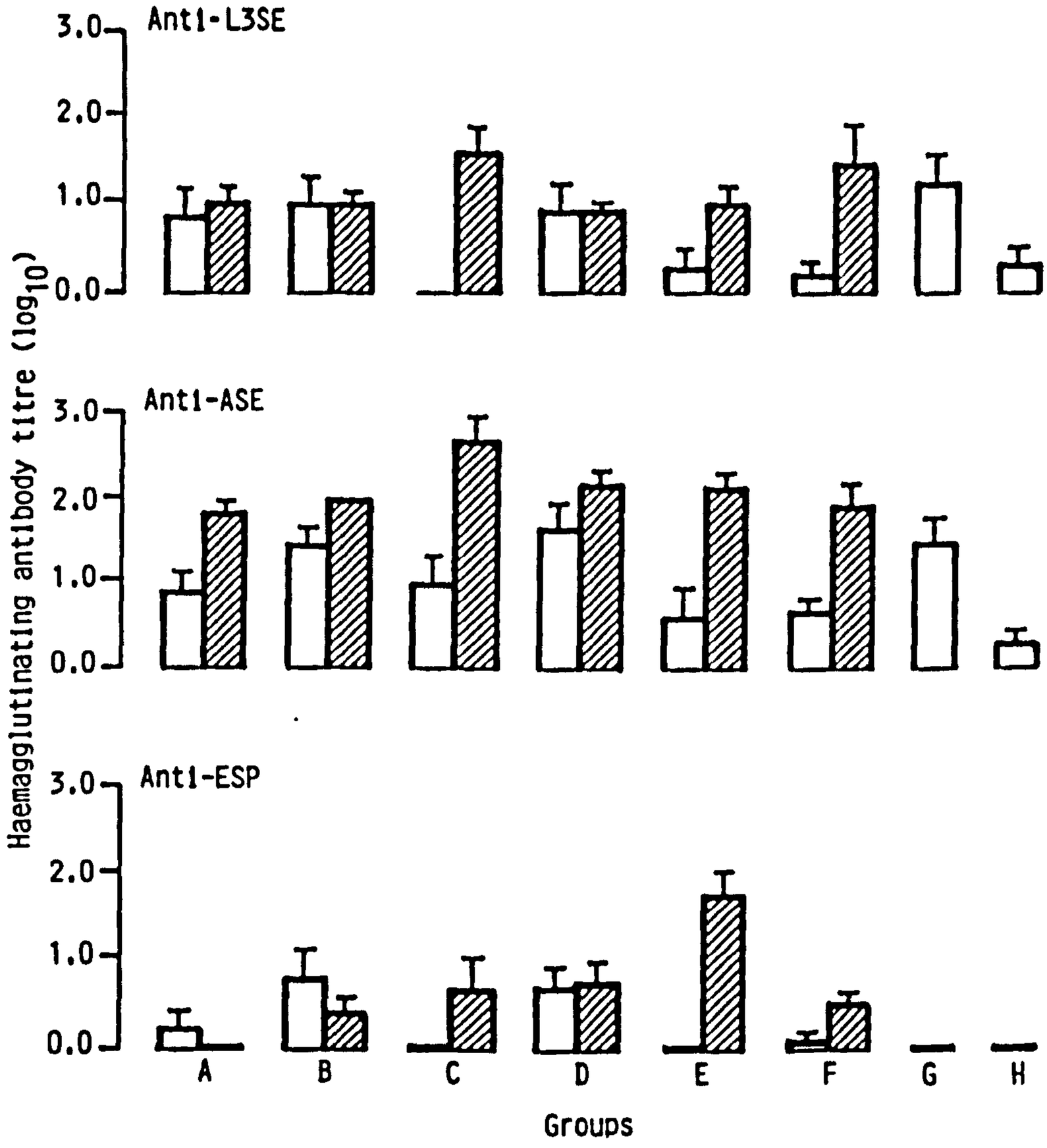

Fig. 3.2. Haemagglutinating antibody titres in serum of gerbils (mean of 5 gerbils ± S.E.) following vaccination with irradiated or non-irradiated larvae and after challenge with 1,500 T. colubriformis larvae.

□ 25 days after vaccination ▨ 24 days after challenge

Anti-ASE Antibody Titres

Serum anti-ASE antibody levels were relatively high 25 days after vaccination in all groups and markedly increased in all vaccinates 24 days after challenge. Post-vaccination anti-ASE titres were negatively correlated with the estimated post-challenge worm burdens ($r = -0.706$, $P < 0.02$).

The results for individual gerbils are shown in Appendix I, Table 2.

Anti-ESP Antibody Titres

Haemagglutinins reacting with ESP antigens were generally low in serum, following vaccination and could not be detected in Groups C and E vaccinated with larvae irradiated at 40 krad and 80 krad respectively or in the challenge control group. After challenge a marked increase in serum anti-ESP antibody level was observed only in Group E gerbils (vaccinated with larvae irradiated at 80 krad).

Results for individual gerbils are shown in Appendix I, Table 3.

Haemagglutinating Antibody Titres in Small Intestine Mucosal Extracts of Gerbils Following Vaccination and Challenge

Small intestine mucosal antibody titres against T. colubriformis L3SE, ASE and ESP antigens are shown in Figure 3.3. Results for individual gerbils are shown inAppendix I, Tables 4, 5 and 6.

In general, the anti-L3SE and anti-ASE antibody titres in the mucosal extracts were high in all groups of gerbils 25 days following vaccination compared with the titres in the worm-free

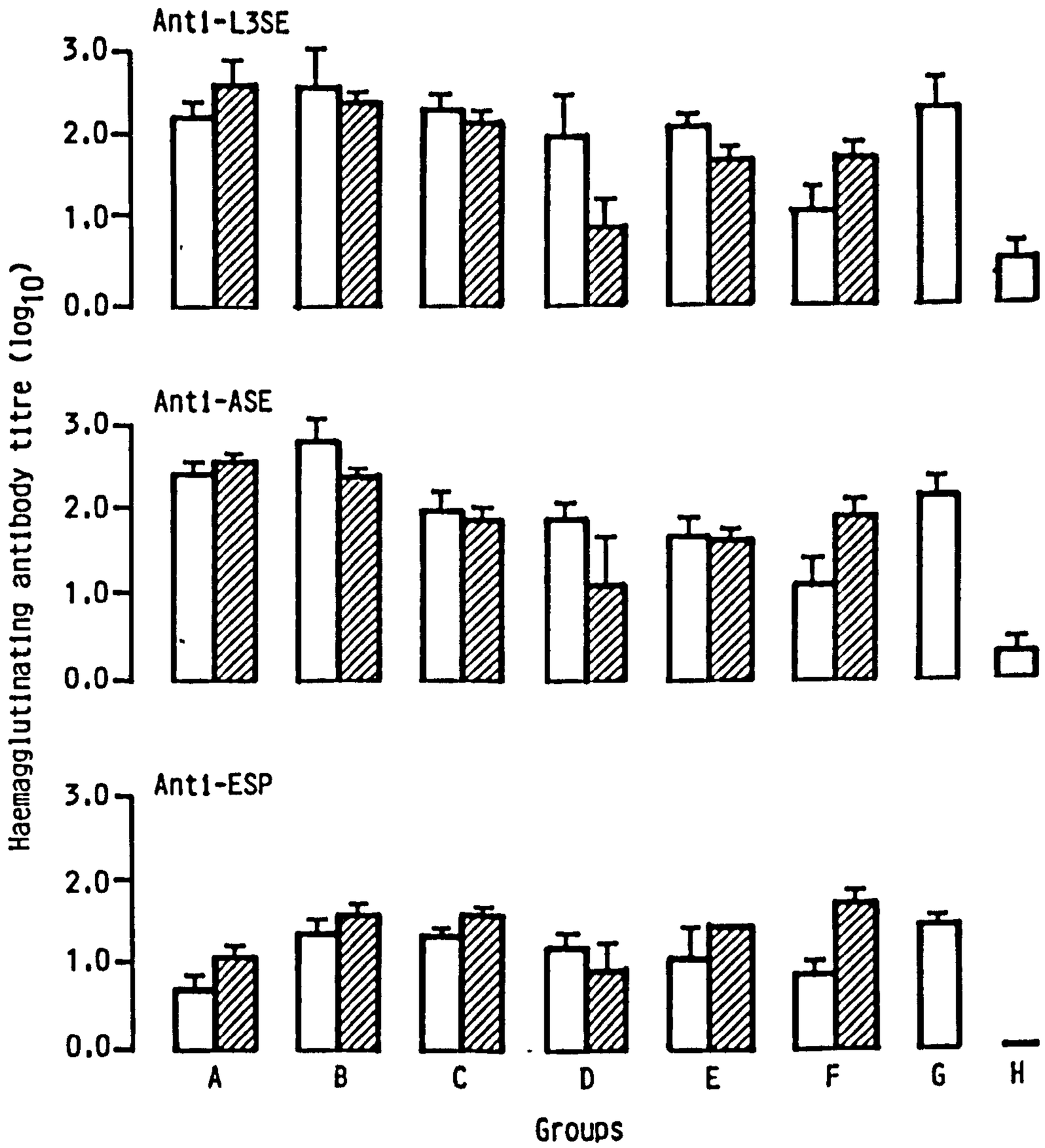

Fig. 3.3. Haemagglutinating antibody titres in small intestine mucosal extracts of gerbils (mean of 5 gerbils ± S.E.) following vaccination with irradiated or non-irradiated larvae and after challenge with 1,500 T. colubriformis larvae.

□ 25 days after vaccination ▨ 24 days after challenge

controls (Group H). The post-vaccination anti-ASE titres showed a significant positive correlation with the worm burdens 25 days after vaccination ($r = 0.842$, $P < 0.001$). It is perhaps noteworthy that Group F, vaccinated with larvae irradiated at 120 krad, had the highest estimated establishment for the challenge infection and also showed the lowest post-vaccination anti-L3SE and anti-ASE titres. Anti-L3SE and anti-ASE mucosal extract antibody titres in the challenge control gerbils, Group G, were similar to those recorded in the animals vaccinated with non-irradiated larvae, Group A.

Following challenge anti-L3SE and anti-ASE titres showed little change except in Group D in which post-challenge anti-L3SE and anti-ASE titres showed a sharp decrease, which was however not statistically significant, and in Group F where both anti-L3SE and anti-ASE titres showed an increase. In the latter case the increase was statistically significant.

Anti-ESP mucosal antibody titres were lower than similar anti-L3SE or anti-ASE measurements. The maximum post-vaccination value (1.4 x the level in the worm-free controls) was observed in Group B gerbils (vaccinated with larvae at 20 krad). Post-challenge anti-ESP titres were increased in all experimental groups except Group D, vaccinated with larvae at 120 krad.

There was no correlation between mucosal extract haemagglutinating antibody titres and the estimated establishment of the challenge infection.

Haemagglutinating Antibody Titres in Small Intestine Contents of Gerbils Following Vaccination and Challenge

Anti-L3SE, anti-ASE and anti-ESP haemagglutinating titres in small intestine contents of all experimental groups are shown in Fig. 3.4.

Results for individual gerbils are shown in Appendix I, Tables 7, 8 and 9.

Compared with the uninfected control gerbils, Group H, anti-L3SE titres in all groups were high 25 days after vaccination. Following challenge titres remained at about the post-vaccination level or were at lower levels.

Anti-ASE titres in all experimental groups 25 days after vaccination were significantly higher than in the non-infected gerbils, Group H. After challenge titres in all groups showed an increase, particularly in Groups D and E, vaccinated with larvae at 60 and 80 krad respectively.

Anti-ESP titres in small intestine contents were remarkably similar to those observed in mucosal extracts and except for Group D showed post-challenge increases. Post-vaccination titres of anti-ESP antibodies in the small intestine contents were negatively correlated with the estimated post-challenge worm burdens ($r = -0.725$, $P < 0.02$).

Haemagglutinating Antibody Titres in Large Intestine Contents of Gerbils Following Vaccination and Challenge

The results of the haemagglutinating antibody tests on the large intestine contents of the gerbils following vaccination and challenge are shown in Fig. 3.5.

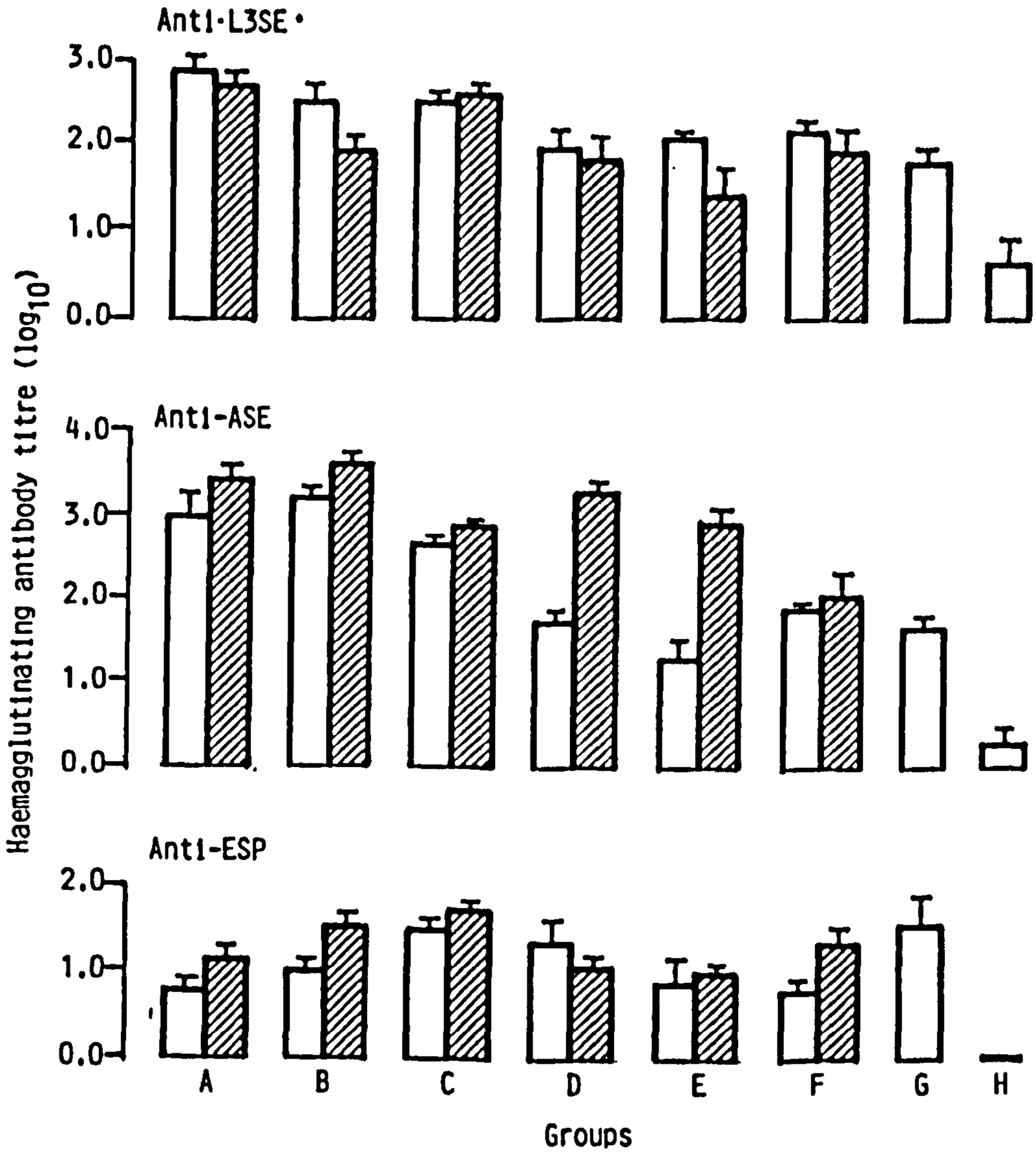

Table 3.4. Haemagglutinating antibody titres in small intestine contents of gerbils (mean of 5 gerbils ± S.E.) following vaccination with irradiated or non-irradiated larvae and after challenge with 1,500 T. colubriformis larvae

□ 25 days after vaccination ▨ 24 days after challenge

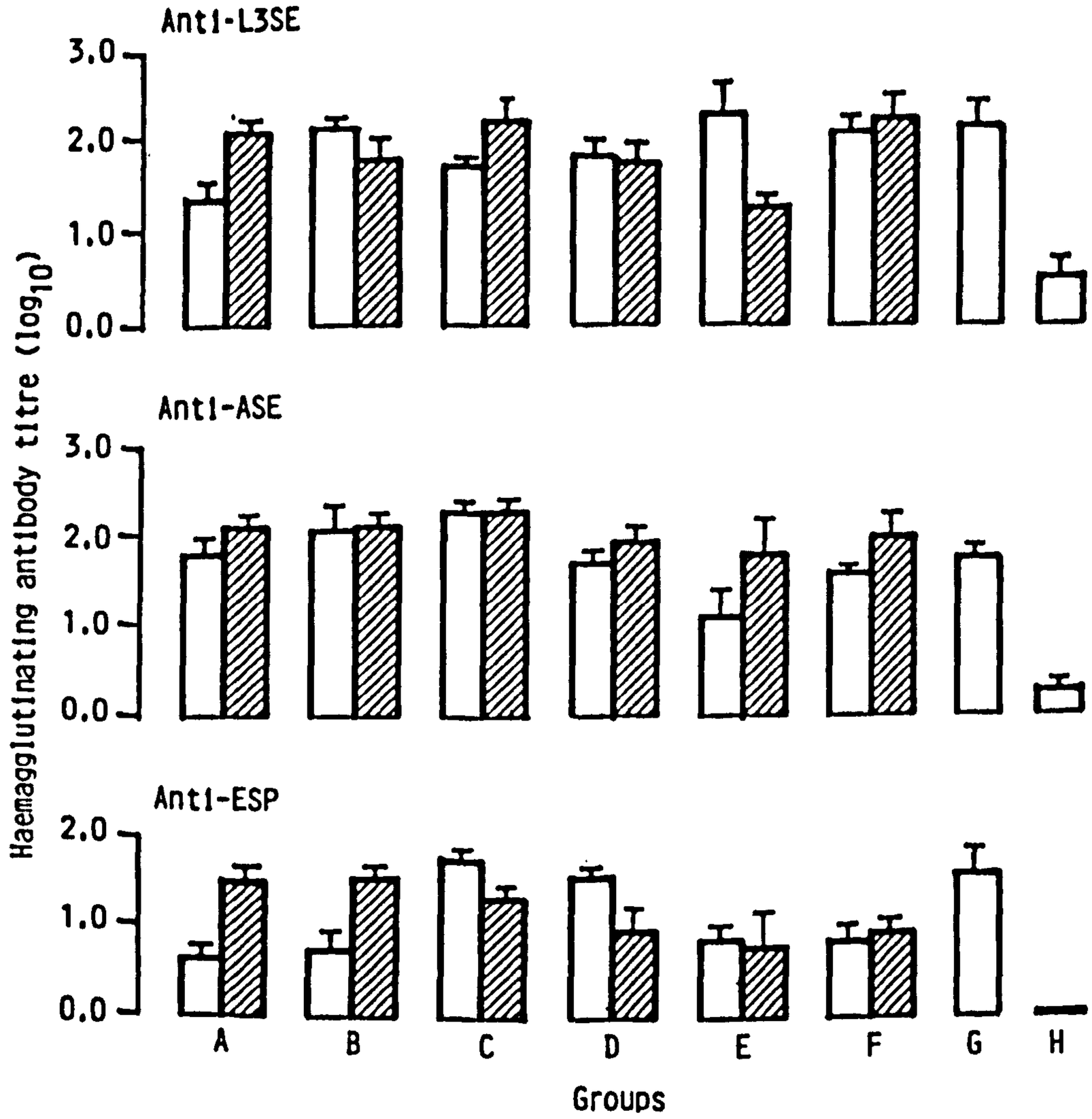

Fig. 3.5. Haemagglutinating antibody titres in large intestine contents of gerbils (mean of 5 gerbils ± S.E.) following vaccination with irradiated or non-irradiated larvae and after challenge with 1,500 T. colubriformis larvae.

□ 25 days after vaccination ▨ 24 days after challenge

Results for individual gerbils are shown in Tables 10, 11 and 12.

Except for Group A, vaccinated with non-irradiated T. colubriformis larvae, changes in anti-L3SE antibody levels were similar to those observed in small intestine contents.

In Group A large intestine contents antibody levels were lower 25 days after vaccination compared with the post-challenge levels. Changes in anti-ASE haemagglutinating levels in large intestine contents were similar to those observed in the small intestine contents following vaccination and challenge. Antibodies specific to ESP antigens showed a similar pattern after vaccination to that observed in small intestine contents. After challenge, however, antibody levels in the large intestine contents in Groups C and D were lower than the post-vaccination levels. Anti-ESP antibody titres 25 days post-vaccination were negatively correlated with post-challenge worm burdens ($r = -0.662$, $P < 0.05$).

Haemagglutinating Antibody Levels in Faecal Extracts of Gerbils Following Vaccination and Challenge

Anti-L3SE Antibody Titres

Anti-L3SE faecal antibody titres are shown in Figure 3.6. Maximum antibody titres were observed at about 15 days after vaccination and there was a significant positive correlation at this time between the titre and the number of worms recovered 25 days after vaccination ($r = 0.634$, $P < 0.05$).

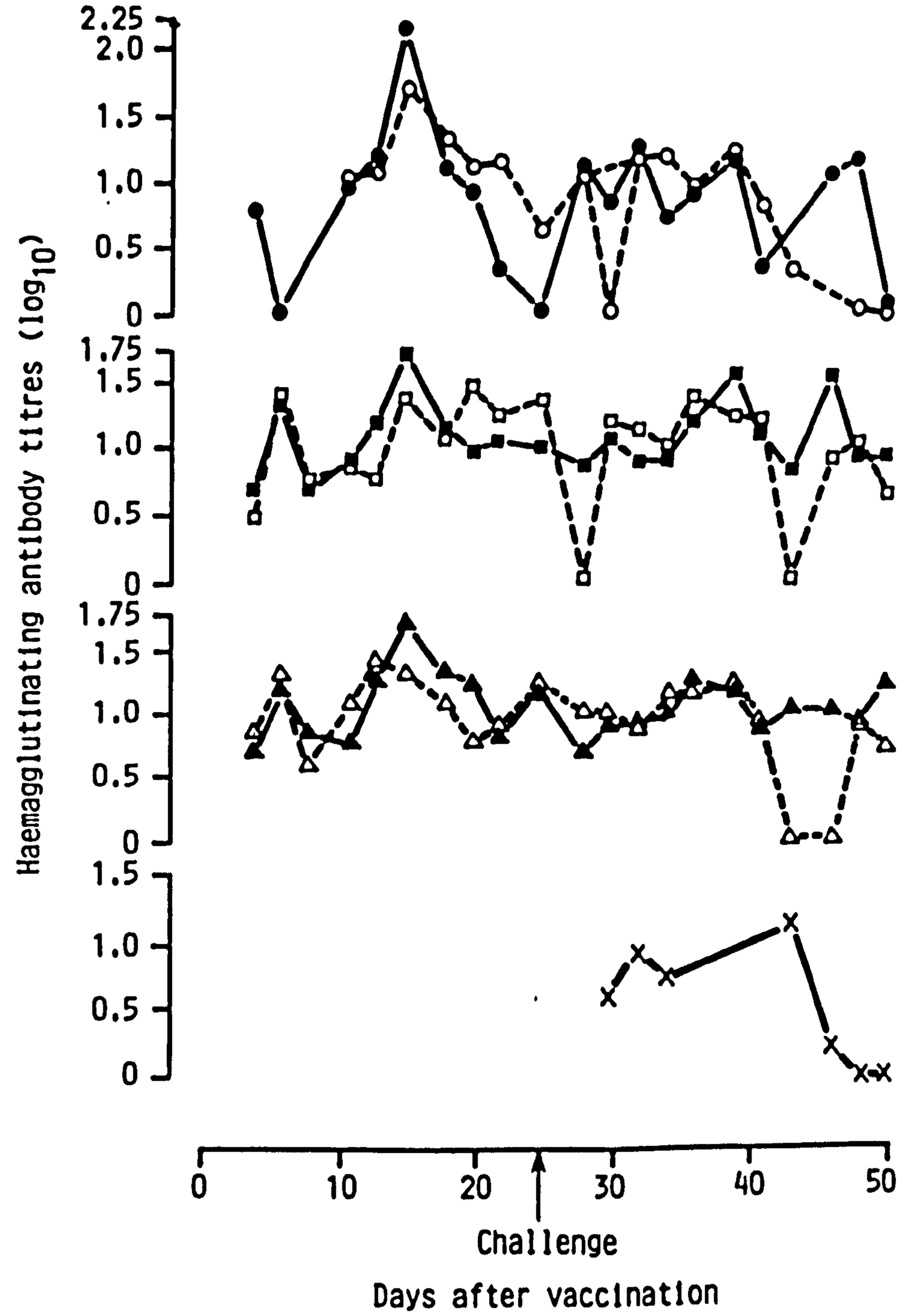

Fig. 3.6. Anti-L3SE haemagglutinating antibody titres in faecal extracts of gerbils following vaccination with irradiated or non-irradiated larvae and after challenge with 1,500 T. colubriformis larvae.

-o- A (0 krad) —●— B (20 krad) -□- C (40 krad)
—■— D (60 krad) —▲— E (80 krad) -△- F (120 krad)
—✳— G (Challenge controls)

Anti-ASE Antibody Titres

Anti-ASE faecal antibody titres are shown in Figure 3.7.

The maximum post-vaccination titres appeared between days 13 and 20 after vaccination and all groups showed moderate increases in post-challenge titres.

Anti-ESP Antibody Titres

Anti-ESP faecal antibody titres are shown in Figure 3.8.

Following vaccination there was a direct correlation between the dose of radiation used to attenuate the larvae and the time interval between vaccination and the appearance of faecal anti-ESP antibodies ($r = 0.942$, $P < 0.001$). Thus in Groups A and B, vaccinated with larvae irradiated at 0 krad and 20 krad respectively, anti-ESP antibodies were detected 6 days after vaccination. In Group C, vaccinated with larvae irradiated at 40 krad, anti-ESP antibodies appeared 11 days after vaccination and in the group vaccinated with larvae irradiated at 60 krad (Group D) anti-ESP antibodies appeared at 13 days after vaccination. In the groups vaccinated with larvae irradiated at 80 and 120 krad (Groups E and F) anti-ESP faecal antibodies were not detected until 15 days after vaccination. Maximum anti-ESP faecal antibody titres in Groups A and B were observed 18 days after vaccination and in the remaining groups, 20 days after vaccination.

There was a significant negative correlation between doses of radiation used to attenuate the vaccinating larvae and the post-vaccination anti-ESP coproantibody levels ($r = -0.843$, $P < 0.001$).

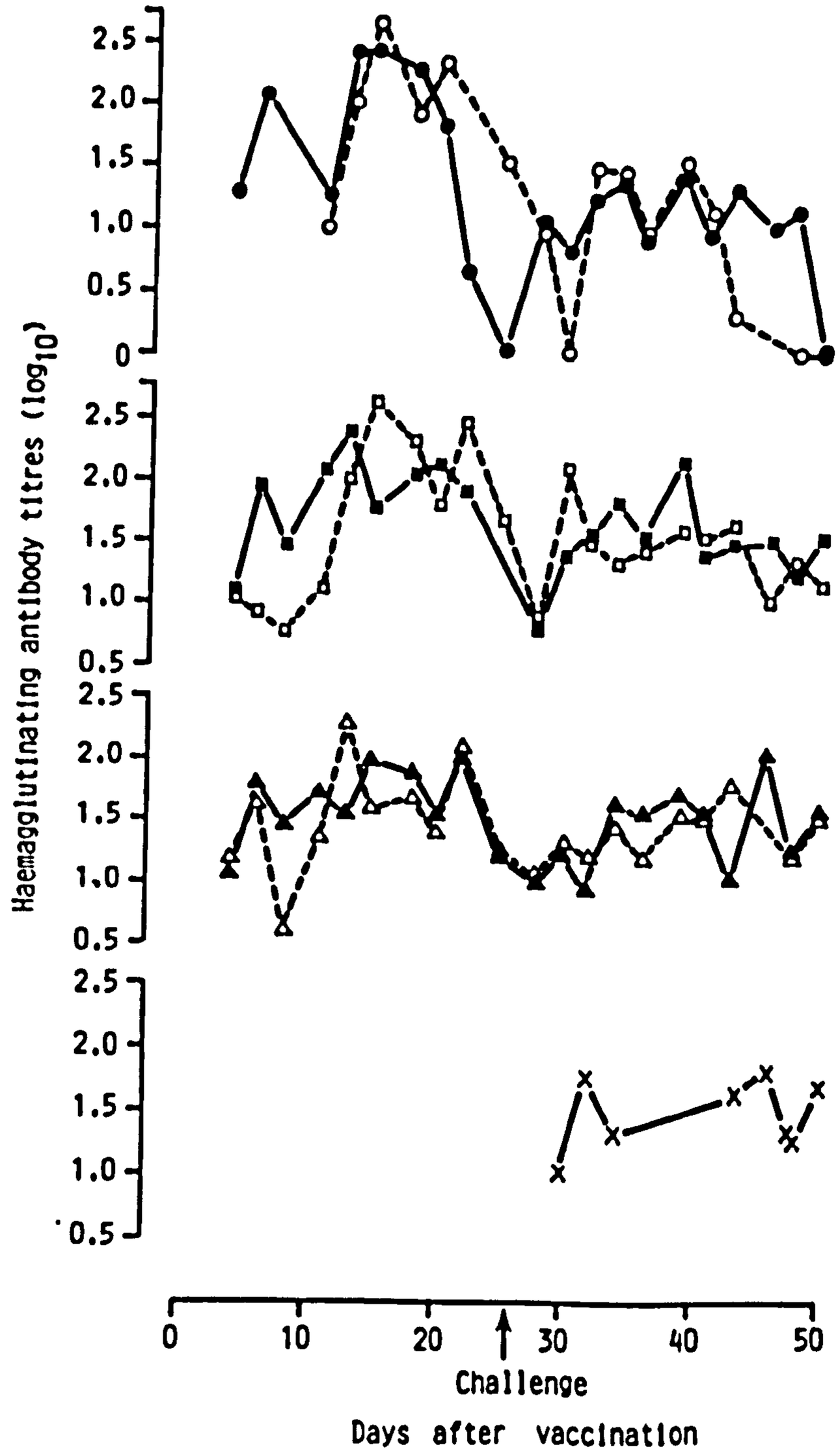

Fig. 3.7. Anti-ASE haemagglutinating antibody titres in faecal extracts of gerbils following vaccination with irradiated or non-irradiated larvae and after challenge with 1,500 T. colubriformis larvae.

-○- A (0 krad) —●— B (20 krad) -□- C (40 krad)
—■— D (60 krad) —▲— E (80 krad) -△- F (120 krad)
—✱— G (Challenge controls)

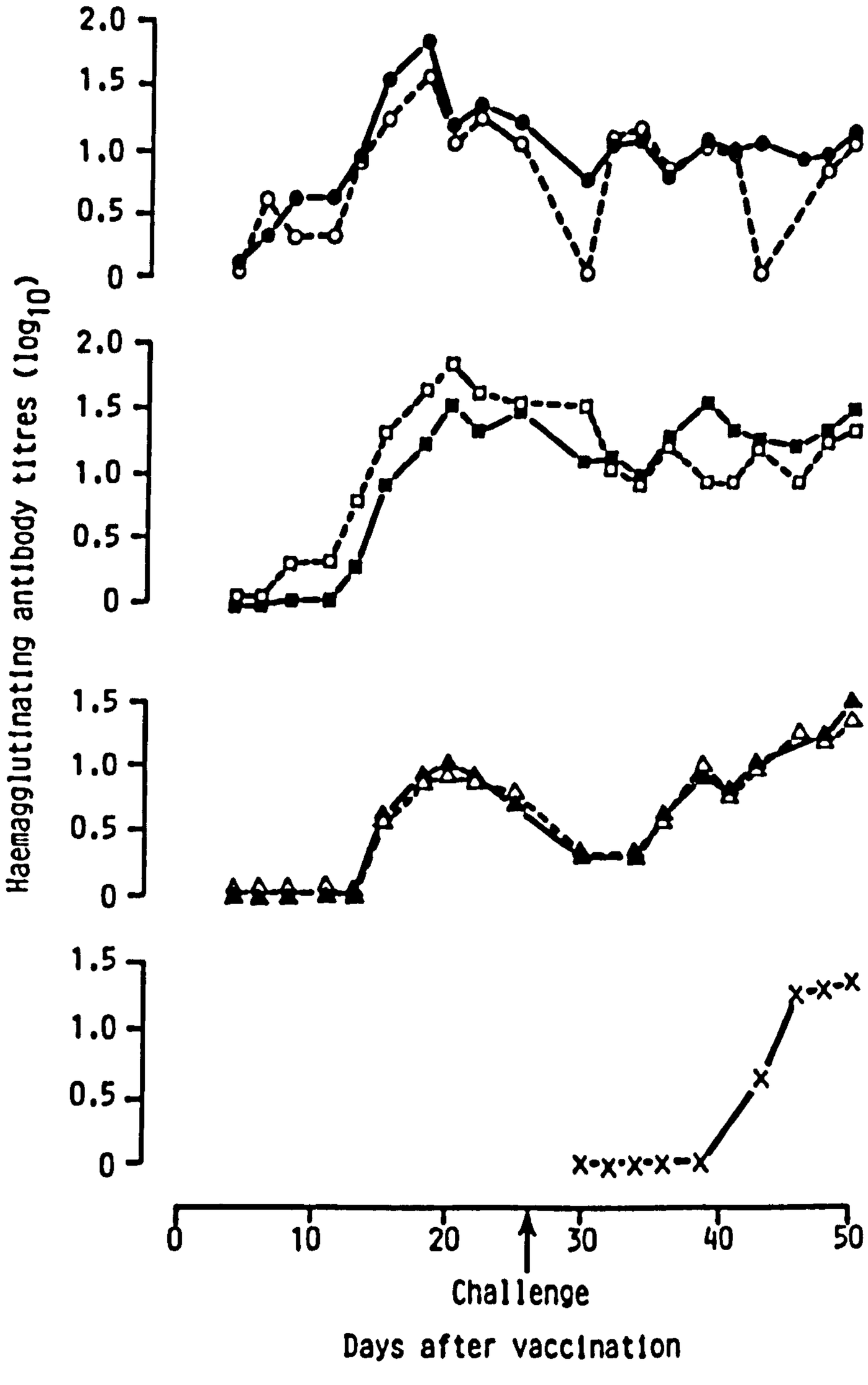

Fig. 3.8. Anti-ESP haemagglutinating antibody titres in faecal extracts of gerbils following vaccination with irradiated or non-irradiated larvae and after challenge with 1,500 T. colubriformis larvae.

-o- A (0 krad) —●— B (20 krad) -□- C (40 krad)
—■— D (60 krad) —▲— E (80 krad) —Δ- F (120 krad)
—✕— G(Challenge controls)

There was some suggestion of a correlation between peak post-vaccination faecal anti-ESP antibody titres and the percentage of worms established from the vaccination dose but this was not statistically significant ($r = 0.439$, $P > 0.1$).

In Groups E and F, anti-ESP faecal antibody titres 24 days after challenge had reached similar levels to the other groups.

In the challenge control gerbils, anti-ESP faecal antibodies were detected about the time of patency and reached similar levels to other groups 24 days after challenge.

Discussion

The results presented in this chapter showed that gerbils vaccinated with T. colubriformis larvae attenuated by gamma radiation developed degrees of resistance to reinfection. This resistance was manifest both in terms of reduced T. colubriformis eggs in the faeces of vaccinated gerbils, compared with challenge control animals, and in reductions in the estimated post-challenge worm establishment in the vaccinated gerbils. Serum, local intestinal antibody and coproantibody levels were monitored in groups of gerbils following vaccination with irradiated or non-irradiated larvae and after challenge infection. With a few important exceptions there was little evidence of correlation between haemagglutinating antibody titres and worm establishment either after vaccination or after challenge.

Worm establishment of 22.6% of the infecting larval dose 25 days after vaccination in Group A gerbils, vaccinated with 1,500 non-irradiated T. colubriformis larvae, was similar to the percentage of infecting larval doses established in gerbils in the experiments described in Chapters 1 and 2. In the former experiment a drug-susceptible strain of T. colubriformis showed a 20% establishment and in Chapter 2 the establishment of a drug-resistant strain of the parasite was 22.6%. However a higher percentage (44%) of the drug-susceptible strain of T. colubriformis established following infection in the experiment discussed in Chapter 2.

The infections in Group A gerbils became patent on the 17th day after vaccination and egg production remained high until challenge at day 25 after vaccination. Again this was similar to the pattern observed in Chapter 1 and 2 when patency occurred at 17 days after infection and egg production remained at high levels beyond the 25th day after infection.

Following vaccination, Group A gerbils showed losses in body weight. A similar pattern emerged in the experiments discussed in Chapters 1 and 2 in which T. colubriformis infected gerbils showed either reduced weight gains or weight losses compared with non-infected control animals.

The above evidence indicates that the pathogenesis of the strain of T. colubriformis used in this experiment (Chapter 3) was similar to the strains used in the previous experiments.

Worm burdens 25 days after vaccination in the groups of gerbils vaccinated with irradiated T. colubriformis larvae were significantly negatively correlated with the radiation doses (22.6% to 0.2% establishment following irradiation with 0 to 120 krad). Similar results were obtained in Section I of this study in the experiments involving rats vaccinated with irradiated N. brasiliensis.

Compared with the Group A gerbils, vaccinated with non-irradiated larvae, the effect of radiation on the vaccinating larvae was to diminish greatly faecal egg output. In Group B vaccinated with larvae irradiated at 20 krad, eggs first appeared 20 days after vaccination and only reached a similar level to Group A at 25 days after vaccination. Output of T. colubriformis eggs in the faeces of gerbils in Groups C, D and E in the pre-challenge period was very low and eggs were detected only on days 25, 22 and 25 after vaccination in Groups C, D and E respectively. Although a few worms (3 ± 1) were found in the gerbils of Group F 25 days after vaccination with larvae irradiated at 120 krad, eggs were not detected in the faeces until the challenge infection became patent.

In previous studies male T. colubriformis worms were found to be more radiosensitive than females (Gordon et al, 1960; Mulligan, Gordon, Stewart and Wagland, 1961). It is also possible that the relatively low worm populations in Groups C, D,E and F contained a high proportion of sterile female worms. These factors could account for the relatively low egg count in the faeces of Group D

gerbils, in which 5.9% of the infecting dose became established and for the total absence of T. colubriformis eggs in the faeces of Group F gerbils following vaccination.

The percentage of worms establishing in the challenge controls was about 7% lower than that observed in Group A following vaccination with non-irradiated larvae. The same dose of larvae was used in both cases and the difference in establishment may have been due to aging of the T. colubriformis larvae used in the challenge infections. The percentage of worms established after challenge was estimated by subtracting the numbers of worms found in the vaccinated groups 25 days after vaccination from the totals recovered 24 days after challenge. It should be noted that it is most probable that some of the worms from the vaccinating infections would have been lost during the challenge phase and that the percentage establishment recorded 24 days after challenge may be an underestimate of the true establishment. There was no correlation between estimated establishment of the challenge infection and irradiation doses used to attenuate the larvae in Groups B to F.

It has been shown in N. brasiliensis infections in the rat that as few as 10 adult female worms in the intestine can stimulate protective immunity (Ogilvie, 1965a). Similarly, the results presented in this present chapter indicate that a small number of T. colubriformis worms (3 ± 1) in Group F may have induced a degree of protection against establishment of a challenge infection.

There have been conflicting reports concerning the antigens involved in protection against larval challenge with T. colubriformis. Herlich (1966) showed that T. colubriformis infections terminated

2 DAI at the L3 larval stage resulted in a high degree of immunity to challenge and this immunity was not enhanced by terminating the infection after the L4 or L5 larval stages. It is therefore possible that irradiated L3 larvae which failed to reach maturity may, nevertheless, have contributed to the development of protective immunity in the present study.

Double diffusion tests for precipitating antibodies were negative in serum, large and small intestine contents, intestinal mucosal and faecal extracts against all three antigens L3SE, ASE and ESP. Several explanations can be offered for this situation. The method may not be sufficiently sensitive to detect low levels of precipitating antibody. Furthermore it is known that the quantitative relationship between antibody and antigen is critical for precipitation reactions to occur and concentrations of antibody and antigens used in the tests may not have been in the correct ratio. However agglutination tests are more sensitive than precipitation tests and anti-worm antibodies were detected in serum, small and large intestinal contents and in mucosal and faecal extracts by the passive haemagglutination test.

Haemagglutinating antibody levels detected in serum of all vaccinated groups by L3SE, ASE and ESP antigens were low and only anti-ASE antibody levels consistently showed an anamnestic response. Post-vaccination anti-L3SE serum antibody titres showed a significant positive correlation with post-vaccination worm establishment. Low antibody levels in serum of gerbils infected with T. colubriformis is not a surprising observation since T. colubriformis worms develop and

mature in the small intestine of the gerbil. It has previously been shown by several workers (reviewed in Section I) that resistance to mucosal infection can exist in the absence of demonstrable serum antibodies. Furthermore the experiments described in Section I showed that measurement of local immune responses to N. brasiliensis infections in the rat provided a good index of immunity to the nematode, where detectable systemic responses were poor.

Local intestinal haemagglutinating antibodies to L3SE, ASE and ESP antigens were detected in all groups of gerbils both after vaccination and after challenge. Post-challenge estimated worm establishment was negatively correlated with anti-ESP antibody titres in small and large intestine contents.

In studies of local immune responses to T. colubriformis in sheep Cripps and Adams (1978) found that significant levels of antibody were present in serum and intestinal lymph of immune sheep and these antibody levels increased after challenge. Most of the antibody activity in the serum was associated with IgM, whereas in the intestinal lymph the antibody was associated with IgA. These observations indicated the occurrence of a local antibody response to T. colubriformis in the intestine of immune sheep.

Adams, Merritt and Cripps (1980) also demonstrated local immune responses to T. colubriformis in sheep. These workers showed that a response to challenge with T. colubriformis produced a proliferation of IgG and IgA secreting cells in the small intestine lamina propria, although the appearance of IgA secreting cells was transient. Adams et al (1980) also found high levels of anti-L4SE

and anti-ASE haemagglutinating antibody in serum and in small intestine mucosal extracts in immune sheep before and after challenge. Antibody titres in small intestine contents were lower and the authors attributed this to the low protein content of the samples. These results are in broad agreement with the findings of the present study. However the latter authors failed to detect any significant anti-L3SE haemagglutinating antibodies in their tests.

The highest anti-L3SE faecal haemagglutinating antibody titres were observed 15 days after vaccination. There was a significant positive correlation between anti-L3SE faecal antibody levels 15 days after vaccination and the percentage establishment of the vaccinating dose of T. colubriformis. Group D, vaccinated with larvae irradiated at 60 krad showed a slightly higher titre than Group C, vaccinated with larvae irradiated at 40 krad. In all groups of vaccinated gerbils (except Group C) anti-L3SE faecal antibody titres were higher 15 days after vaccination than at 25 days after vaccination, the time when post-mortem intestinal material was collected. These observations lead to the suggestion that coproantibody measurement, at daily intervals if necessary, provides a much more sensitive index of changes in local immune responses at intestinal surfaces than similar measurement on post-mortem intestinal extracts or contents. All groups of gerbils showed small anamnestic increases in coproantibody levels detected with L3SE antigens.

Anti-ASE coproantibody levels detected by haemagglutination tests reached peak post-vaccination levels at 15 - 20 days after

vaccination. Groups of gerbils vaccinated with larvae given lower radiation doses tended to have higher titres than gerbils vaccinated with larvae given higher radiation doses. Anti-ASE coproantibody levels 15 - 20 days after vaccination were higher than on day 20 after vaccination and the coproantibody levels reflected similar measurements in intestinal contents and mucosal extracts. In all groups there were post-challenge increases in anti-ASE coproantibody titres.

Peak post-vaccination titres in faecal extracts against ESP antigens were observed on day 18 after vaccination in Groups A and B vaccinated with non-irradiated larvae and larvae irradiated at 20 krad respectively. With increasing doses of attenuating radiation anti-ESP coproantibodies appeared later after vaccination and remained at lower levels, i.e. there was a negative correlation between radiation doses used to attenuate the vaccinating larvae and anti-ESP coproantibody levels.

The results obtained in the present investigation of local immune responses in gerbils following vaccination with irradiated T. colubriformis larvae are somewhat different to the results obtained in Section I when local immune responses to N. brasiliensis in the rat were studied. In the N. brasiliensis-infected rats high anti-ESP haemagglutinating antibody titres were detected in intestinal contents and in mucosal extracts. Furthermore there was a significant positive correlation between anti-ESP haemagglutinating coproantibody titres and resistance to reinfection with N. brasiliensis in the rat.

The results of this experiment show that strong local antibody responses can be detected by the passive haemagglutinating test in gerbils following vaccination with irradiated or non-irradiated T. colubriformis larvae. In particular strong anti-L3SE and anti-ASE antibody levels were detected in small intestine mucosal extracts, small and large intestine contents and in faecal extracts. Copro-antibodies were shown to reflect antibody levels in intestinal contents and mucosal extracts. This experiment gave further support to two of the main findings of this thesis. Firstly that coproantibody measurement is a sensitive index of immunity to intestinal parasitic infections at mucosal surfaces and secondly that the Mongolian gerbil (Meriones unguiculatus) is a suitable host for the study of T. colubriformis infections. The gerbil-T. colubriformis model may also be superior to the rat-N. brasiliensis model for the study of helminth immunity since the former parasite is non-migratory and of major veterinary importance.

In retrospect the protocol adopted in this study makes interpretation of the results difficult. In particular, difficulty was experienced in estimating the level of protection against challenge because of the persistence of worms from the vaccination phase of the experiment. Treatment with an anthelmintic prior to challenge may therefore have been advisable. It was shown by Gregg, Dineen and Griffiths (1976) that two doses of attenuated larvae followed by treatment with an anthelmintic prior to challenge was a satisfactory protocol for the study of guinea pigs infected with T. colubriformis and this protocol has been extended to numerous studies

in sheep (Dineen and Windon, 1980; Dineen and Wagland, 1982; Wagland, Steel, Windon and Dineen, 1984).

In conclusion it should be stated that the experiment discussed in this chapter was essentially preliminary in nature, and local immunity to T. colubriformis in the gerbil, in general, and the role of secretory-IgA in particular in this immunity, is a subject worthy of further detailed study.

GENERAL DISCUSSION

The results reported in Chapters 1 and 2 of this section show clearly that the Mongolian gerbil (Meriones unguiculatus) is a suitable host for the laboratory study of T. colubriformis infections. The gerbil was shown to be superior to other small animals which have been used for the study of T. colubriformis in terms of both the relatively high level of establishment and in the duration of infection. Guinea pigs have previously been widely used as an experimental host for T. colubriformis. However they have two important disadvantages. Firstly guinea pigs show variable susceptibility to infection (see review by Williams and Palmer, 1964) and secondly the infections are transient in nature and terminate soon after patency (Herlich, 1958; Connan, 1966; and Rothwell and Griffiths, 1975). A further advantage of the gerbil as an experimental host was that the pathogenic effects of T. colubriformis infections in this small animal host were very similar to changes observed in sheep infected with the same parasite.

The experiment described in Chapter 2 showed that the gerbil infected with T. colubriformis provided a good model for comparing the pathogenesis of drug-susceptible and drug-resistant strains of T. colubriformis. The results obtained in this study showed that the drug-susceptible strain of T. colubriformis was more pathogenic than the drug-resistant strain. These results were in agreement with previous findings of Prichard et al (1978) who found a mixed culture of drug-susceptible T. colubriformis and H. contortus more pathogenic than a mixed culture of drug-resistant strains of the

two parasites. However other workers (Drudge et al, 1957 and Kelly et al, 1978) found that drug-resistant strains of H. contortus were more pathogenic than drug-susceptible strains of H. contortus.

A further study of the relative pathogenicity of drug-susceptible and drug-resistant strains of T. colubriformis in the gerbil might clarify the situation and eliminate (or otherwise) the possibility that differences in pathogenicity were merely reflections of a higher parasite establishment which might not be an inherent feature of the strain of parasite.

The problems of anthelmintic resistance in nematodes has been reviewed by Kelly and Hall (1979) and by Prichard et al (1980). The availability of a suitable small animal laboratory host for T. colubriformis provides an opportunity for increased research into the growing problem of the development of drug-resistant strains of T. colubriformis without incurring costs which might become prohibitive. Ostlind and Cifelli (1981) used the gerbil (Meriones unguiculatus) to test the efficacy of anthelmintics against 6 day T. colubriformis infections. Panitz and Shum (1981a) also successfully used the same experimental model to test the efficacy of anthelmintics against 23 day T. colubriformis infections. Following the results reported in Chapters 1 and 2 of Section II it is obvious that T. colubriformis infections in the Mongolian gerbil may be studied over longer periods of up to 50 days post-infection.

Methods have previously been developed, using the rat-N. brasiliensis system as a model, in which the uptake of radiolabelled substances from the tissue fluids of the host can be accurately measured (Henney, Maclean and Mulligan, 1971 and 1973). This radio-

isotope technique was used by Henney and Tewari (1973) to measure the *in vivo* uptake by adult (7 DAI) *N. brasiliensis* in rats, of ^{75}Se from intravenously injected ^{75}Se-selenomethionine following treatment with different doses of anthelmintic. Worm damage resulting from the action of the anthelmintic drug was reflected in terms of reduced uptake of the ^{75}Se label by the worms. Both time-response and dose-response of the *N. brasiliensis* worms to the anthelmintic were measured by this technique.

The above technique could probably be used to study the *in vivo* effects of anthelmintics on the metabolic activity of *T. colubriformis* worms in the gerbil. Time-response and dose-response experiments could be conducted and any successful trials could be repeated in large animal hosts if necessary. The method described by the above workers could also be adapted to study the relative uptake of radiolabelled drugs by drug-susceptible and drug-resistant strains of *T. colubriformis* in the gerbil. A pilot experiment conducted by the author suggested that the uptake from the host's tissue fluids of radioactive tracers by *T. colubriformis* worms could be measured provided the specific radioactivity of the radio-labelled drug was sufficiently high. Studies on drug-resistance in *T. colubriformis* infected gerbils could provide useful information which might lead to a greater understanding of the problems involved in the development of drug-resistant strains of helminths.

The rat-*N. brasiliensis* model has been widely used over a long period in the study of immunity to gastrointestinal parasites. This model has been used extensively primarily because the host animals

are relatively cheap to obtain and maintain, the pre-patent period is short and the parasite population is expelled dramatically by the host's immune response at about 13 DAI. N. brasiliensis however is different from the intestinal nematodes of major veterinary significance in that the parasite has a migratory phase, commencing with percutaneous penetration and then migration to the small intestine via the lungs (see Section I).

Trichostrongylus colubriformis on the other hand experiences only an intestinal phase in the host and, in this respect, is similar to other important intestinal nematodes such as H. contortus, Ostertagia spp which infect sheep and cattle and T. axei which occurs in lambs. Gerbils infected with T. colubriformis may therefore provide a better opportunity for the laboratory study of immunity to gastrointestinal parasites than the rat-N. brasiliensis model.

. The investigations described in the final chapter of this section showed clearly that some of the techniques used to detect local antibody responses to N. brasiliensis in the rat can also be applied to monitor local antibody responses to T. colubriformis in the gerbil. Local intestinal haemagglutinating antibodies were detected against T. colubriformis antigens in all groups of gerbils both after vaccination and following challenge. However one difference between the results of measurements of local antibody responses to T. colubriformis infections in the gerbil and similar tests on rats infected with N. brasiliensis was the apparently low levels of anti-ESP antibodies in the T. colubriformis infected gerbils.

Excretory-secretory products (ESP antigens) have been shown by several workers to play a significant part in the development of immunity to N. brasiliensis in the rat (Denham, 1969; Poulain et al, 1976b; Day et al, 1979; Bolla and Weinstein, 1980) and the results obtained in Section I of this study were in agreement with these findings. However because of the experimental protocol followed in the study of local antibody responses to T. colubriformis antigens in the gerbils it was not possible to determine the percentage of protection induced by the vaccinating larvae but there was, in general, no obvious relationship between local antibody responses and the estimated establishment of the challenge infection in the groups of gerbils tested. Nevertheless it is interesting to note that there were significant negative correlations between anti-ESP post-vaccination antibody titres in both small and large intestine contents and estimated post-challenge worm establishment. These findings may indicate a relationship between anti-ESP antibodies and protective immunity but further investigations are required before any firm conclusions can be made.

There is little documented information on the significant antigens responsible for immunity to T. colubriformis. The results obtained in the present study showed strong local anti-L3SE and anti-ASE antibody responses to T. colubriformis in the gerbil. Herlich (1966) demonstrated the protective capacity of the L3 larval stage of T. colubriformis in the guinea pig. However, in the present experiment, there was no obvious relationship between high anti-L3SE local antibody titres and protection as measured by examining estimated post-challenge worm burdens.

It is appreciated that the experiment discussed in the final chapter of this study was preliminary in nature and local immunity to T. colubriformis in the gerbil and the role of secretory IgA in such immunity requires further study using an improved experimental protocol. In addition, vaccination of gerbils with larval and adult T. colubriformis somatic antigens and with parasite excretory-secretory antigens prior to challenge infection might elicit useful information on the role of parasite antigens in protective immunity to T. colubriformis.

In conclusion the three experiments described in Section II have shown the Mongolian gerbil to be a suitable laboratory host for T. colubriformis and to be superior in many ways to other small laboratory hosts previously used in the study of this parasite. Furthermore the gerbil-T. colubriformis model would appear to be eminently suitable for the study of resistance to anthelmintics. Finally gerbils infected with T. colubriformis may provide a good model for the study of local immunity to intestinal parasites.

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APPENDIX A

Appendix to Chapter 1

Table 1

Numbers (Mean + S.E.) of adult N. brasiliensis recovered from irradiated and non-irradiated rats infected with 3,000 larvae

Days After Infection	Group I (Infected)	Group II (Infected-Irradiated)	P**
	Mean ± S.E. *	Mean ± S.E. *	
3	0		
6	1290 ± 143	1580 ± 202	N.S.
9	1366 ± 224	1672 ± 285	N.S.
13	0	1344 ± 190	
16	0	796 ± 163	
20	0	2	
24	0	3	
27	0	0	

* Five rats per group

** Results of analysis by Student's T-Test: I vs II

Table 2

N. brasiliensis egg count in faeces of irradiated and non-irradiated rats infected with 3,000 larvae

Day of Infection	Group I (Infected)	Group II (Infected-Irradiated)
6 - 7	2,900	8,100
7 - 8	16,350	25,450
8 - 9	15,000	33,500
9 - 10	300	13,300
10 - 11	0	8,525
11 - 12		7,700
12 - 13		4,500
13 - 14		2,950
14 - 15		1,450
15 - 16		250
16 - 17		0

Table 3

Numbers (Mean ± S.E.) of Peyers patches in the first 30 cm of small intestine in irradiated and non-irradiated rats infected with 3,000 N. brasiliensis. (Five rats per group.)

Days After Infection	Group I (Infected)	Group II (Infected/ Irradiated)	P **	Group III (Irradiated non-infected)	P ***
	Mean ± S.E.	Mean ± S.E.		Mean ± S.E.	
3	7.2 ± 0.5	0.6 ± 0.4	< 0.001	0.6 ± 0.9	N.S.
6	9.0 ± 0.8	0.6 ± 0.4	< 0.001	0	N.S.
9	14.4 ± 0.8	1.2 ± 0.6	< 0.001	0	N.S.
13	6.4 ± 0.8	3.0 ± 0.4	< 0.01	3.6 ± 1.0	N.S.
16	7.0 ± 0.4	2.8 ± 0.8	< 0.01	2.2 ± 0.4	N.S.
20	5.4 ± 0.4	2.2 ± 1.1	< 0.05	4.0 ± 0.7	N.S.
24	7.5 ††±0.9	4.0 ††±0.7	< 0.05	4.6 ††±0.7	N.S.
27	5.6 †±0.4	3.7 †±0.3	N.S.		

†† 4 rats per group

† 3 rats per group

Result in worm-free control rats (Group IV) - 6 ± 0.4

P** Results of analysis by Student's T-test: Group I vs Group II

P*** Results of analysis by Student's T-test: Group II vs Group III

Table 4

Haemagglutinating antibody titres (mean for 5 rats ± S.E.) against L3SE antigens in serum from irradiated and non-irradiated rats following infection with 3,000 N. brasiliensis

Days After Infection	Group I (Infected) Mean ± S.E.	Group II (Infected/ Irradiated) Mean ± S.E.	P **	Group III (Irradiated Non-infected) Mean ± S.E.
3	510 ± 175	133 ± 60	< 0.01	0
6	320 ± 0	22 ± 10	< 0.001	0
9	768 ± 485	16 ± 6	< 0.01	0
13	400 ± 160	12 ± 4	< 0.001	0
16	208 ± 107			
20	288 ± 72	2 ± 5	< 0.001	0
24	440 †† ± 240	23 †† ± 39	< 0.02	0
27	475 † ± 185	7 † ± 6	< 0.02	0

†† 4 rats per group

† 3 rats per group

Mean ± S.E. titre in 15 worm free controls (Group IV); 11 ± 10

P ** Results of analysis by Student's T-test Group I vs Group II

Table 5

Haemagglutinating antibody titres (mean for 5 rats ± S.E.) against L3SE antigens in small intestine mucosal extracts in irradiated and non-irradiated rats following infection with 3,000 *N. brasiliensis*

Days After Infection	Group I (Infected)	Group II (Infected/ Irradiated)	P **	Group III (Irradiated/ Non-infected)	P ***
	Mean ± S.E.	Mean ± S.E.		Mean ± S.E.	
3	30 ± 13	12 ± 2	N.S.	12 ± 2	N.S.
6	176 ± 39	5 ± 4	< 0.01	2 ± 2	N.S.
9	336 ± 89	20 ± 15	< 0.01	24 ± 14	N.S.
13	672 ± 178	0		8 ± 2	
16	304 ± 96	0		30 ± 6	
20	272 ± 48	68 ± 25	< 0.01	28 ± 15	N.S.
24	220 †† ± 54	35 †† ± 5	< 0.05	13 †† ± 8	N.S.
27	120 † ± 40	10 † ± 6	N.S.		

†† 4 rats per group

† 3 rats per group

Mean ± S.E. titre in 15 worm-free controls (Group IV); 30 ± 20

P** Results of analysis by Student's T-test Group II vs Group I

P*** Results of analysis by Student's T-test Group III vs Group II

APPENDIX B

Appendix to Chapter 2

Table 1

Immunoglobulin Levels in Faecal Extracts of Rats Following Infection with 5,000 N. brasiliensis

Day After Infection	IgA		IgG		IgM	
	µg/g *	% **	µg/g *	% **	µg/g *	% **
0	132 ± 20	0.30	408 ± 70	0.90	1.6 ± 0.5	0.009
3	150 ± 35	0.46	1006 ± 150	3.11	36 ± 10	0.11
6	185 ± 40	1.00	1020 ± 145	5.50	87 ± 25	0.47
12	230 ± 30	1.35	810 ± 98	4.80	45 ± 10	0.26
18	275 ± 130	1.40	2200 ± 300	5.30	16 ± 5	0.04
24	265 ± 45	1.70	950 ± 140	6.00	10 ± 4	0.06
30	165 ± 30	0.56	688 ± 105	4.70	9 ± 4	0.03

* µg immunoglobulin per 1 g faeces ± S.E. (mean values for 5 rats)

** Immunoglobulin as % of total globulin in extract of 1 g faeces (mean values for 5 rats)

Table 2

Immunoglobulin Levels in Intestinal Mucosal Extracts of Rats Following Infection with 5,000 *N. brasiliensis*

Day After Infection	IgA		IgG		IgM	
	µg/g *	% **	µg/g *	% **	µg/g *	% **
0	122 ± 25	0.35	508 ± 59	2.40	83 ± 20	0.35
3	155 ± 13	1.50	1040 ± 46	9.00	175 ± 30	1.70
6	425 ± 38	3.10	2494 ± 109	18.70	290 ± 48	2.20
12	980 ± 135	7.30	1626 ± 81	12.10	245 ± 72	1.90
18	925 ± 105	7.10	3260 ± 220	27.10	260 ± 53	2.20
24	510 ± 62	5.70	1779 ± 78	20.40	610 ± 93	6.90
30	220 ± 24	2.50	752 ± 66	8.50	420 ± 79	4.70

* µg immunoglobulin per 1 g tissue ± S.E. (mean values for 5 rats)

** Immunoglobulin as % of total globulin in extract of 1 g tissue (mean values for 5 rats)

Table 3

Immunoglobulin Levels in Serum of Rats Following Infection with 5,000 N. brasiliensis

Day After Infection	IgA µg/ml *	IgA % **	IgG mg/ml *	IgG % **	IgM µg/ml *	IgM % **
0	89 ± 2.0	0.1	14.7 ± 0.24	23.3	584 ± 66.3	0.94
3	131 ± 7.8	0.23	15.0 ± 0.30	24.7	738 ± 19.2	1.27
6	179 ± 12.8	0.28	15.5 ± 0.24	27.8	878 ± 20.7	1.51
12	275 ± 26.0	0.43	18.5 ± 0.30	28.6	901 ± 23.2	1.40
18	399 ± 24.0	0.57	20.4 ± 0.32	29.3	924 ± 63.2	1.32
24	510 ± 50.0	0.75	20.8 : 0.47	30.6	785 ± 43.4	1.17
30	529 ± 69.5	0.78	18.2 ± 0.38	26.9	738 ± 28.4	1.09

* Immunoglobulin per 1 ml serum ± S.E. (mean value for 5 rats)

** Immunoglobulin as % of total protein in 1 ml of serum (mean value for 5 rats)

Table 4

Haemagglutinating Antibody Titres in Faecal Extracts of Rats Following Infection with 5,000 N. brasiliensis

Days After Infection	Antigen Used	Antibody Titres (log 10)
3	ESP	1.30
	ASE	1.30
	L3SE	0.00
6	ESP	1.60
	ASE	2.20
	L3SE	1.30
9	ESP	3.41
	ASE	3.71
	L3SE	2.51
12	ESP	4.01
	ASE	2.51
	L3SE	2.51
18	ESP	1.00
	ASE	3.71
	L3SE	1.90
21	ESP	1.90
	ASE	2.81
	L3SE	1.90
24	ESP	2.81
	ASE	1.90
	L3SE	1.90
30	ESP	2.81
	ASE	1.60
	L3SE	1.60

Table 5

Haemagglutinating Antibody Titres in Serum and Intestinal Mucosal Extracts of Rats Following Infection with 5,000 N. brasiliensis

Days After Infection	Antigen Used	Antibody Titre (log 10)	
		Serum	Intestinal Mucosa
3	ESP	0	0
	ASE	0	1.00
	L3SE	0	1.00
6	ESP	2.51	1.60
	ASE	2.51	1.90
	L3SE	1.00	1.00
12	ESP	1.90	4.01
	ASE	3.01	3.41
	L3SE	1.78	2.20
18	ESP	1.78	3.11
	ASE	3.41	4.01
	L3SE	1.90	2.20
24	ESP	3.11	3.41
	ASE	3.41	3.41
	L3SE	3.01	1.90
30	ESP	2.51	3.11
	ASE	3.11	2.81
	L3SE	2.71	1.90
Hyperimmune rats 6 days after 3rd infection	ESP	2.71	3.11
	ASE	3.61	2.81
	L3SE	3.01	1.90

Table 6

Haemagglutinating Antibody Titres in Faecal Extracts of Rats After First, Second and Third Infections with N. brasiliensis

Days after First Infection	Antigen Used	Antibody Titre(log 10)
12	ESP	4.01
	ASE	2.51
	L3SE	2.51
15 (Second Infection)	ESP	4.01
	ASE	2.51
	L3SE	2.20
18	ESP	2.20
	ASE	1.40
	L3SE	1.60
21	ESP	2.81
	ASE	2.51
	L3SE	1.30
24	ESP	2.81
	ASE	2.51
	L3SE	1.90
27 (Third Infection)	ESP	3.11
	ASE	3.11
	L3SE	1.90
30	ESP	3.71
	ASE	3.11
	L3SE	1.60
34	ESP	3.11
	ASE	2.51
	L3SE	0.00

Table 7

Total Immunoglobulin Levels in Faecal Extracts of Rats Following Infection with 5,000 N. brasiliensis

Day After Infection	Total Globulins mg/g faeces* + SE	IgA µg/g* + SE	IgA %**	IgG µg/g* + SE	IgG %**	IgM µg/g* + SE	IgM %**
0	30.1 ± 0.48	78.3 ± 2.46	0.26	873 ± 38	2.9	2.4 ± 0.80	0.01
1	27.2 ± 0.65	80.1 ± 2. 3	0.21	797 ± 41	2.9	8.4 ± 0.94	0.03
2	26.1 ± 0.81	81.0 + 3.31	0.34	778 ± 36	3.0	9.5 ± 0.58	0.04
3	24.2 ± 1.12	120.4 ± 3.48	0.49	847 ± 45	3.3	21.3 ± 1.25	0.09
4	15.9 ± 0.49	124.9 ± 4.12	0.78	636 ± 33	3.9	21.6 ± 1.07	0.14
5	14.0 ± 1.65	94.1 ± 3.35	0.67	840 ± 42	6.0	14.0 ± 1.25	0.10
6	18.0 ± 1.74	120.6 ± 4.29	0.65	1224 ± 61	6.8	29.5 ± 1.48	0.16
7	14.3 ± 0.34	177.6 ± 6.31	1.14	1001 ± 30	6.9	71.2 ± 0.56	0.50
8	20.0 ± 2.00	180.0 ± 6.22	0.94	1398 ± 51	7.1	101.1 ± 3.98	0.52
9	25.7 ± 1.56	208.2 ± 3.35	0.81	1901 ± 66	7.4	102.8 ± 3.40	0.40
10	20.4 ± 1.88	224.1 ± 3.94	1.10	1303 ± 61	6.8	69.2 ± 3.31	0.34
11	7.9 ± 1.52	85.3 ± 1.70	1.08	420 ± 17	5.5	66.9 ± 2.53	0.24
12	23.6 ± 1.39	287.9 ± 6.35	1.22	1109 ± 26	4.7	20.1 ± 1.07	0.08
13	14.3 ± 1.83	203.5 ± 4.83	1.42	643 ± 28	4.6	18.5 ± 1.48	0.05
14	21.0 ± 2.15	170.6 ± 4.29	0.81	945 ± 32	4.5	16.0 ± 2.28	0.07
15	18.7 ± 2.37	170.5 ± 4.29	0.91	953 ± 25	5.1	14.7 ± 2.15	0.08
16	17.3 ± 2.37	166.1 ± 3.49	0.96	951 ± 25	5.6	14.1 ± 1.07	0.08
17	13.0 ± 1.21	124.8 ± 5.01	0.95	923 ± 22	7.1	14.8 ± 1.30	0.11
18	30.0 ± 2.37	288.0 ± 7.29	0.94	2520 ± 51	8.5	35.3 ± 2.15	0.12
19	46.1 ± 3.62	793.0 ± 8.85	1.72	3411 ± 81	7.5	56.5 ± 3.18	0.12
20	45.1 ± 2.28	816.9 ±17.35	1.51	3382 ± 87	7.1	61.2 ± 3.62	0.14
21	48.1 ± 2.73	683.1 ±17.98	1.42	3174 ± 66	6.7	25.7 ± 2.64	0.05
22	43.2 ± 4.43	600.5 ±16.23	1.39	2721 ± 47	6.3	71.0 ± 3.76	0.11
24	37.9 ± 3.67	770.5 ±15.65	2.03	2425 ± 73	6.5	68.0 ± 3.09	0.11
26	19.4 ± 2.15	149.4 ± 4.43	0.77	562 ± 48	2.9	16.1 ± 1.30	0.03
28	44.1 ± 3.62	120.0 ± 4.38	0.2	1274 ± 51	2.8	12.3 ± 1.70	0.03
30	32.5 ± 3.22	71.5 ± 2.15	0.22	1121 ± 56	2.6	0	0
Control	28.8 ± 0.67	95.3 ± 3.53	0.32	792 ± 20	2.7	4.3 ± 0.80	0.02

* Mean value for 5 samples (25 rats)

** Percentage of total globulins in 1 g faeces

Table 8

Haemagglutinating Antibody Titres (Mean of 5 Groups, Log 10 ± SE) Against ESP, ASE and L3SE Antigens in Faecal Extracts of Rats Following Infection with 5,000 N. brasiliensis

Days After Infection	Anti-ESP Titres Mean Log 10 ± SE	Anti-ASE Titres Mean Log 10 ± SE	Anti-L3SE Titres Mean Log 10 ± SE
0			0.12 ± 0.07
3	2.40 ± 0.03	2.23 ± 0.07	1.02 ± 0.07
4	2.92 ± 0.20	2.50 ± 0.15	1.14 ± 0.06
5	2.50 ± 0.13	2.29 ± 0.12	1.32 ± 0.07
6	3.21 ± 0.17	2.11 ± 0.19	1.30 ± 0.23
7	3.14 ± 0.21	2.17 ± 0.29	1.81 ± 0.10
8	3.03 ± 0.31	2.23 ± 0.24	1.99 ± 0.07
9	3.41 ± 0.18	3.08 ± 0.31	2.59 ± 0.11
10	3.40 ± 0.16	3.11 ± 0.20	2.41 ± 0.10
11	3.63 ± 0.15	2.75 ± 0.13	2.47 ± 0.06
12	3.45 ± 0.19	2.38 ± 0.30	2.49 ± 0.11
13	3.30 ± 0.23	2.89 ± 0.12	2.41 ± 0.10
14	3.36 ± 0.19	3.19 ± 0.18	2.25 ± 0.07
15	3.35 ± 0.10	3.61 ± 0.00	1.99 ± 0.07
16	3.19 ± 0.17	3.56 ± 0.10	1.99 ± 0.07
17	2.71 ± 0.17	3.30 ± 0.19	1.89 ± 0.11
18	2.56 ± 0.21	3.33 ± 0.19	1.93 ± 0.12
19	2.26 ± 0.22	3.55 ± 0.18	1.99 ± 0.07
20	2.38 ± 0.12	3.31 ± 0.13	1.92 ± 0.15
21	2.44 ± 0.06	3.13 ± 0.07	1.87 ± 0.11
22	2.68 ± 0.15	2.78 ± 0.35	1.74 ± 0.11
23	2.51 ± 0.00	2.59 ± 0.24	
24	2.63 ± 0.07	2.35 ± 0.11	1.66 ± 0.13
25	2.38 ± 0.12	2.11 ± 0.10	
26	1.72 ± 0.07	1.84 ± 0.11	1.62 ± 0.07
27	1.84 ± 0.11	1.72 ± 0.12	
28	1.90 ± 0.10	1.34 ± 0.35	1.50 ± 0.10
29	1.48 ± 0.12	0.96 ± 0.60	
30	1.04 ± 0.13	0.72 ± 0.30	1.06 ± 0.10

APPENDIX C

Appendix to Chapter 3

Table 1

N. brasiliensis faecal egg counts in rats following infection with normal or irradiated larvae

Group	Irradiation Dose (krad)	Days after Infection					Days after Challenge		
		7	8	9	10		7	8	9
A	0	13,700	13,800	12,175	3,825	All rats challenged on 15 DAI 5×10^3 L3/ rat	200	160	150
B	10	10,400	12,100	6,225	2,950		360	300	300
C	20	5,850	4,200	4,675	1,375		0	100	0
D	40	1,750	1,350	975	625		0	0	0
E	80	650	125	100	25		0	300	500
F	120	300	0	0	0		0	0	0
G	*						84,600	64,500	ND

* Challenge control rats

Table 2

Haemagglutinating antibody titres (mean log 10) against L3SE antigens in lung extracts of rats following infection with normal or irradiated N. brasiliensis larvae and after challenge on 15 DAI.

	No. of Rats Necropsied Per Group	Group A	B	C	D	E	F
10 Days After Infection	5	0	0	0	2.107	1.806	2.709
		0	0	0	0.301	3.010	2.107
		0	0	0	1.504	2.408	2.408
		0	0	0	0.301	1.806	2.107
		0	0	0	0.0	2.408	1.806
	Mean	0	0	0	0.843	2.348	2.227
	± SE	0	0	0	0.408	0.241	0.153
10 Days After Challenge	5	0.301	0.602	2.408	2.107	2.408	2.709
		0.0	0.301	1.806	1.806	2.107	2.709
		0.602	2.709	1.505	2.107	2.408	2.408
		0.903	0.0	3.311	1.806	2.107	2.709
		2.709	0.602	2.408	2.107	2.408	2.408
	Mean	0.904	0.843	2.288	1.987	2.288	2.589
	± SE	0.478	0.480	0.310	0.074	0.074	0.074

Table 3

Serum haemagglutinating antibody titres(mean log 10) against L3SE antigens 10 DAI with normal or irradiated N. brasiliensis larvae

No. of Rats necropsied per group	Group					
	A	B	C	D	E	F
5	2.107	2.408	2.107	1.806	0.602	0.903
	1.806	1.204	1.806	1.806	0.301	0.301
	2.408	3.010	2.408	0.301	0.602	0.602
	2.709	2.408	2.107	2.107	0.602	0.602
	2.408	3.010	2.107	3.010	0.602	0.602
Mean	2.408	2.408	2.107	1.806	0.599	0.602
± SE	0.153	0.295	0.085	0.413	0.059	0.095

Table 4

Haemagglutinating antibody titres (log 10) against ESP antigens in faecal extracts of rats following infection with normal or irradiated N. brasiliensis larvae and after challenge on 15 DAI

Group	Day after infection									
	0	3	6	9	12	15*	18	21	24	25
A	0.903	2.158	3.063	2.847	1.455	1.602	1.556	3.111	2.760	2.505
B	1.070	1.414	2.482	2.380	1.380	1.602	2.858	3.186	3.440	2.505
C	0.602	1.806	1.505	1.176	1.380	1.602	1.778	2.857	3.283	2.806
D	0.903	1.505	2.245	1.301	1.643	1.602	1.832	3.158	3.311	2.806
E	1.079	1.477	1.505	1.204	1.698	1.700	1.748	3.010	2.709	2.806
F	1.250	1.477	1.204	1.414	1.792	1.700	1.556	1.880	3.361	2.505

* All groups were challenged on 15 DAI

Table 5

Haemagglutinating antibody titres (log 10) against L3SE antigens in faecal extracts of rats following infection with normal or irradiated N. brasiliensis larvae and after challenge on 15 DAI

Group	Days after infection									
	0	3	6	9	12	15*	18	21	24	25
A	0.903	2.760	3.334	2.350	3.061	3.107	2.458	2.049	1.857	1.980
B	0.903	2.301	3.048	2.584	2.584	3.107	3.365	1.982	2.245	2.000
C	0.903	2.301	2.408	3.584	2.584	1.903	2.447	1.982	2.380	2.280
D	0.903	1.806	2.702	3.408	2.847	3.107	3.298	2.245	2.408	2.500
E	0.903	3.146	2.107	2.408	2.158	2.010	2.049	1.857	1.602	1.480
F	0.903	3.107	2.408	3.522	2.584	-	2.459	2.434	2.158	1.980

* All groups were challenged on 15 DAI

APPENDIX D

Appendix to Chapter 4

Table 1

Numbers of adult *N. brasiliensis* recovered (mean ± S.E.) from Group I rats (*N. brasiliensis* + *T. brucei* infected) and from Group II rats (*N. brasiliensis* infected) after primary infection and after challenge on day 28 after primary infection

Group	No. of rats necropsied per day	Days after primary infection					
		14	18	24	28	32	42 †
I	5	81	16	18	71	1054	0
		59	24	20	7	1280	24
		182	42	7	1	323	3
		178	7	45	8	1198	53
		202	23	34	13	323	16
	Mean	140	23	25	20	853	17
	± S.E.	29	6	7	13	205	9
II	5	15	5	6	15	49	0
		160	16	34	49	241	0
		53	12	5	31	90	5
		58	37	25	0	173	0
		194	17	4	3	204	0
	Mean	96	18	15	18	151	1
	± S.E.	35	5	6	9	36	1
	P $\frac{I}{II}$	N.S.	N.S.	N.S.	N.S.	< 0.02	N.S.

† Six rats necropsied

Table 2

Haemagglutinating antibody titres (log 10) against L3SE antigens in intestinal mucosal extracts of Group I rats (infected with N. brasiliensis + T. brucei) and of Group II rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI

Group	No. of Rats Used per day	Day after primary infection					
		14	18	24	28	32	42
		2.709	2.408	1.806		2.107	1.806
		1.806	2.408	1.806		2.408	2.107
I	5	2.709	2.709	2.107		2.107	1.806
		3.010	2.408	1.806		1.806	2.107
		2.709	2.709	2.107		2.408	1.806
	Mean	2.586	2.528	1.926		2.167	1.920
	± S.E.	0.204	0.074	0.074		0.112	0.074
		1.806	3.010	2.107	1.806	2.107	2.107
		2.709	2.709	2.107	1.806	2.107	2.709
II	5	2.709	2.709	2.107	2.107	1.806	2.107
		2.107	2.408	1.806	2.107	2.107	2.709
		3.010	3.010	2.107	1.806	2.107	2.107
	Mean	2.468	2.769	2.047	1.926	2.046	2.348
	± S.E.	0.221	0.113	0.061	0.074	0.061	0.147
	P	N.S.	N.S.	N.S.		N.S.	< 0.05

Titre (mean log 10 ± S.E.) in Group III rats (uninfected controls):-
0.3 ± 0.1 (n = 10)

Table 3

Haemagglutinating antibody titres (log 10) against L3SE antigens in faecal extracts of Group I rats (infected with N. brasiliensis + T. brucei) and of Group II rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI

Group	No. of Rats Used per day	Days after primary infection					
		14	18	24	28	32	42
		2.709	1.806	1.204	0.903	1.204	0.903
		2.107	1.505	1.204	1.204	1.505	0.602
I	5	2.709	1.806	1.505	0.903	1.204	1.204
		3.010	2.107	1.806	1.505	1.204	0.803
		2.107	1.505	1.204	0.903	0.903	0.602
	Mean	2.528	1.746	1.385	1.084	1.204	0.882
	± S.E.	0.181	0.113	0.120	0.120	0.095	0.112
		3.010	2.107	1.505	1.505	2.107	2.408
		2.709	1.806	1.806	1.204	1.807	1.806
II	5	2.709	2.107	1.806	1.505	2.107	1.505
		2.709	1.505	1.505	1.204	2.107	2.107
		3.010	1.806	1.806	1.806	1.807	1.807
	Mean	2.829	1.866	1.686	1.445	1.987	1.927
	± S.E.	0.074	0.113	0.074	0.112	0.074	0.153
	P	N.S.	N.S.	N.S.	N.S.	< 0.001	0.001

Titre (mean log 10 ± S.E.) in Group III rats (uninfected controls):
0.9 ± 0.1 (n = 10)

Table 4

Haemagglutinating antibody titres (log 10) against L3SE antigens in serum of Group I rats (infected with N. brasiliensis + T. brucei) and of Group II rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI

Group	No. of Rats Used per day	Day after primary infection: 14	18	24	28	32	42
I	5	1.505	2.709	1.806	1.505	1.806	1.806
		1.806	2.107	1.505	1.204	1.806	2.107
		1.505	2.107	1.204	1.505	1.806	2.107
		1.806	1.806	1.505	1.204	2.107	1.806
		1.204	2.107	1.204	1.204	1.806	1.505
	Mean	1.565	2.167	1.445	1.324	1.866	1.866
	± S.E.	0.113	0.147	0.113	0.074	0.061	0.113
II	5	2.107	3.010	2.704	2.107	3.010	2.107
		2.704	2.704	2.408	2.704	2.709	1.806
		1.806	3.010	3.010	2.107	3.010	2.107
		2.107	2.704	2.408	2.704	2.709	1.806
		2.704	3.311	3.010	2.704	3.010	2.107
	Mean	2.086	2.948	2.708	2.465	2.890	1.990
	± S.E.	0.300	0.114	0.135	0.146	0.074	0.074
	P	N.S.	<0.02	<0.001	<0.001	<0.001	N.S.

Titre (mean log 10 ± S.E.) in Group III rats (uninfected controls):
0.2 ± 0.1 (n = 10)

Table 5

Haemagglutinating antibody titres (log 10) against ESP antigens in intestinal mucosal extracts of Group I rats (infected with *N. brasiliensis* + *T. brucei*) and of Group II rats (infected with *N. brasiliensis*) after primary infection and after challenge at 28 DAPI

Group	No. of Rats Used per Day	Day after primary infection 14	18	24	28	32	42
		2.107	1.807	1.807		2.709	1.807
		2.505	1.807	1.807		2.107	2.505
I	5	2.107	2.107	1.505		2.107	2.505
		1.807	1.505	1.505		1.802	1.807
		2.107	1.204	1.505		1.802	1.505
	Mean	2.126	1.686	1.625		2.105	2.056
	± S.E.	0.111	0.154	0.073		0.166	0.203
		2.107	2.505	1.807	1.204	1.807	2.107
		1.807	1.807	1.807	1.505	2.107	2.709
II	5	2.107	2.709	2.505	1.204	1.807	2.709
		1.807	2.505	1.204	1.806	2.204	2.709
		2.107	1.807	1.807	1.204	2.107	2.704
	Mean	1.987	2.267	1.826	1.385	2.006	2.586
	± S.E.	0.073	0.192	0.206	0.120	0.083	0.120
	P	N.S.	< 0.05	N.S.		N.S.	< 0.05

Titre (mean log 10 ± S.E.) in Group III rats (uninfected controls):
0.3 ± 0.1 (n = 10)

Table 6

Haemagglutinating antibody titres (log 10) against ESP antigens in faecal extracts of Group I rats (infected with *N. brasiliensis* + *T. brucei*) and of Group II rats(infected with *N. brasiliensis*) after primary infection and after challenge at 28 DAPI

Group	No. of Rats Used per Day	Day after primary infection 14	18	24	28	32	42
		2.107	2.107	1.204	1.505	3.010	2.408
		2.408	1.681	1.505	1.204	3.010	2.107
I	5	1.681	1.681	1.505	1.204	2.408	2.408
		2.107	1.681	1.681	1.204	2.709	2.408
		1.681	2.107	1.204		2.709	1.806
	Mean	1.997	1.851	1.420	1.279	2.769	2.227
	± S.E.	0.104	0.104	0.094	0.075	0.113	0.120
		2.710	2.709	2.408	1.806	3.010	2.709
		2.710	2.107	2.709	1.806	3.311	2.709
II	5	2.110	2.408	1.806	2.107	3.311	3.010
		2.710	1.806	1.505	2.107	3.010	3.010
		1.810	1.505		2.107	3.010	2.709
	Mean	2.410	2.107	2.107	1.987	3.130	2.829
	± S.E.	0.190	0.213	0.275	0.074	0.074	0.074
	P	N.S.	N.S.	N.S.	< 0.001	< 0.05	< 0.01

Titre (mean log 10 ± S.E.) in Group III rats (uninfected controls)
0.8 ± 0.15 (n = 10)

Table 7

Haemagglutinating antibody titres (log 10) against ESP antigens in serum of Group I rats (infected with N. brasiliensis + T. brucei) and of Group II rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI

Group	No. of Rats Used per day	Day after primary infection 14	18	24	28	32	42
		1.505	0.302	1.807	1.504	1.807	2.107
		0.602	0.602	1.505	1.504	1.807	2.107
I	5	1.204	1.207	1.505	1.807	1.204	1.505
		0.301	0.302	1.505	1.807	2.807	2.505
		0.602	0.302	1.204	1.505	2.107	1.204
	Mean	0.843	0.543	1.505	1.625	1.942	1.886
	± S.E.	0.221	0.176	0.095	0.074	0.260	0.234
		1.807	1.807	2.107	1.807	2.709	2.505
		1.807	2.505	2.107	1.807	2.505	2.107
II	5	1.204	2.709	1.807	2.107	2.709	2.505
		1.807	1.807	1.807	1.807	2.505	2.107
		1.204	1.807	2.107	1.807	2.709	1.505
	Mean	1.566	2.127	1.987	1.867	2.627	2.346
	± S.E.	0.330	0.199	0.073	0.060	0.050	0.097
	P	< 0.05	< 0.001	< 0.01	< 0.05	< 0.01	N.S.

Titres (mean log 10 ± S.E.) in Group III rats (uninfected controls):
0.2 ± 0.1 (n = 10)

Table 8

Numbers of adult N. brasiliensis recovered from Group A rats (infected with N. brasiliensis + T. brucei) and from Group B rats (infected with N. brasiliensis) and from Group C rats (challenge control group)

Group	No. of Rats Necropsied per day	Days after primary infection						
		8	12	14*	18	28	32	42
		2,880	43	12	4	0	2,368	6
		3,114	673	6	7	23	3,464	28
A	5	4,780	697	2	0	65	3,294	4
		4,020	55	2	0	2	2,576	4
		4,990	-	24	56	12	1,854	2
	Mean	3,957	367	9	13	20	2,709	9
	± S.E.	425	184	4	10	12	298	5
		1,225	30	8	45	5	968	0
		1,639	20	0	17	14	1,024	0
B	5	2,060	30	8	10	15	876	2
		2,023	11	8	10	11	548	0
		2,525	37	124	52	32	-	0
	Mean	1,894	26	30	27	15	854	0.4
	± S.E.	219	5	24	9	5	106	0.4
	P B/A	< 0.01	N.S.	N.S.	N.S.	N.S.	< 0.001	N.S.

* No. of worms recovered from intestine wall. On day 5 after challenge the following numbers of worms were recovered from the challenge control rats, Group C:
2410, 3830, 3840, 3690, 3900 and 3110 - mean 3463 ± 241

Table 9

Haemagglutinating antibody titres (log 10) against L3SE antigens in lung extracts of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI

Group	No. of Rats Necropsied per Day	Day after primary infection							Uninfected Controls
		8	12	14	18	28	32	42	
		0.903	0.903	1.505	0.301	0.301	0.301	0.301	0
		0.903	1.505	0.903	0.602	0.301	0	0.602	0.301
A	5	0.602	0.903	1.505	0.602	0.301	0.301	0.903	0.602
		0.602	0.903	0.903	0.301	0.301	0.602	0.602	0.301
		0.903	0.903	1.204	0.301	0.301	0.301	0.602	0.301
	Mean	0.783	1.024	1.204	0.421	0.301	0.301	0.602	
	± S.E.	0.074	0.121	0.135	0.074	0	0.095	0.095	
		1.505	1.806	2.107	1.204	0.903	1.806	1.806	0
		1.505	1.204	1.806	1.505	0.903	1.505	2.107	0.301
B	5	1.204	1.505	1.806	0.903	1.204	1.204	1.806	0.602
		1.505	1.204	2.107	1.204	0.903	1.505	1.806	0.301
		1.505	1.505	2.107	1.204	1.204	1.204	1.806	0.301
	Mean	1.444	1.444	1.987	1.204	1.023	1.445	1.866	
	± S.E.	0.059	0.112	0.374	0.091	0.074	0.113	0.066	
	P B/A	< 0.001	< 0.05	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	

Titres (mean log 10 ± S.E.) in group D rats (uninfected controls): 0.301 ± 0.095 (n = 10)

Table 10

Haemagglutinating antibody titres (log 10) against L3SE antigens in small intestine mucosal extracts of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis)after primary infection and after challenge at 28 DAPI

Group	No. of Rats Necropsied per Day	Days after primary infection						
		8	12	14	18	28	32	42
A	5	2.107	1.806	1.806	2.170	2.107	1.204	1.505
		1.806	1.806	1.806	2.170	2.107	1.204	1.505
		2.107	0.903	0.903	1.806	1.806	1.505	1.505
		1.806	1.806	2.408	1.806	1.806	1.505	1.505
		2.107	-	2.408	2.408	2.408	1.204	1.505
	Mean	1.987	1.580	1.866	2.047	2.047	1.324	1.505
	± S.E.	0.074	0.226	0.276	0.113	0.113	0.074	0.0
B	5	2.709	2.408	2.407	2.408	2.408	1.807	2.107
		1.505	2.107	1.806	2.408	2.408	1.807	2.107
		3.612	2.408	2.408	2.408	2.408	1.505	2.107
		3.612	-	2.107	2.408	2.408	1.807	2.107
	Mean	2.950	2.182	2.107	2.408	2.408	1.807	2.107
	± S.E.	0.397	0.144	0.134	0.0	0.0	0.095	0
	P B/A	<0.05	N.S.	N.S.	< 0.02	< 0.02	< 0.01	

Titres (mean log 10 ± S.E.) in Group D rats (uninfected controls). 0.06 + 0.2 (n = 10)

Table 11

Haemagglutinating antibody titres (log 10) against L3SE antigens in serum of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI

Group	No. of rats Necropsied per Day	Days after primary infection						
		8	12	14	18	28	32	42
		0.0	1.806	1.806	2.408	0.0	0.0	1.505
		1.505	1.806	1.204	1.505	1.806	0.0	0.0
A	5	0.0	0.903	1.806	0.0	0.0	1.204	0.0
		0.0	0.903	1.505	0.0	0.0	1.204	1.505
		0.903	-		0.0	1.505	1.107	1.505
	Mean	0.481	1.355	1.580	0.783	0.662	0.703	0.903
	± S.E.	0.311	0.261	0.144	0.144	0.408	0.288	0.369
		2.408	2.408	2.408	2.709	3.311	3.010	1.806
		0.903	2.408	2.408	2.709	2.107	3.010	1.806
B	5	2.408	0.903	1.806	2.408	0.903	2.107	1.806
		2.408	2.408	1.505	2.408	3.311	2.709	1.806
		2.408	2.408		2.709	2.407	-	1.806
	Mean	2.107	2.107	2.032	2.587	2.408	2.709	1.806
	± S.E.	0.284	0.301	0.226	0.074	0.446	0.213	± 0
	P B/A	< 0.01	N.S.	N.S.	< 0.01	< 0.05	< 0.001	< 0.05

Titres (mean log 10 ± S.E.) in Group D rats (uninfected controls): 0.2 ± 0.1 (n = 10)

Table 12

Haemagglutinating antibody titres (log 10) against L3SE antigens in small intestine contents of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI

Group	No.of Rats Necropsied per day	Days after primary infection						
		8	12	14	18	28	32	42
A	5	1.204	0.602	2.709	2.709	2.409	1.204	3.612
		0.301	2.709	1.806	2.709	3.311	4.214	3.612
		0.903	2.408	3.913	3.010	1.806	0.612	3.913
		0.903	0.0	1.806	3.010	3.311	4.214	3.010
		0.602	-	4.214	2.709	1.506	4.816	3.311
	Mean	0.782	1.430	2.889	2.829	2.469	3.012	3.491
	± S.E.	0.153	0.666	0.509	0.074	0.373	0.871	0.153
B	5	2.107	-	3.311	4.214	3.311	3.913	4.214
		2.408	1.806	3.612	3.913	3.612	4.214	4.214
		2.408	2.408	3.311	4.214	3.010	3.913	4.214
		2.709	1.806	3.010	4.214	4.214	3.612	4.214
		2.709	-	3.010	3.913	2.408	3.913	-
	Mean	2.468	2.007	3.251	4.093	3.311	3.913	4.214
	± S.E.	0.113	0.201	0.113	0.074	0.301	0.095	0.0
	P B/A	< 0.001	N.S.	N.S.	< 0.001	N.S.	N.S.	< 0.01

Titres (mean log 10 ± S.E.) in Group D rats (uninfected controls); 0.6 ± 0.24 (n = 10)

Table 13

Haemagglutinating antibody titres (log 10) against L3SE antigens in large intestine contents of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI

Group	No.of Rats Necropsied per day	Days after primary infection						
		8	12	14	18	28	32	42
A	5	3.010	3.010	3.612	2.107	1.505	2.107	1.806
		2.107	0.903	3.612	2.107	2.709	2.107	1.505
		1.204	1.806	0.903	2.107	1.204	2.709	1.505
		1.806	3.010	0.000	2.107	2.709	0.903	0
		1.204	0.903	3.612	2.107	1.806	2.107	0
	Mean	1.866	1.926	2.348	2.107	1.987	1.987	0.963
	± S.E.	0.355	0.472	0.787	-	0.310	0.295	0.397
B	5	2.408	2.644	4.212	3.010	3.111	2.709	3.178
		3.010	3.010	3.010	2.708	2.709	2.408	3.098
		3.010	3.010	1.505	3.010	3.010	2.709	3.010
		3.010	2.170	3.111	3.010	1.505	2.408	3.214
		2.708	-	3.512	3.010	0.903	2.107	-
	Mean	2.829	2.708	3.070	2.946	2.248	2.468	3.125
	± S.E.	0.120	0.199	0.446	0.060	0.442	0.113	0.045
	P B/A	< 0.05	N.S.	N.S.	N.S.	N.S.	N.S.	< 0.01

Titre (mean log 10 ± S.E.) in Group D rats (uninfected controls): 0.4 ± 0.1 (n = 10)

Table 14

Haemagglutinating antibody titre† (log 10) against L3SE and ESP antigens in faecal extracts of Group A rats (infected with N. brasiliensis and T. brucei) and of Group B rats (infected with N. brasiliensis) after primary infection and after challenge on 28 DAPI

Antigen	Group	Days after primary infection													
		0	3	6	8	12	14	18	21	25	28	32	35	38	42
L3SE	A	0.301	0.903	1.204	1.602	1.903	2.204	1.903	1.602	1.408	1.301	1.408	1.408	1.301	1.204
	B	0.301	1.505	2.408	2.709	2.107	2.408	2.107	2.107	1.806	1.505	1.806	2.107	2.408	2.408
	D	0.301			0		0.602		0.301		0		0.301		0.408
ESP	A	0.903	1.204	2.107	2.408	2.408	2.408	2.107	2.107	2.408	2.408	2.719	2.408	2.408	2.408
	B	0.903	1.505	2.408	2.719	2.107	2.408	2.719	2.408		1.806	2.107	2.709	3.011	3.311
	D	0.903	0.602		0.301			0.301			0.602		0.301		0.602

† Tests were carried out on pooled samples from 5 rats

Table 15

Haemagglutinating antibody titres (log 10) against ESP antigens in small intestine mucosal extracts of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI

Group	No.of Rats Necropsied per day	Days after primary infection						
		8	12	14	18	28	32	42
A	5	2.408	1.806	2.408	2.408	1.602	2.107	2.107
		2.107	1.505	2.107	2.107	1.204	2.107	2.709
		1.806	1.806	2.709	1.806	1.806	1.807	2.408
		2.408	2.107	2.107	2.107	1.602	2.107	2.408
		2.408	2.107	3.010	2.107	1.602	2.107	2.408
	Mean	2.188	1.866	2.468	2.107	1.563	2.047	2.408
	± S.E.	0.156	0.133	0.176	0.095	0.098	0.060	0.951
B	5	3.010	2.408	2.107	2.107	2.107	2.107	2.408
		2.709	2.107	2.709	2.408	1.806	2.107	2.709
		3.612	2.709	1.806	2.709	2.107	2.107	2.709
		3.311	2.709	1.612	1.806	1.806	2.107	2.408
		2.709	2.408	2.709	2.709	2.107	2.107	2.709
	Mean	3.070	2.468	2.189	2.348	1.987	2.107	2.589
	± S.E.	0.176	0.133	0.226	0.176	0.074	0.0	0.074
	P B/A	< 0.01	< 0.01	< 0.01	N.S.	< 0.01	N.S.	N.S.

Titres (mean log 10 ± S.E.) in Group D rats (uninfected controls): 0.6 ± 0.2 (n = 10)

Table 16

Haemagglutinating antibody titres (log 10) against ESP antigens in serum of Group A rats (infected with N. brasiliensis + T. Brucei) and of Group B rats (infected with N. brasiliensis) after primary infection and after challenge at 28 DAPI

Group	No.of Rats Necropsied per day	Day after primary infection						
		8	12	14	18	28	32	42
		1.204	2.408	1.806	0.0	2.107	0.0	2.107
		0.602	1.505	1.204	1.505	1.806	1.505	2.107
A	5	0.0	2.204	0.903	2.807	1.505	1.806	2.107
		0.0	0.0	1.505	0.0	1.505	1.204	1.806
		0.0	-	-	0.0	1.806	1.806	2.107
	Mean	0.361	1.529	1.355	0.662	1.746	1.264	2.047
	± S.E.	0.241	0.545	0.192	0.408	0.113	0.335	0.060
		1.806	1.204	0.903	2.510	2.107	2.709	2.408
		1.806	1.000	2.408	1.204	1.806	2.709	2.107
B	5	1.806	1.681	1.806	2.120	2.107	2.709	2.709
		1.806	1.681	0.0	1.204	1.806	2.709	2.408
		1.806	1.681	-	2.120	2.107	-	-
	Mean	1.806	1.449	1.279	1.832	1.987	2.709	2.483
	± S.E.	0	0.145	0.527	0.266	0.074	0	0.144
	P B/A	< 0.001	N.S.	N.S.	< 0.05	N.S.	< 0.01	< 0.05

Titres (mean log 10 ± S.E.) in Group D rats (uninfected controls): 0.2 ± 0.1 (n = 10)

Table 17

Haemagglutinating antibody titres (log 10) against antigens in small intestine contents of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis after primary infection and after challenge at 28 DAPI

Group	No.of Rats Necropsied per day	Day after primary infection						
		8	12	14	18	28	32	42
		3.010	3.010	3.311	4.212	3.612	3.612	3.311
		0.0	1.806	3.913	3.612	4.212	3.612	3.311
A	5	3.010	2.408	2.107	4.212	4.212	3.612	3.311
		2.709	2.709	3.612	2.408	4.212	3.612	3.612
		0.0	-	3.612	3.913	1.806	3.311	3.311
	Mean	1.745	2.483	3.311	3.672	3.611	3.552	3.371
	± S.E.	0.715	0.257	0.316	0.335	0.466	0.062	0.060
		2.709	2.107	3.612	3.913	3.010	3.913	4.214
		3.010	2.107	3.311	3.913	3.311	3.612	4.214
B	5	2.709	2.408	3.612	3.913	3.010	3.913	4.214
		2.709	1.806	3.913	4.212	3.311	3.612	4.214
		2.709	-	3.612	4.212	3.311	3.913	4.214
	Mean	2.769	2.107	3.612	4.033	3.191	3.792	4.214
	± S.E.	0.060	0.123	0.095	0.074	0.074	0.074	0
	P B/A	N.S.	N.S.	N.S.	N.S.	N.S.	< 0.05	< 0.001

Titres (mean log 10 ± S.E.) in Group D rats (uninfected controls): 0.6 ± 0.1 (n = 10)

Table 18

Haemagglutinating antibody titres (log 10) against ESP antigens in large intestine contents of Group A rats (infected with N. brasiliensis + T. brucei) and of Group B rats (infected with N. brasiliensis after primary infection and after challenge at 28 DAPI

Group	No.of Rats Necropsied per day	Day after primary infection						
		8	12	14	18	28	32	42
		2.709	3.010	3.010	3.111	2.709	1.807	2.107
		2.408	1.505	2.709	3.111	2.709	1.807	1.806
A	5	3.010	1.806	3.311	3.111	2.408	1.807	1.505
		1.806	3.010	3.010	2.107	2.709	1.807	0.903
		2.709	1.806	3.010	2.709	2.709	1.807	0.0
	Mean	2.528	2.227	3.010	2.890	2.649	1.807	1.264
	± S.E.	0.204	0.034	0.952	0.197	0.060	0	0.374
		2.709	2.885	3.612	3.311	3.311	2.709	3.062
		3.010	3.010	3.311	2.408	1.204	2.408	3.065
B	5	3.010	3.010	2.107	3.612	1.505	2.408	3.010
		3.010	2.709	3.010	2.709	3.010	2.709	3.010
		3.010	3.010	4.214	3.612	2.709	3.010	-
	Mean	2.950	2.925	3.251	3.130	2.348	2.646	3.034
	± S.E.	0.060	0.059	0.348	0.245	0.419	0.113	0.142
	P B/A	N.S.	N.S.	N.S.	N.S.	N.S.	< 0.001	< 0.01

Titre (mean log 10 ± S.E.) in Group D rats (uninfected controls): 0.3 ± 0.3 (n = 10)

Table 19

Immunoglobulin levels (µg/ml and % of total globulins) in serum of rats in Group A (infected with N. brasiliensis and T. brucei) and in Group B (infected with N. brasiliensis) following primary infection and after challenge at 28 DAPI and in Group D (worm-free controls)

Group	Days after Primary Infection	IgA * µg/ml	IgA %	IgG * µg/ml	IgG %	IgM * µg/ml	IgM %
A	8	193 ± 24	0.16	11,347 ± 300	29.9	1,264 ± 517	4.2
	12	295 ± 24	0.67	10,680 ± 300	29.7	1,054 ± 565	2.9
	14	335 ± 12	0.85	12,015 ± 600	33.0	880 ± 28	2.7
	18	335 ± 26	0.96	11,347 ± 900	25.0	1,054 ± 54	4.2
	28	372 ± 8	0.32	12,015 ± 470	25.5	632 ± 54	1.4
	32	335 ± 18	0.43	10,860 ± 300	20.5	630 ± 88	1.2
	42	372 ± 68	0.95	14,710 ± 210	35.1	357 ± 16	1.2
B	8	465 ± 42	1.30	11,340 ± 300	36.4	632 ± 56	2.30
	12	558 ± 59	0.95	12,015 ± 470	38.1	1,054 ± 54	2.60
	14	744 ± 17	1.70	13,350 ± 300	39.2	890 ± 23	2.31
	18	744 ± 23	1.70	13,350 ± 600	41.5	1,054 ± 54	2.73
	28	367 ± 83	0.80	12,806 ± 136	36.1	354 ± 19	1.09
	32	372 ± 47	0.55	12,015 ± 340	35.0	421 ± 0	1.00
	42	374 ± 60	0.45	13,350 ± 350	41.5	1,054 ± 87	2.78
D		169 ± 6	0.18	8,740 ± 240	20.0	421 ± 74	0.8

* Mean ± S.E. (n = 5)

Table 20

Immunoglobulin levels (µg/ml and % of total globulins) in lung extracts of rats in Group A (infected with N. brasiliensis + T. brucei) and for Group B (infected with N. brasiliensis) following primary infection and after challenge at 28 DAPI and in Group D (worm-free controls)

Group	Days after Primary Infection	IgA		IgG		IgM	
		* µg/ml	%	*µg/ml	%	*µg/ml	%
A	8	93 ± 8	2.53	1,001 ± 68	27	42 ± 7	1.14
	12	93 ± 8	2.04	2,781 ± 176	61	42 ± 7	1.00
	14	92 ± 9	1.55	3,337 ± 292	56	632 ± 94	10.53
	18	37 ± 4	0.10	3,893 ± 536	89	295 ± 63	7.17
	28	93 ± 5	0.90	2,670 ± 68	52	295 ± 35	5.65
	32	193 ± 12	1.95	3,893 ± 176	45	295 ± 36	3.38
	42	137 ± 16	1.04	3,738 ± 68	53	126 ± 19	1.77
B	8	93 ± 5	4.84	1,112 ± 153	46	189 ± 19	4.00
	12	186 ± 10	3.60	2,225 ± 223	42	142 ± 5	1.05
	14	409 ± 53	7.69	2,781 ± 125	67	17 ± 4	0.50
	18	447 ± 46	9.80	3,337 ± 176	63	126 ± 9	2.76
	28	207 ± 11	4.01	1,118 ± 176	41	49 ± 4	1.80
	32	186 ± 7	2.06	3,337 ± 160	37	210 ± 7	2.30
	42	93 ± 6	1.24	3,337 ± 300	22	89 ± 24	2.20
D		12 ± 7	0.21	893 ± 205	18	34 ± 3	0.07

* Mean ± S.E. (n = 5)

Table 21

Immunoglobulin levels (µg/ml and % of total globulins) in intestinal mucosal extracts of rats in Group A (infected with N. brasiliensis + T. brucei) and in Group B (infected with N. brasiliensis) following primary infection and after challenge on 28 DAPI and in Group D (worm-free controls)

Group	Days after Primary Infection	IgA		IgG		IgM	
		* µg/ml	%	*µg/ml	%	*µg/ml	%
A	8	74	1.55	810	14.1	0	0
	12	260	8.12	667	20.8	0	0
	14	186	4.33	1,068	24.9	204	4.75
	18	335	8.40	1,335	33.4	295	7.40
	28	93	5.90	1,201	32.4	843	9.20
	32	186	5.02	1,201	31.4	210	5.67
	42	186	3.38	1,335	24.3	247	4.50
B	8	186	3.10	1,335	22.0	0	0
	12	297	10.20	801	27.6	0	0
	14	558	11.60	667	13.9	253	5.27
	18	409	8.69	1,602	34.0	295	6.27
	28		6.90	1,200	30.0	208	5.20
	32	335	10.15	1,201	36.3	210	6.36
	42	186	4.65	1,468	36.7	463	8.60
D		117	1.54	344	8.3	10	0.25

* Tests were carried out on pooled samples from 5 rats.

Table 22

Immunoglobulin levels (µg/ml and % of total globulins) in small intestine contents of rats in Group A (infected with N. brasiliensis + T. brucei) and in Group B (infected with N. brasiliensis) following primary infection and after challenge at 28 DAPI and in Group D (worm-free controls)

Group	Days after Primary Infection	IgA * µg/ml	IgA %	IgG * µg/ml	IgG %	IgM * µg/ml	IgM %
A	8	335±59	10.5	1,228±140	38.0	224±53	7.0
	12	807±57	14.0	2,781±301	57.0	281±53	8.6
	14	1,676±263	10.2	3,337±306	24.7	1,634±528	9.2
	18	1,040±37	4.0	2,225±364	9.4	217±22	1.8
	28	744±42	6.2	4,003±178	18.4	28±17	0.2
	32	837±103	4.4	2,781±301	17.4	217±35	3.1
	42	290±34	1.0	5,340±403	18.4	84±38	0.8
B	8	419±53	10.4	1,112±105	27.8	280±30	4.0
	12	837±53	13.2	3,337±426	41.7	280±47	2.5
	14	837±76	5.9	3,337±71	21.5	140±34	1.2
	18	837±103	4.1	3,893±146	16.0	196±33	0.8
	28	669±38	4.5	2,120±329	14.0	84±27	0.6
	32	837±69	3.7	3,337±57	15.5	817±493	6.0
	42	1,247±249	3.9	5,340±593	17.0	420±49	1.9
D		76 ± 8	0.5	2,020±439	13.6	112±13	0.7

* Mean ± S.E. (n = 5)

Table 23

Immunoglobulin levels (µg/ml and % of total globulin) in large intestine contents of rats inGroup A (infected with N. brasiliensis + T. brucei) and in Group B (infected with N. brasiliensis) following primary infection and after challenge at 28 DAPI and in Group D (worm-free controls)

Group	Days after Primary Infection	IgA		IgG		IgM	
		* µg/ml	%	* µg/ml	%	* µg/ml	%
A	8	351	6.3	2,002	36.5	556	7.5
	12	644	10.2	667	12.2	1,062	9.0
	14	585	3.6	1,068	6.5	1,518	9.0
	18	402	2.4	667	3.9	1,012	6.0
	28	930	3.9	4,005	16.7	1,012	3.5
	32	270	1.8	133	1.2	735	4.2
	42	837	4.4	133	1.0	294	1.4
B	8	558	7.5	1,468	19.7	357	4.9
	12	1,117	9.7	1,602	13.9	354	3.1
	14	968	5.7	2,002	11.8	423	2.5
	18	585	5.0	14,685	13.5	1,958	1.8
	28	-	5.0	842	5.1	168	1.0
	32	372	2.0	1,068	6.0	1,137	4.9
	42	837	3.8	133	4.6	130	4.5
D		42	0.3	482	3.4	51	0.4

* Tests were carried out on pooled samples from 5 rats

Table 24

Immunoglobulin levels (µg/ml and % of total globulins) in faecal extracts of rats in Group A (infected with N. brasiliensis + T.brucei) and in Group B (infected with N. brasiliensis) following primary infection and after challenge at 28 DAPI and in Group D (worm-free controls)

Group	Days after Primary Infection	IgA		IgG		IgM	
		* µg/ml	%	* µg/ml	%	* µg/ml	%
A	8	137	2.7	2,520	18.4	367	2.8
	12	123	4.5	3,173	11.6	888	3.3
	14	132	3.2	4,152	10.1	1,162	3.8
	18	535	2.3	2,120	9.1	652	2.8
	28	198	3.1	780	12.1	1,438	2.3
	32	253	3.4	953	12.8	241	3.3
	42	112	2.8	407	10.2	180	3.2
B	8	315	2.3	1,820	9.0	404	2.0
	12	1,039	3.8	1,342	8.2	163	1.5
	14	883	2.5	1,415	4.3	394	1.2
	18	687	1.5	2,520	5.5	458	1.0
	28	321	2.0	643	4.0	121	0.8
	32	153	1.48	882	8.5	238	2.3
	42	138	1.38	707	7.0	107	1.0
D		68	0.6	841	2.1	35	0.3

* Tests were carried out on pooled samples from 5 rats

APPENDIX E

Appendix to Chapter 5

Table 1

Haemagglutinating antibody titres (mean log 10) against L4SE antigens in serum of rats after vaccination with N. brasiliensis antigens and after challenge with 5 x 10³ N. brasiliensis

	Group					
	I	II	III	IV	V	VI
9 Days After Vaccination	2.709	1.505	1.806	1.806	0.903	
	2.408	0.903	1.504	1.806	0.903	
	2.107	0.903	2.107	1.806	0.903	
	2.408	0.602	1.806	1.505	0.903	
	2.408	0.903	1.806	2.107	0.903	
Mean	2.408	0.903	1.806	1.806	0.903	
± S.E.	0.095	0.147	0.100	0.010	0.000	
9 Days After Challenge	0.903	1.204	0.301	1.505	1.806	1.505
	1.505	0.903	0.301	1.505	1.806	1.204
	0.903	1.505	0.301	1.505	1.806	1.505
	1.806	0.602	0.301	1.806	1.806	1.204
	0.602	1.204	0.301	1.204	1.806	1.204
Mean	1.204	1.084	0.301	1.505	1.806	1.324
± S.E.	0.220	0.150	0.000	0.096	0.000	0.010

Table 2

Haemagglutinating antibody titres (mean log 10) against ESP antigens in serum of rats after vaccination with N. brasiliensis antigens and after challenge with 5 x 10^3 N. brasiliensis

	Group					
	I	II	III	IV	V	VI
9 Days After Vaccination	0.602	0.301	0.602	0.602	0	
	0.903	0.301	0.301	0.602	0	
	0.602	0.301	0.301	0.602	0	
	0.301	0.301	0.602	0.602	0	
	0.602	0.301	0.602	0.602	0	
Mean	0.602	0.301	0.481	0.602	0	
± S.E.	0.095	0.000	0.070	0.000	0	
9 Days After Challenge	0.903	0.301	0.602	0.301	0.301	0.301
	0.602	0.301	0.903	0.000	0.301	0.301
	0.301	0.301	0.602	0.602	0.301	0.602
	0.903	0.301	0.903	0.000	0.301	0.301
	0.602	0.301	0.602	0.301	0.301	0.301
Mean	0.662	0.301	0.702	0.204	0.301	0.361
± S.E.	0.112	0.000	0.070	0.110	0.000	0.060

Table 3

Mucosal antibody isotypes (mean O.D. ± S.E.) against fourth stage larval somatic antigens (L4SE) and adult *Nippostrongylus brasiliensis* excretory secretory products (ES)

Group		L4SE IgA	L4SE IgG	L4SE IgM	Adult ESp IgA	Adult ESp IgG	Adult ESp IgM
I	a	1.001 ± 0.21	0.733 ± 0.074	0.825 ± 0.036	0.528 ± 0.09	0.376 ± 0.042	0.705 ± 0.060
	b	0.639 ± 0.1	0.805 ± 0.131	0.7 ± 0.079	0.618 ± 0.037	0.459 ± 0.066	0.413 ± 0.08
II	a	0.796 ± 0.075	0.684 ± 0.089	0.789 ± 0.059	0.632 ± 0.03	0.430 ± 0.084	0.541 ± 0.111
	b	0.56 ± 0.095	0.882 ± 0.041	0.667 ± 0.084	0.578 ± 0.085	0.514 ± 0.088	0.297 ± 0.081
III	a	0.642 ± 0.13	0.873 ± 0.098	0.781 ± 0.044	0.378 ± 0.097	0.554 ± 0.058	0.570 ± 0.112
	b	0.546 ± 0.06	0.683 ± 0.142	0.637 ± 0.09	0.622 ± 0.113	0.436 ± 0.093	0.384 ± 0.083
IV	a	0.470 ± 0	0.445 ± 0.047	0.828 ± 0.054	0.160 ± 0	0.525 ± 0.044	0.357 ± 0.129
	b	0.717 ± 0.1	0.506 ± 0.114	0.72 ± 0.183	0.546 ± 0.077	0.374 ± 0.045	0.431 ± 0.056
V	a	0.404 ± 0	0.778 ± 0.032	0.883 ± 0.02	0.512 ± 0	0.476 ± 0.006	0.617 ± 0.219
	b	0.607 ± 0.07	0.445 ± 0.11	0.672 ± 0.067	0.446 ± 0.08	0.496 ± 0.06	0.385 ± 0.022
VI		0.651 ± 0.06	1.001 ± 0.088	0.684 ± 0.092	0.559 ± 0.1	0.418 ± 0.022	0.570 ± 0.067
VII		0.156 ± 0.03		0.372 ± 0.14	0.199 ± 0.001	0.31 ± 0.02	0.262 ± 0.02

a = antibody level on day 9 after infection measured by ELISA

b = antibody level on day 9 after challenge measured by ELISA

(n = 5)

Table 4

Haemagglutinating antibody titres (mean log 10) against L4SE antigens in small intestine contents of rats after vaccination with N. brasiliensis antigens and after challenge with 5 x 10³ N.brasiliensis

	Group					
	I	II	III	IV	V	VI
9 Days After Vaccination	3.610	3.310	3.010	3.310	3.310	
	3.610	3.310	3.310	2.709	3.010	
	3.610	3.310	2.709	3.010	3.310	
	3.610	3.010	3.301	3.610	3.620	
	3.610	2.709	2.709	2.408	3.010	
Mean	3.610	3.060	3.008	3.010	3.252	
± S.E.	0.0	0.110	0.133	0.200	0.110	
9 Days After Challenge	3.610	1.204	3.310	3.310	2.709	3.310
	3.610	1.505	3.010	3.010	2.709	3.010
	3.610	1.806	3.310	3.610	2.709	3.310
	3.610	1.806	3.602	2.709	2.709	3.310
	3.610	1.204	3.010	3.410	2.709	3.010
Mean	3.610	1.505	3.248	3.209	2.709	3.190
± S.E.	0.0	0.010	0.111	0.016	0.000	0.070

Table 5

Haemagglutinating antibody titres (mean log 10) against ESP antigens in small intestine contents of rats after vaccination with N. brasiliensis antigens and after challenge with 5 x 10³ N. brasiliensis

	Groups					
	I	II	III	IV	V	VI
9 Days After Vaccination	1.806	1.204	2.107	1.506	0.602	
	1.806	1.806	2.408	1.506	0.602	
	1.806	0.903	2.107	1.506	0.602	
	1.806	0.903	2.709	1.506	0.602	
	1.806	1.204	1.806	1.506	0.602	
Mean	1.806	1.204	2.280	1.506	0.602	
± S.E.	0.0	0.165	0.150	0.000	0.000	
9 Days After Challenge	2.709	0.301	0.602	1.204	2.107	0.602
	2.408	0.000	0.602	1.204	2.406	0.301
	3.010	0.903	0.602	1.204	2.709	0.602
	2.709	0.000	0.602	1.204	2.107	0.903
	2.709	0.903	0.602	1.204	2.408	0.903
Mean	2.709	0.421	0.602	1.204	2.347	0.661
± S.E.	0.095	0.200	0.000	0.000	0.110	0.110

Table 6

Antibodies against N. brasiliensis fourth stage larval surface antigens after vaccination with N. brasiliensis antigens and following challenge with 5 x 10³ N. brasiliensis

Group	Percentage of antibody bound by Intestinal Contents		Faecal Extracts		Serum	
	9 DAV *	9 DPCh †	9 DAV *	9 DPCh †	9 DAV *	9 DPCh †
I	3.02 ± 0.8	8.12 ± 2.3	6.3 ± 3.1	12.3 ± 3.1	33.3 ± 4.3	21.7 † 5.1
II	13.2 ± 4	7.3 ± 1.4	13.5 ± 1.8	3.1 ± 1.7	30.7 ± 4.3	36.6 ± 5.4
III	18.1 ± 2.3	4.01 ± 1.9	3.5 ± 2.8	3.1 ± 1.7	18.5 ± 8.1	23 ± 8.9
IV	3.05± 1.4	25.7 ± 3.0	3.12± 3	6.4 ± 2.4	11.7 ± 7.2	20 ± 3.8
V	11.9 ± 2.9	3.1 ± 2.9	22.5 ± 3.1	10.6 ± 3.8	7.8 ± 3.2	10.1 ± 3.1
VI	6.13 ± 1.9		7.6		29.1 ± 2.3	
VII	2.8 ± 2.1		3.8		4.5 ± 2.1	

* DAV = day after vaccination

† DPCh = day post-challenge

Results show means from 5 animals. Data calculated as percentage TCA-precipitate input cpm per assay tube.

APPENDIX F

Appendix to Chapter 6

Table 1

Anti-L4SE IgA levels† in lung extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. (A = 0krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls and J = worm-free controls

	Group									
	A	B	C	D	E	F	G	H	I	*J
	0.600	0.742	0.410	0.986	0.228	0.340	0.304	0.560		0.202
9 Days	0.290	0.554	0.470	0.986	0.200	0.560	0.560	0.804		0.258
After	0.290	0.516	0.450	0.854	0.226	0.304				0.212
Vaccination	0.290	0.509	0.800	0.670	0.320	0.712				0.198
	0.220	0.472	0.701	0.670	0.256	0.380				0.254
Mean	0.338	0.559	0.566	0.830	0.246	0.459	0.432	0.682		0.224
± S.E.	0.066	0.050	0.077	0.077	0.020	0.080	0.080	0.077		0.010
	0.602	0.827	0.510	0.854	0.200	0.650	0.390	0.443	0.543	
9 days	0.150	0.650	0.670	0.854	0.248	0.720	0.600	0.220	0.671	
After	0.402	0.903	0.810	0.730	0.720	0.240	0.820	0.340	0.543	
Challenge	0.402	0.720	0.850	0.730	0.552	0.250	0.230	0.610	0.453	
	0.600	0.720	0.911	0.620	0.552	0.210	0.980	0.670	0.381	
Mean	0.430	0.768	0.760	0.756	0.454	0.414	0.604	0.456	0.518	
± S.E.	0.080	0.050	0.066	0.044	0.100	0.110	0.137	0.080	0.049	

* Worm-free control group † ELISA O.D. Values

Table 2

Anti-L4SE IgG levels† in lung extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls; J = worm-free controls)

	Groups									
	A	B	C	D	E	F	G	H	I	*J
Days After Vaccination	0.193	0.386	0.145	0.478	0.496	0.465	0.517	0.444		0.158
	0.171	0.209	0.223	0.337	0.443	0.464	1.013	0.797		0.212
	0.669	0.287	0.338	0.384	0.436	0.554				0.176
	0.149	0.255	0.330	0.405	0.548	0.457				0.136
	0.243	0.177	0.418	0.413	0.443	0.481				0.140
Mean	0.285	0.257	0.301	0.403	0.473	0.484	0.765	0.620		0.164
± S.E.	0.097	0.037	0.051	0.023	0.022	0.018	0.157	0.111		0.010
Days After Challenge	0.132	0.107	0.357	0.395	0.462	0.572	0.310	0.172	0.203	
	0.176	0.408	0.866	0.476	0.419	0.595	0.527	0.236	0.266	
	0.218	0.530	0.299	0.361	0.601	0.371	0.708	0.155	0.349	
	0.268	0.471	0.233	0.363	0.461	0.430	0.287	0.727	0.788	
	0.142	0.106	0.340	0.689	0.427	0.530	0.311	0.664		
Mean	0.187	0.324	0.419	0.457	0.474	0.500	0.429	0.391	0.402	
± S.E.	0.026	0.091	0.114	0.062	0.033	0.043	0.082	0.126	0.132	

* Worm-free controls. † ELISA O.D. Values

Table 3

Anti-L4SE IgM levels† in lung extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls and J = worm-free controls)

	Group									
	A	B	C	D	E	F	G	H	I	*J
	0.564	0.156	0.660	0.793	0.189	0.448	0.811	0.663		0.282
Days	0.826	0.210	0.489	0.866	0.763	0.302	0.390	0.643		0.312
After	0.902	0.541	0.644	0.253	0.978	0.749				0.258
Vaccination	0.490	0.717	0.590	0.590	0.910	0.629				0.213
	0.811	0.846	0.215	0.464	0.715	0.016				0.282
Mean	0.719	0.494	0.520	0.593	0.711	0.629	0.600	0.653		0.269
± S.E.	0.081	0.136	0.082	0.111	0.139	0.123	0.133	0.006		0.020
	0.813	0.078	0.395	0.687	0.425	0.873	0.000	0.008	0.754	
Days	0.723	0.712	0.709	0.136	0.748	0.917	0.000	0.009	0.765	
After	0.499	0.205	0.574	0.885	0.187	0.620	0.910	0.010	0.851	
Challenge	0.110	0.316	0.605	0.644	1.011	0.227	0.713	0.006	0.000	
	0.541	0.111	0.333	0.258	0.609	1.009	0.563	0.008		
Mean	0.537	0.284	0.523	0.522	0.569	0.729	0.437	0.008	0.593	
± S.E.	0.121	0.115	0.069	0.140	0.140	0.141	0.117	0.000	0.197	

* Worm-free Controls † ELISA O.D. Values

Table 4

Anti-ESP IgA levels† in lung extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls; J = worm-free controls)

	A	B	C	D	E	F	G	H	I	*J
9 Days After Vaccination	0.186	0.160	0.160	0.189	0.200	0.210	0.419	0.420		0.189
	0.180	0.200	0.060	0.196	0.318	0.096	0.419	0.480		0.194
	0.110	0.180	0.120	0.230	0.160	0.170				0.171
	0.250	0.180	0.140	0.130	0.222	0.210				0.156
	0.110	0.250	0.140	0.180	0.080	0.210				0.223
Mean	0.167	0.194	0.124	0.175	0.196	0.179	0.419	0.450		0.186
± S.E.	0.026	0.015	0.017	0.020	0.040	0.020	0.000	0.018		0.011
9 Days After Challenge	0.226	0.282	0.105	0.201	0.200	0.255	0.170	0.180	0.186	
	0.104	0.340	0.107	0.240	0.140	0.170	0.130	0.300	0.228	
	0.220	0.320	0.134	0.060	0.104	0.170	0.504	0.252	0.293	
	0.105	0.280	0.182	0.230	0.214	0.170	0.116	0.202	0.313	
	0.110	0.320	0.204	0.240	0.200	0.170	0.658	0.301	0.186	
Mean	0.161	0.312	0.146	0.194	0.171	0.187	0.310	0.247	0.241	
± S.E.	0.034	0.010	0.020	0.034	0.020	0.017	0.110	0.025	0.026	

* worm-free controls

† ELISA O.D. values

Table 5

Anti-ESP IgG levels† in lung extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls and J = worm-free controls)

	A	B	C	D	E	F	G	H	I	*J
	0.241	0.297	0.403	0.472	0.345	0.052	0.842	0.820		0.212
Days	0.167	0.466	0.435	0.292	0.151	0.031	0.684	0.815		0.256
After	0.248	0.268	0.147	0.150	0.236	0.434				0.238
Vaccination	0.319	0.203	0.093	0.204	0.161	0.525				0.270
	0.320	0.415	0.188	0.059	0.436	0.585				0.212
Mean	0.259	0.330	0.253	0.235	0.266	0.468	0.763	0.817		0.237
± S.E.	0.028	0.048	0.070	0.070	0.055	0.065	0.097	0.002		0.010
	0.331	0.205	0.420	0.277	0.467	0.151	0.261	0.216	0.366	
Days	0.228	0.336	0.474	0.270	0.172	0.392	0.381	0.053	0.369	
After	0.448	0.384	0.333	0.288	0.216	0.162	0.590	0.252	0.437	
Challenge	0.411	0.432	0.302	0.335	0.454	0.079	0.391	0.199	0.289	
	0.514	0.388	0.259	0.192	0.098	0.443	0.546	0.213		
Mean	0.386	0.349	0.358	0.272	0.282	0.245	0.434	0.187	0.365	
± S.E.	0.049	0.039	0.039	0.023	0.076	0.072	0.060	0.034	0.030	

* worm-free controls † ELISA O.D. values

Table 6

Anti-ESP IgM levels † in lung extracts of rats following vaccination and after challenge with 5 x 10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls and J = worm-free controls)

	A	B	C	D	E	F	G	H	I	*J
Days After Vaccination	0.309	0.157	0.235	0.299	0.246	0.214	0.783	0.643		0.265
	0.138	0.376	0.134	0.112	0.417	0.087	0.617	0.215		0.282
	0.143	0.323	0.179	0.203	0.481	0.153				0.258
	0.214	0.296	0.168	0.203	0.411	0.468				0.312
	0.165	0.120	0.185	0.137	0.279	0.520				0.241
Mean	0.194	0.254	0.180	0.191	0.367	0.288	0.700	0.425		0.272
± S.E.	0.032	0.049	0.016	0.033	0.045	0.087	0.052	0.132		0.010
Days After Challenge	0.302	0.270	0.525	0.262	0.264	0.496	0.501	0.203	0.481	
	0.166	0.314	0.229	0.158	0.197	0.696	0.527	0.195	0.300	
	0.329	0.090	0.173	0.161	0.090	0.152	0.298	0.400	0.401	
	0.352	0.157	0.267	0.363	0.367	0.328	0.468	0.286	0.514	
	0.145	0.286	0.223	0.237	0.515	0.162	0.165	0.392		
Mean	0.259	0.306	0.283	0.236	0.287	0.361	0.392	0.295	0.424	
± S.E.	0.043	0.077	0.062	0.038	0.073	0.102	0.069	0.044	0.048	

* worm-free controls

† ELISA O.D. values

Table 7

Anti-L4SE IgA levels† in small intestine mucosal extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls and J = worm-free controls)

	Group									
	A	B	C	D	E	F	G	H	I	*J
	0.165	0.342	0.360	0.210	0.782	0.442	0.222	0.208		0.202
9 Days	0.400	0.160	0.508	0.248	0.540	0.706	0.246	0.224		0.116
after	1.580	0.106	0.472	0.312	0.690	0.446				0.212
Vaccination	1.265	0.104	0.716	0.210	0.646	0.290				0.200
	0.595	0.422	0.472	0.255	0.230	0.440				0.050
Mean	1.001	0.226	0.506	0.247	0.577	0.385	0.234	0.216		0.156
± S.E.	0.210	0.020	0.058	0.118	0.100	0.100	0.012	0.006		0.030
	0.672	0.420	0.354	0.610	0.732	0.632	0.498	0.444	0.810	
9 Days	0.800	0.808	0.504	0.940	0.934	1.108	0.646	0.684	0.580	
After	0.760	0.932	0.472	0.888	0.796	0.960	0.474	0.721	0.740	
Challenge	0.260	0.508	0.716	0.600	0.646	0.886	0.998	0.521	0.648	
	0.740	0.506	0.472	0.544	0.852	0.852	0.730	0.696	0.480	
Mean	0.639	0.634	0.503	0.716	0.792	0.887	0.670	0.613	0.651	
± S.E.	0.100	0.100	0.059	0.082	0.005	0.080	0.100	0.050	0.060	

* worm-free controls † ELISA O.D. values

Table 8

Anti-L4SE IgG levels† in small intestine mucosal extracts of rats following vaccination and after challenge with 5 x 10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls and J = worm-free controls)

	Group									
	A	B	C	D	E	F	G	H	I	*J
	0.799	0.051	0.605	0.734	0.866	0.470	0.592	0.274		
9 Days	0.464	0.678	0.613	0.077	0.835	0.310	0.700	0.916		
After	0.829	0.525	0.688	0.715	0.910	0.619				
Vaccination	0.895	0.719	0.416	0.591	0.822	0.825				
	0.729	0.455	0.390	0.632	0.778	0.854				
Mean	0.733	0.486	0.542	0.750	0.842	0.616	0.646	0.595		
± S.E.	0.074	0.119	0.059	0.086	0.022	0.104	0.054	0.321		
	1.077	0.719	0.428	0.499	0.393	1.134	1.100	0.860	1.223	
9 Days	0.945	0.622	0.513	0.801	0.166	0.879	1.020	0.894	1.057	
After	1.026	0.552	0.130	0.982	1.114	0.655	0.946	0.909	0.869	
Challenge	0.449	0.577	0.380	0.687	0.941	1.111	0.990	0.724	1.122	
	0.530	0.421	0.642	0.570	0.482	0.879	0.789	0.466	0.736	
Mean	0.805	0.578	0.414	0.709	0.819	0.932	0.969	0.771	1.001	
± S.E.	0.131	0.049	0.085	0.086	0.161	0.088	0.052	0.083	0.988	

* Not Done † ELISA O.D. values

Table 9

Anti-L4SE IgM levels† in small intestine mucosal extracts of rats following vaccination and after challenge with 5 x 10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls and J = worm-free controls)

	Groups									
	A	B	C	D	E	F	G	H	I	*J
	0.736	0.947	1.000	0.897	0.887	0.836	0.952	0.819		
9 Days	0.783	0.872	0.875	0.849	0.835	0.701	0.816	0.833		
After	0.923	0.697	0.947	0.823	0.880	0.857				
Vaccination	0.899	0.742	0.907	0.970	0.916	0.811				
	0.784	0.823	0.921	0.394	0.815	0.904				
Mean	0.825	0.813	0.926	0.787	0.887	0.822	0.884	0.826		0.372
± S.E.	0.036	0.047	0.024	0.101	0.018	0.034	0.068	0.007		0.014
	0.819	0.866	0.784	0.649	0.123	0.966	0.930	0.406	0.535	
9 Days	0.549	0.962	0.725	0.820	0.783	0.934	0.986	0.775	0.605	
After	0.555	0.883	0.401	0.851	0.470	0.900	0.960	0.909	1.042	
Challenge	0.948	0.704	0.804	0.688	0.849	0.945	0.964	0.841	0.572	
	0.630	0.688	0.771	0.854	0.860	0.823	0.846	0.812	0.667	
Mean	0.700	0.821	0.697	0.772	0.617	0.941	0.937	0.732	0.684	
± S.E.	0.079	0.053	0.075	0.043	0.142	0.025	0.024	0.112	0.092	

* Worm-free controls † ELISA O.D. values

Table 10

Anti-ESP IgA levels† in small intestine mucosal extracts of rats following vaccination and after challenge with 5 x 10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls and J = worm-free controls)

	Group									
	A	B	C	D	E	F	G	H	I	*J
	0.325	0.226	0.172	0.175	0.286	0.166	0.202	0.260		0.201
9 Days	0.480	0.200	0.126	0.105	0.020	0.106	0.202	0.288		0.178
After	0.580	0.210	0.122	0.230	0.640	0.140				0.206
Vaccination	0.855	0.274	0.104	0.155	0.220	0.222				0.228
	0.420	0.474	0.128	0.155	0.172	0.200				0.178
Mean	0.528	0.276	0.130	0.164	0.268	0.166	0.202	0.247		0.198
± S.E.	0.090	0.050	0.110	0.020	0.103	0.020	0.000	0.008		0.001
	0.647	0.746	0.472	0.726	0.616	0.906	0.480	0.760	0.828	
9 Days	0.672	0.640	0.826	0.522	0.942	0.688	0.600	0.512	0.299	
After	0.481	0.436	0.622	0.913	0.700	0.874	0.612	0.916	0.580	
Challenge	0.666	0.600	0.408	0.656	0.970	0.760	0.624	0.740	0.370	
	0.604	0.580	0.828	0.332	0.870	0.300	0.283	0.740	0.720	
Mean	0.619	0.600	0.631	0.629	0.819	0.705	0.519	0.733	0.559	
± S.E.	0.037	0.050	0.090	0.100	0.070	0.110	0.004	0.060	0.100	

* worm-free controls † ELISA O.D. values

Table 11

Anti-ESP IgG levels† in small intestine mucosal extracts of rats following vaccination and after challenge with 5 x 10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls and J = worm-free controls)

	Groups									
	A	B	C	D	E	F	G	H	I	*J
	0.336	0.422	0.501	0.528	0.342	0.481	0.500	0.349		
9 Days	0.414	0.488	0.504	0.460	0.429	0.621	0.601	0.439		
After	0.340	0.600	0.455	0.306	0.343	0.532				
Vaccination	0.519	0.420	0.308	0.510	0.594	0.670				
	0.270	0.637	0.300	0.544	0.638	0.526				
Mean	0.376	0.513	0.414	0.470	0.469	0.566	0.551	0.394		0.310
± S.E.	0.042	0.045	0.046	0.043	0.062	0.035	0.050	0.045		0.020
	0.669	0.428	0.287	0.176	0.634	0.736	0.562	0.666	0.478	
9 Days	0.431	0.443	0.335	0.698	0.645	0.550	0.613	0.912	0.447	
After	0.534	0.305	0.515	0.612	0.663	0.606	0.541	0.617	0.415	
Challenge	0.294	0.238	0.467	0.541	0.763	0.526	0.611	0.688	0.378	
	0.367	0.379	0.453	0.653	0.530	0.516	0.818	0.562	0.370	
Mean	0.459	0.359	0.411	0.536	0.647	0.594	0.629	0.689	0.418	
± S,E,	0.066	0.039	0.043	0.094	0.037	0.038	0.049	0.060	0.020	

* worm-free controls † ELISA O.D. values

Table 12

Anti-ESP IgM levels† in small intestine mucosal extracts of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls and J = worm-free controls)

	Groups									
	A	B	C	D	E	F	G	H	I	*J
	0.271	0.260	0.570	0.742	0.770	0.341	0.916	0.311		
9 Days	0.637	0.135	0.240	0.887	0.874	0.342	0.643	0.355		
After	0.774	0.790	0.611	1.033	0.943	0.536				
Vaccination	0.873	0.479	0.574	0.952	0.990	0.235				
	0.520	0.369	0.590	0.421	0.940	0.658				
Mean	0.705	0.407	0.517	0.807	0.903	0.422	0.789	0.333		0.262
± S.E.	0.060	0.116	0.070	0.107	0.038	0.076	0.137	0.022		0.020
	0.279	0.444	0.337	0.319	0.273	0.290	1.016	0.724	0.438	
9 Days	0.273	0.500	0.267	0.248	0.760	0.233	1.070	0.091	0.410	
After	0.332	0.345	0.323	0.381	0.193	0.660	0.940	0.527	0.607	
Challenge	0.491	0.643	0.567	0.297	0.629	0.805	0.940	0.336	0.780	
	0.691	0.605	0.605	0.591	0.328	0.372	1.020	0.591	0.617	
Mean	0.413	0.507	0.420	0.367	0.437	0.472	0.997	0.434	0.570	
± S.E.	0.080	0.054	0.069	0.060	0.109	0.111	0.025	0.110	0.067	

* worm-free controls † ELISA O.D. values

Table 13

Anti-L4SE haemagglutinating antibody titres (log 10) in small intestine contents of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls

	Groups								
	A	B	C	D	E	F	G	H	I
	3.610	3.950	3.612	3.310	3.310	3.010	3.31	3.010	
9 Days	3.610	3.950	3.612	3.310	3.310	3.010	2.709	3.310	
After	3.610	3.950	3.913	3.310	3.011	3.010	2.709	2.709	
Vaccination	3.610	3.950	3.913	3.310	3.602	3.010	3.620	3.310	
	3.610	3.950	3.612	3.310	3.011		3.620	3.010	
Mean	3.610	3.950	3.700	3.310	3.250	3.010	3.313	3.070	
± S.E.	0.000	0.000	0.008	0.000	0.110	0.000	0.240	0.110	
	3.610	3.310	2.709	3.670	3.010	3.670	2.107	2.700	3.310
9 Days	3.610	3.310	3.010	3.670	3.310	3.670	1.806	2.408	3.010
After	3.610	3.310	2.709	3.670	2.709	3.670	2.107	3.010	3.310
Challenge	3.610	3.310	3.010	3.670	3.010	3.670	1.806	2.709	3.310
	3.610	3.310	2.709	3.670	2.709		1.806	3.010	3.010
Mean	3.610	3.310	2.800	3.670	3.000	3.670	1.926	2.770	3.190
± S.E.	0.000	0.000	0.030	0.000	0.112	0.000	0.070	0.110	0.070

Table 14

Anti-ESP haemagglutinating antibody titres(log 10) in small intestine contents of rats following vaccination and after challenge with 5×10^3 N. brasiliensis. (A = 0 krad; B = 80 krad; C = 120 krad; D = 140 krad; E = 160 krad; F = 180 krad; G = L3SE intravenously; H = L3SE intragastrically; I = challenge controls

	Groups								
	A	B	C	D	E	F	G	H	I
	1.806	2.107	2.709	1.804	1.806	1.505	2.107	0.301	
9 Days	1.806	2.709	2.709	2.107	2.107	1.505	2.107	0.301	
After	1.806	1.504	2.408	1.204	1.505	0.903	1.806	0.301	
Vaccination	1.806	3.011	2.408	1.804	2.709	0.903	2.107	0.301	
	1.806	1.203	2.709	2.107	1.806		1.806	0.301	
Mean	1.806	2.100	2.560	1.806	1.990	1.100	1.986	0.301	
± S.E.	0.000	0.340	0.070	0.090	0.204	0.150	0.070	0.000	
	2.709	1.504	2.107	1.505	0.602	0.301	0.301	0.301	0.602
9 Days	2.408	2.107	1.806	1.505	0.903	0.602	0.301	0.000	0.301
After	3.010	1.504	2.107	1.505	0.602	0.602	0.301	0.602	0.602
Challenge	2.709	2.107	1.806	1.505	1.204	0.602	0.301	0.301	0.903
	2.709	1.806	2.107	1.505	0.301		0.301	0.602	0.903
Mean	2.709	1.800	1.980	1.505	0.700	0.450	0.301	0.361	0.661
± S.E.	0.095	0.134	0.090	0.000	0.153	0.110	0.000	0.110	0.110

APPENDIX G

Appendix to Chapter 1

Table 1

Body Weights of gerbils (mean g ± S.E.) following infection with 1,500 T. colubriformis larvae and in worm free controls

	Days after infection							
	11	18	25	32	39	46	53	60
Infected	50 ± 1.7 (n = 23)	46 ± 1.4 (n = 19)	47 ± 1.4 (n = 16)	50 ± 1.4 (n = 16)	51 ± 1.4 (n = 16)	50 ± 1.3 (n = 11)	52 ± 1.4 (n = 8)	47 ± 3.0 (n = 8)
Control	52 ± 1.9 (n = 14)	53 ± 1.9 (n = 14)	52 ± 3.2 (n = 9)	55 ± 2.9 (n = 9)	52 ± 3.2 (n = 9)	58 ± 3.2 (n = 9)	58 ± 2.0 (n = 4)	60 ± 2.8 (n = 4)
P	N.S.	< 0.01	N.S.	N.S.	N.S.	< 0.05	< 0.05	< 0.02

Table 2

Worm burdens, body weights and packed cell volumes in gerbils following infection with 1,500 T. colubriformis

	Days after Infection								
	10	15	20	25	30	40	40 ♂	50	60
Worm Burdens	(1) 155	(6) 349	(11) 59	(16) 297	(21) 412	(26) 622	(91) 472	(33) 16	(38) 75
	(2) 64	(7) 330	(12)109	(17) 144	(22) 494	(27) 93	(92) 227	(34) 29	(39) 293
	(3) 79	(8) 353	(13)373	(18) 147	(23) 360	(28) 8	(93) 201	(35) 0	(40) 90
	(4) 39	(9) 329	(14) 12	(19) 94	(24) 348	(29) 194	(94) 44	(36) 58	(41) 12
	(5) 53	(10) 242	(15)101	(20) 121	(25) 367	(30) 451	(95) 147	(37) 4	(42) 491
Mean ± S.E.	72 ± 20	321 ± 20	131 ± 63	161 ± 35	396 ± 27	274 ± 114	218 ± 71	21 ± 10	192 ± 88
Body Weight g	(1) 73.7	(6) 49.3	(11) 49.6	(16) 37.0	(21) 43.7	(26) 52.4	(91) 52.0	(33) 56.5	(38) 52
	(2) 45.3	(7) 49.1	(12) 53.0	(17) 41.0	(22) 40.0	(27) 40.0	(92) 48.0	(34) 51.4	(39) 44
	(3) 49.3	(8) 48.1	(13) 44.4	(18) 51.0	(23) 53.0	(28) 58.8	(93) 55.0	(35) 46.0	(40) 47
	(4) 47.5	(9) 44.4	(14) 56.0	(19) 40.0	(24) 50.0	(29) 50.4	(94) 64.0	(36) 53.0	(41) 56
	(5) 50.3	(10) 45.8	(15) 41.5	(20) 40.0	(25) 46.5	(30) 43.4	(95) 49.2	(37) 48.5	(42) 33
Mean ± S.E.	53 ± 5	47 ± 1	49 ± 3	41.8 ± 2	46.6 ± 2	49 ± 3	54 ± 3	44 ± 2	46 ± 4
Packed Cell Volume %	(1) 41	(6) 41	(11) 39	(16) 38	(21) 29.5	(26) 41	(91) 32	(33)	(38) 28
	(2) 36	(7) 37	(12) 36	(17) 35	(22) 32	(27) 29	(92) 39	(34)	(39) 36
	(3) -	(8) 34	(13) 28	(18) 43	(23) 30	(28) 42	(93) 43	(35) 43	(40) 36
	(4) 38.5	(9) 37	(14) 38	(19) 39	(24) 37	(29) 43	(94) 44	(36) 44	(41) 45
	(5) 43.5	(10) 36	(15) 28	(20) 32	(25) 33	(30) 41	(95) 39	(37) 45	(42) 33
Mean ± S.E.	40 ± 2	37 ± 1	34 ± 2	38 ± 2	32 ± 1	39 ± 3	39 ± 2	44± 0.5	36 ± 2.5

Numbers in brackets are gerbil numbers.

Table 3

T. colubriformis egg counts in faeces of gerbils (egg/g faeces x 10^3) following infection with 1,500 larvae

Day of Infection	♂ Cage C	Cage D	Cage E	Cage F	Mean ± S.E.
17	0.625	0.267	1.300		0.730 ± 0.30
19	3.975	3.125	7.750		4.950 ± 1.42
21	7.225	7.375	14.150		9.580 ± 2.28
24	8.350	7.075	22.525	17.175	13.781 ± 3.68
26	11.175	7.900	18.600	-	12.558 ± 3.16
28	9.975	6.875	26.200	12.575	13.906 ± 4.26
31	8.500	2.925	12.850	12.725	9.250 ± 2.34
33	2.300	1.400	3.900	7.875	3.870 ± 1.43
35	3.975	2.150	2.750	10.300	4.794 ± 1.87
38	5.075	2.075	8.325	7.425	5.725 ± 1.40
40	6.650	4.475	17.025	11.150	9.825 ± 2.77
42	-	3.475	9.450	6.275	6.400 ± 1.73
45		5.125	15.200	5.175	8.500 ± 3.35
49		1.875	14.500		8.187
52		1.750	14.900		8.325
54		1.325	14.375		7.85
56					
59					
60					

Table 4

Serum total protein, albumin and globulin levels in gerbils, following infection with 1,500 T. colubriformis

	Days after Infection								
	10	15	20	25	30	40	40 ♂	50	60
Total Protein g/100 ml	(1) 5.6 (2) 4.9 (3) 4.6 (4) 5.4 (5) 5.9	(6) 5.0 (7) 8.5 (8) 8.0 (9) 8.3 (10) 6.8	(11) 4.4 (12) 3.9 (13) 3.2 (14) 4.4 (15) 4.2	(16) * (17) 5.0 (18) 5.4 (19) 5.3 (20) 4.3	(21) 5.6 (22) * (23) 4.4 (24) 4.5 (25) 5.3	(26) 5.7 (27) 5.6 (28) 6.5 (29) 6.6 (30) 4.6	(91) 6.1 (92) 3.9 (93) 6.5 (94) 6.0 (95) 5.3	(33) 5.0 (34) 5.4 (35) 4.8 (36) 5.2 (37) 5.3	(38) 6.7 (39) 4.2 (40) 4.2 (41) 5.2 (42) 6.1
Mean	5.3 ± 0.2	7.32 ± 0.65	4.0 ± 0.2	5.0 ± 0.25	5.0 ± 0.3	5.8 ± 0.36	5.6 ± 0.46	5.1 ± 0.11	5.3 ± 0.50
Albumin g/100 ml	(1) 3.0 (2) 3.3 (3) 2.5 (4) 2.9 (5) 2.8	(6) * (7) 2.1 (8) 1.7 (9) 2.1 (10) 1.4	(11) * (12) 1.1 (13) 1.2 (14) 1.9 (15) *	(16) * (17) * (18) * (19) * (20) 1.4	(21) * (22) * (23) * (24)* (25) *	(26) * (27) 1.1 (28) 2.1 (29) * (30) 1.7	(91) 0.9 (92) 0.7 (93) 1.8 (94) 2.4 (95) 1.5	(33) * (34) * (35) * (36) * (37) 2.2	(38) 1.2 (39) 1.7 (40) * (41) 2.1 (42) 1.5
Mean	2.9 ± 0.13	1.8 ± 0.17	1.4 ± 0.25			1.63 ± 0.29	1.46 ± 0.31		1.6 ± 0.19
Globulins g/100 ml	(1) 2.6 (2) 1.6 (3) 2.1 (4) 2.6 (5) 3.1	(6) * (7) 6.4 (8) 6.3 (9) 6.2 (10) 2.8	(11) * (12) 2.8 (13) 2.0 (14) 2.5 (15) *	(16) * (17) * (18) * (19) * (20) 3.0	(21) * (22) * (23) * (24) * (25) *	(26) * (27) 4.5 (28) 4.4 (29) * (30) 2.9	(91) 5.2 (92) 3.2 (93) 4.7 (94) 3.6 (95) 2.9	(33) * (34) * (35) * (36) * (37) 3.1	(38) 5.5 (39) 5.0 (40) * (41) 3.1 (42) 4.6
Mean	2.4 ± 0.78	5.4 ± 0.87	2.4 ± 0.23			3.9 ± 0.52	3.9 ± 0.44		4.5 ± 0.52
A/G Ratios	(1) 0.87 (2) 0.48 (3) 0.84 (4) 1.12 (5) 0.90	(6) * (7) 0.33 (8) 0.27 (9) 0.34 (10) 0.50	(11) * (12) 0.39 (13) 0.60 (14) 0.76 (15) *	(16) * (17) * (18) * (19) * (20) 0.47	(21) * (22) * (23) * (24) * (25) *	(26) * (27) 0.24 (28) 0.48 (29) * (30) 0.59	(91) 0.17 (92) 0.22 (93) 0.38 (94) 0.67 (95) 0.52	(33) * (34) * (35) * (36) * (37) 0.71	(38) 0.22 (39) 0.34 (40) * (41) 0.68 (42) 0.33
Mean	0.84 ± 0.10	0.36 ± 0.05	0.58 ± 0.11			0.44 ± 0.10	0.39 ± 0.17		0.39 ± 0.10

Numbers in brackets are gerbil numbers

* Insufficient serum to complete tests

Table 5

Serum total protein, albumin and globulin levels in non-infected (control) gerbils

	Day of Experiment	
	18	25
Total Protein g/100 ml	(1) 6.0	(6) 5.4
	(2) *	(7) 2.7
	(3) 5.0	(8) 5.6
	(4) 5.0	(9) 6.6
	(5) 5.0	(10) 5.8
Mean ± S.E.	5.2 ± 0.25	5.2 ± 0.66
Albumin g/100 ml	(1) 2.4	(6) *
	(2) *	(7) *
	(3) 0.9	(8) 2.2
	(4) 1.7	(9) 2.5
	(5) 1.8	(10) *
Mean ± S.E.	1.7 ± 0.31	2.4 ± 0.1
Globulin g/100 ml	(1) 3.6	(6) *
	(2) *	(7) *
	(3) 4.1	(8) 3.1
	(4) 3.3	(9) 4.1
	(5) 3.2	(10) *
Mean ± S.E.	3.6 ± 0.20	3.6 ± 0.50
A/G ratio	(1) 0.67	(6) *
	(2) *	(7) *
	(3) 0.22	(8) 0.71
	(4) 0.51	(9) 0.61
	(5) 0.56	(10) *
Mean ± S.E.	0.49 ± 0.10	0.66 ± 0.05

Numbers in brackets are gerbil numbers.

APPENDIX H

Appendix to Chapter 2

Table 1

Faecal egg counts in gerbils following infection with 1,500 T. colubriformis larvae, drug-susceptible or drug-resistant strain

Strain of T. colubriformis	Cage	Days after Infection														
		17	20	22	24	27	29	31	34	36	38	41	43	45	48	50
Susceptible	J	3,270	10,170	11,970	16,530	9,100	7,560	8,100	14,030	12,250	10,400	12,400	6,700	-	-	-
	L	470	7,830	8,670	15,800	5,900	9,160	9,000	8,960	3,300	18,800	2,700	4,400	4,500	3,900	1,800
	M	260	5,870	12,300	15,930	7,850	14,000	11,100	11,830	8,250	13,000	-	-	-	-	-
	O	1,233	15,330	15,770	27,130-	24,800	17,700	-	-	-	-	-	-	-	-	-
	Mean	1,310	9,800	12,180	18,850	11,910	12,105	9,400	11,600	7,930	14,060	7,750	5,550			
	± SE	587	2,042	1,451	2,765	4,350	2,310	890	1,465	2,590	2,480	1,150	1,150			
Resistant	E	330	4,230	7,500	8,430	9,230	13,300	5,100	10,060	9,050	13,000	8,400	18,200	-	-	-
	G	100	4,930	4,370	16,930	10,900	5,230	20,100	14,600	6,050	18,000	16,000	7,400	9,200	14,300	5,000
	H	260	3,700	1,670	15,800	9,130	10,600	9,600	5,400	8,850	15,000	-	-	-	-	-
	I	66	2,770	8,030	12,200	12,800	9,930	17,000	9,600	6,900	10,000	8,800	-	11,800	10,700	7,600
	Mean	190	3,910	5,390	13,340	10.515	10,140	12,950	9,940	7,710	14,000	11,060	11,760	11,499	12,500	6,300
	± SE	60	455	1,480	1,922	860	1,251	3,420	1,630	736	1,683	2,470	3,510	2,940	1,800	1,300

Table 2

Numbers of worms recovered from gerbils following infection with 1,500 drug-susceptible or drug-resistant T. colubriformis

Group	Days after Infection				
	10	20	30	40	50
Drug Susceptible Strain Infections (DS)	88	622	354	156	326
	63	560	361	401	420
	54	743	411	399	525
	49	531	404	389	154
		552			400
Mean ± S.E.	64 ± 9	602 ± 34	383 ± 14	336 ± 60	365 ± 62
Drug Resistant Strain Infections (DR)	63	365	331	231	392
	-	224	320	164	349
	114	265	299	395	566
	231	345	256	318	550
		498			69
					295
Mean ± S.E.	136 ± 43	339 ± 47	302 ± 17	277 ± 50	370 ± 75
't' test DS/DR P	N.S.	< 0.01	< 0.01	N.S.	N.S.

Table 3

Body weights of individual gerbils following infection with 1,500 drug-susceptible or drug-resistant strain of T. colubriformis immediately prior to necropsy

	Days after infection				
	10	20	30	40	50
Drug Susceptible Strain Infections	49.0	39.0	50.0 *	55.0	60.0
	40.0	43.0	66.6	59.0	55.0
	47.0	38.0	49.9	59.0	60.0
	54.0	36.1	58.3	50.0	64.0
		41.0	49.6		69.0
Mean ± S.E.	47.5 ± 2.9	39.4 ± 1.2	54.9 ± 3.4	55.8 ± 2.1	61.6 ± 2.3
Drug Resistant Strain Infections	45.0	49.0	54.8 *	71.0	50.0
	42.0	41.0	43.3	59.0	50.0
	50.0	45.0	45.5	55.0	51.0
	44.0	43.0	40.6	62.0	60.0
		48.0	49.3		70.0
					70.0
Mean ± S.E.	45.3 ± 1.7	45.2 ± 1.5	46.7 ± 2.5	61.7 ± 3.4	58.7 ± 4.0

* Worm burdens not assessed

Table 4

Body weights of groups of gerbils (mean + S.E.) following infection with 1,500 T. colubriformis larvae, drug-susceptible or drug-resistant strain, and weights of worm-free controls (mean + S.E.)

Group		Days after Infection					
		14	21	28	35	42	49
T.colubriformis (drug susceptible strains) infected	Mean	50.4	52.1	52.7	56.3	61.0	61.6
	± S.E.	1.4	1.4	1.6	1.7	0.6	2.3
	Number in Group	29	14	12	10	4	5
T.colubriformis (drug resistant strain) infected	Mean	50.0	49.6	52.1	55.9	56.2	57.9
	± S.E.	1.4	1.7	1.7	1.9	2.7	3.1
	Number in Group	33	27	28	24	17	10
Worm-free controls	Mean	58.1	56.6	57.1	59.2	58.5	56.8
	± S.E.	1.7	1.3	1.4	1.5	1.8	1.6
	Number in Group	27	26	24	21	14	12

Table 5

Total protein concentrations (g/100 ml) in gerbils following infection with drug-susceptible or drug-resistant T. colubriformis and in worm-free controls

	Days after Infection				
	10	20	30	40	50
Drug Susceptible Strain T.colubriformis Infections	7.2	4.8	5.1	5.2	5.0
	6.5	4.8	4.1	3.8	5.4
	7.9	4.4	4.5	4.0	4.8
	5.4	4.8	4.5	5.4	5.1
		5.2			4.9
Mean	6.8	4.8	4.6	4.6	5.1
± S.E.	0.53	0.13	0.21	0.41	0.1
Drug Resistant Strain T. colubriformis Infections	5.7	5.1	4.5	6.2	4.3
	4.9	4.6	3.8	5.6	7.1
	5.1	4.5	4.4	5.6	4.3
	5.0	5.4		3.9	5.5
		4.7			6.3
					6.1
Mean	5.2	4.1	4.2	4.9	5.6
± S.E.	0.18	0	0.22	0.4	0.4

Mean total protein concentration in worm-free control gerbils:

6.0 ± 0.32 g/100 ml (n = 9)

Table 6

Serum albumin concentrations (g/100 ml) in gerbils following infection with drug-susceptible or drug-resistant strains of T. colubriformis and in worm-free controls

Group	Days after Infection				
	10	20	30	40	50
Drug Susceptible Strain T.colubriformis Infections	3.2	3.0	3.2	1.8	1.6
	2.6	2.9	2.4	2.1	2.6
	3.4	2.0	2.3	1.7	2.4
	1.7	2.9	2.7	2.3	1.8
		2.1			2.4
Mean	2.7	2.6	2.7	2.0	2.2
± S.E.	0.38	0.22	0.20	0.14	0.19
Drug Resistant Strain T.colubriformis Infection	2.2	2.2	3.2	2.9	2.1
	2.2	2.5	2.1	3.3	1.6
	2.9	2.4	2.7	2.5	2.7
	2.5	2.8		1.6	2.1
		2.5			3.6
					2.7
					2.7
Mean	2.5	2.5	2.7	2.6	2.5
± S.E.	0.17	0.10	0.32	0.36	0.28

Mean serum albumin concentration in worm-free control gerbils:

3.3 ± 0.14 g/100 ml (n = 8)

APPENDIX I

Appendix to Chapter 3

Table 1

Haemagglutinating antibody titres (log 10) against L3SE antigens in serum of gerbils after vaccination with T. colubriformis antigens and after challenge with 1.5 x 10³ T. colubriformis

	Group						
	A	B	C	D	E	F	G
25 Days After Vaccination	0.903	1.806	0.0	1.505	0.0	0.0	
	0.903	0.903	0.0	0.0	1.204	0.301	
	0.0	0.903	0.0	1.806	0.0	0.301	
	1.806	1.505	0.0	0.602	0.0	0.602	
	0.602	0.0	0.0	0.903	0.301	0.0	
Mean	0.842	1.023	0.0	0.963	0.301	0.241	
± S.E.	0.292	0.310	0.0	0.320	0.233	0.112	
24 Days After Challenge	1.505	0.903	2.107	0.903	1.204	1.505	1.505
	0.903	0.903	2.408	1.204	0.602	0.903	1.505
	0.603	1.204	1.204	0.903	0.903	0.903	0.0
	0.903	0.903	1.204	0.903	1.505	3.311	1.806
	-	1.204	1.204	0.903	-	0.903	1.806
Mean	0.988	1.023	1.625	0.963	1.054	1.505	1.324
± S.E.	0.189	0.074	0.262	0.060	0.194	0.466	0.338

Table 2.

Haemagglutinating antibody total (log 10) against ASE antigens in serum of gerbils after vaccination with T. colubriformis antigens and after challenge with 1.5 x 10^3 T. colubriformis

	Group						
	A	B	C	D	E	F	G
25 Days After Vaccination	0.903	1.806	0.0	1.806	1.204	1.204	
	0.903	0.903	2.107	1.806	1.204	0.602	
	0.903	1.806	0.903	0.903	0.0	0.602	
	0.0	1.204	0.903	2.408	0.0	0.602	
	1.505	1.505	1.204	1.505	0.903	0.602	
Mean	0.843	1.449	1.023	1.686	0.662	0.722	
± S.E.	0.241	0.176	0.320	0.245	0.276	0.120	
	2.107	2.107	3.010	1.806	1.806	2.107	1.505
24 Days After Challenge	1.505	2.107	2.709	1.806	2.408	1.505	1.505
	1.806	2.107	2.709	2.408	2.408	1.806	0.602
	1.806	2.107	1.806	3.010	2.107	2.709	2.107
	-		3.311	1.806	-	2.408	2.107
Mean	1.806	2.107	2.709	2.167	2.182	2.107	1.565
± S.E.	0.123	0.0	0.252	0.241	0.144	0.213	0.276

Table 3

Haemagglutinating antibody titre (log 10) against ESP antigens in serum of gerbils after vaccination with T. colubriformis antigens and after challenge with 1.5 x 10³ T. colubriformis

	Group						
	A	B	C	D	E	F	G
25 Days After Vaccination	0.0	0.0	0.0	0.602	0.0	0.602	
	0.0	0.0	0.0	0.602	0.0	0.0	
	0.0	1.204	0.0	1.505	0.0	0.0	
	0.0	1.204	0.0	0.0	0.0	0.0	
	0.903	1.505	0.0	0.903	0.0	0.0	
Mean	0.180	0.783	0.0	0.722	0.0	0.120	
± S.E.	0.180	0.324	0.0	0.244	0.0	0.120	
	0.0	0.0	1.806	0.0	0.602	0.602	0.0
24 Days After Challenge	0.0	0.0	1.505	0.602	2.107	0.602	0.0
	0.0	0.602	0.0	0.602	2.107	0.602	0.0
	0.0	1.204	0.0	0.903	2.107	0.903	0.0
	0.0	0.0	0.0	1.806	2.107	0.0	0.0
Mean	0.0	0.361	0.662	0.783	1.806	0.542	0.0
± S.E.	0.0	0.241	0.408	0.295	0.300	0.147	0.0

Table 4

Haemagglutinating antibody titre (log 10) against L3SE antigens in small intestine mucosal extracts in gerbils after vaccination with T. colubriformis antigens and after challenge with 1.5 x 10³ T. colubriformis

	Group						
	A	B	C	D	E	F	G
25 Days After Vaccination	1.806	3.612	3.010	2.408	1.806	1.505	
	2.107	3.010	2.107	2.408	1.806	1.505	
	2.107	1.806	2.107	0.0	2.107	1.505	
	2.408	1.806	2.107	2.709	2.107	1.204	
	2.709	-	2.408	2.709	2.709	0.0	
Mean	2.227	2.559	2.348	2.047	2.107	1.144	
± S.E.	0.153	0.451	0.176	0.516	0.165	0.292	
24 Days After Challenge	2.107	2.408	2.107	1.505	1.505	0.903	
	3.311	2.408	2.107	1.204	1.505	2.408	2.408
	3.010	2.408	2.107	1.806	1.505	1.806	1.806
	2.107	1.806	2.408	0.0	2.107	1.806	3.311
	-	3.010	2.408	0.0	2.107	2.107	2.107
Mean	2.634	2.408	2.227	0.903	1.746	1.806	2.408
± S.E.	0.310	0.190	0.074	0.381	0.147	0.252	0.325

Table 5

Haemagglutinating antibody titre (log 10) against ASE antigens in small intestine mucosal extracts in gerbils after vaccination with T. colubriformis antigens and after challenge with 1.5 x 10³ T. colubriformis

	Group						
	A	B	C	D	E	F	G
25 Days After Vaccination	2.408	3.612	2.107	2.408	1.505	1.204	
	2.107	1.806	2.107	2.107	1.204	1.204	
	3.010	2.408	2.107	2.107	1.806	1.505	
	2.107	3.311	2.408	1.806	1.806	1.806	
	2.408	3.010	1.204	1.204	2.408	0.0	
Mean	2.408	2.829	1.987	1.926	1.746	1.144	
± S.E.	0.156	0.324	0.204	0.204	0.197	0.307	
24 Days After Challenge	2.107	1.408	1.505	2.107	1.806	1.505	1.505
	2.107	2.408	1.806	0.0	1.806	2.408	2.709
	2.408	2.408	1.806	2.408	1.806	2.408	2.107
	2.408	2.107	2.107	1.204	1.505	1.806	2.408
	-	2.709	2.408	0.0	-	1.806	2.408
Mean	2.258	2.408	1.926	1.144	1.731	1.987	2.227
± S.E.	0.087	0.095	0.153	0.507	0.075	0.181	0.204

Table 6

Haemagglutinating antibody titre (log 10) against ESP antigens in small intestine mucosal extracts in gerbils after vaccination with T.colubriformis antigens and after challenge with 1.5 x 10^3 T. colubriformis

	Group						
	A	B	C	D	E	F	G
25 Days After Vaccination	0.602	1.204	1.505	0.602	0.602	0.602	
	0.602	1.505	1.505	1.204	1.505	0.602	
	0.301	1.505	1.505	1.204	1.806	1.204	
	0.903	0.903	1.204	1.505	1.806	1.204	
	1.204	1.806	1.204	1.806	0.0	1.204	
Mean	0.722	1.385	1.385	1.264	1.144	0.963	
± S.E.	0.153	0.153	0.074	0.200	0.361	0.147	
24 Days After Challenge	1.204	1.505	1.505	0.602	1.505	2.107	2.107
	1.204	1.505	1.505	1.505	1.505	2.107	1.806
	1.204	1.505	1.505	1.505	1.505	2.107	1.505
	0.903	1.806	1.806	1.806	1.505	1.505	1.505
	-	1.806	1.806	0.0	-	1.204	0.903
Mean	1.129	1.625	1.625	1.084	1.505	1.806	1.565
± S.E.	0.075	0.074	0.074	0.338	0.0	0.190	0.189

Table 7

Haemagglutinating antibody titre (log 10) against L3SE antigens in small intestine contents of gerbils after vaccination with T. colubriformis antigens and after challenge with 1.5 x 10³ T.

	Group						
	A	B	C	D	E	F	G
25 Days After Vaccination	2.408	3.010	2.709	1.505	2.408	3.010	
	3.311	3.010	2.709	1.505	2.107	3.010	
	3.010	2.408	3.010	2.107	2.107	3.010	
	3.010	2.408	2.107	2.107	2.107	2.408	
	2.709	1.505	2.408	2.709	2.107	2.408	
Mean	2.890	2.508	2.589	1.987	2.167	2.769	
± S.E.	0.153	1.291	0.153	0.225	0.060	0.147	
24 Days After Challenge	2.408	1.806	2.107	1.505	2.107	3.311	3.010
	3.311	1.806	3.010	2.408	2.107	1.505	3.010
	2.107	1.806	3.010	0.903	1.505	1.806	3.311
	3.010	1.505	2.408	1.806	0.301	1.806	2.408
	2.709	2.709	2.709	2.709	1.204	2.107	2.709
Mean	2.709	1.926	2.649	1.866	1.445	2.107	2.890
± S.E.	0.213	0.204	0.156	0.320	0.355	0.316	0.153

Table 8

Haemagglutinating antibody titre (log 10) against ASE antigens in small intestine contents of gerbils after vaccination with *T. colubriformis* antigens and after challenge with 1.5×10^3 *T. colubriformis*

	Group						
	A	B	C	D	E	F	G
25 Days After Vaccination	2.408	3.612	3.010	2.107	1.505	2.107	
	2.408	3.311	2.709	2.107	1.505	2.107	
	3.311	3.311	2.709	1.806	1.505	1.806	
	3.908	3.311	2.709	1.806	0.602	1.806	
	3.010	2.709	2.408	1.204	1.806	2.408	
Mean	3.009	3.251	2.709	1.806	1.355	2.047	
± S.E.	0.285	0.147	0.095	0.165	0.233	0.113	
24 Days After Challenge	3.612	3.612	3.010	3.612	3.612	3.311	3.010
	3.612	3.010	3.010	3.612	3.311	1.806	3.311
	3.612	3.913	3.010	3.612	2.709	1.505	2.107
	3.010	3.913	3.010	3.010	2.709	2.107	2.709
	-	3.913	2.709	3.010	-	2.107	2.709
Mean	3.462	3.672	2.950	3.371	3.085	2.167	2.769
± S.E.	0.151	0.176	0.060	0.147	0.202	0.307	0.200

Table 9

Haemagglutinating antibody titre (log 10) against ESP antigens in small intestine contents of gerbils after vaccination with *T. colubriformis* antigens and after challenge with 1.5×10^3 *T. colubriformis*

	Group						
	A	B	C	D	E	F	G
	0.602	0.903	1.806	1.505	0.602	0.602	
25 Days	1.204	0.903	0.903	1.505	0.903	0.602	
After	0.301	0.602	1.505	1.204	1.204	0.602	
Vaccination	0.903	1.505	1.505	2.107	1.806	1.204	
	0.602	1.204	1.505	0.602	0.0	1.204	
Mean	0.722	1.023	1.449	1.385	0.903	0.843	
± S.E.	0.153	0.153	0.147	0.245	0.300	0.147	
	1.204	1.204	1.806	1.806	0.903	0.602	0.602
24 Days	1.505	0.903	1.806	1.806	0.903	0.602	1.505
After	0.903	1.505	1.806	1.806	1.204	0.903	1.505
Challenge	0.903	2.107	2.107	1.204	1.204	1.505	1.806
	-	1.806	1.204	1.505	-	2.107	2.709
Mean	1.129	1.505	1.746	1.625	1.054	1.144	1.625
± S.E.	0.144	0.202	0.165	0.120	0.078	0.292	0.338

Table 10

Haemagglutinating antibody titre (log 10) against L3SE antigens in large intestine contents of gerbils after vaccination with *T. colubriformis* antigens and after challenge with 1.5×10^3 *T. colubriformis*

	Group						
	A	B	C	D	E	F	G
	0.903	2.408	1.806	2.107	3.612	2.107	
25 Days	1.806	2.408	1.806	2.107	2.107	2.107	
After	1.806	2.408	1.806	2.408	2.408	3.010	
Vaccination	1.505	1.806	1.806	1.806	1.505	2.408	
	0.903	1.806	1.505	1.204	-	1.806	
Mean	1.385	2.168	2.746	1.926	2.408	2.288	
± S.E.	0.204	0.140	0.060	0.204	0.443	0.204	
	2.408	1.806	2.107	2.408	1.505	3.612	2.408
24 Days	2.408	1.806	2.107	2.408	0.903	1.806	1.505
After	1.806	1.204	2.107	2.107	1.204	2.107	3.010
Challenge	1.806	2.709	2.408	1.806	1.806	2.107	2.107
	-	1.505	2.709	1.204	-	2.408	2.709
Mean	2.107	1.806	2.288	1.987	1.354	2.408	2.348
± S.E.	0.174	0.252	0.120	0.213	0.194	0.316	0.259

Table 11

Haemagglutinating antibody titre (log 10) against ASE antigens in large intestine contents of gerbils after vaccination with 3 T. colubriformis antigens and after challenge with 1.5 x 10^3 T. colubriformis

	Group						
	A	B	C	D	E	F	G
	2.107	2.408	2.107	1.806	0.301	1.806	
25 Days	2.107	2.408	2.107	1.505	0.602	1.806	
After	2.107	2.709	2.408	1.505	1.505	1.806	
Vaccination	1.505	2.107	2.408	2.107	1.505	1.505	
	1.204	0.903	2.709	2.408	1.806	1.505	
Mean	1.806	2.107	2.348	1.731	1.144	1.686	
± S.E.	0.180	0.299	0.113	0.129	0.292	0.074	
	2.408	1.806	2.107	2.107	1.806	3.311	1.806
24 Days	2.408	1.806	2.107	2.408	2.709	1.505	1.806
After	1.806	2.709	2.408	2.408	2.107	1.505	1.806
Challenge	1.806	2.107	2.408	1.806	1.903	2.107	1.505
	-	2.408	2.709	1.505	-	2.107	2.408
Mean	2.107	2.167	2.348	2.047	1.881	2.107	1.866
± S.E.	0.173	0.166	0.113	0.176	0.376	0.330	0.147

Table 12

Haemagglutinating antibody titre (log 10) against ESP antigens in large intestine contents of gerbils after vaccination with *T. colubriformis* antigens and after challenge with 1.5×10^3 *T. colubriformis*

	Group						
	A	B	C	D	E	F	G
	0.602	0.602	1.505	1.204	0.602	0.602	
25 Days	0.602	0.903	1.806	1.505	0.602	0.602	
After	0.903	0.903	1.806	1.806	0.903	0.602	
Vaccination	0.903	1.204	1.806	1.806	1.505	1.204	
	0.0	0.0	2.107	1.806	0.0	1.505	
Mean	0.602	0.722	1.806	1.625	0.903	0.903	
± S.E.	0.165	0.204	0.090	0.120	0.190	0.190	
	1.204	1.204	0.903	0.903	1.505	0.602	0.903
24 Days	1.204	1.204	1.204	1.204	1.806	0.903	1.505
After	1.806	2.107	1.505	1.505	0.0	0.903	1.806
Challenge	1.806	1.505	1.505	1.505	0.0	1.505	2.107
	-	1.806	1.806	0.0	-	1.505	2.107
Mean	1.505	1.565	1.385	1.023	0.828	1.084	1.686
± S.E.	0.174	0.176	0.153	0.279	0.408	0.181	0.225