GENETIC RESISTANCE TO HELMINTH INFECTIONS OF SHEEP.

Summary of a Thesis Submitted for the Degree of Doctor of Philosophy of the University of Glasgow

by

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That some breeds of sheep and individuals within these breeds thrive better than others in parasite-infested localities has probably been recognised by stockmen for centuries, but it is only within the past 50 years, and largely as a result of field surveys that the existence of genetically-determined differences in host resistance to parasitic infections has become widely recognised. As yet remarkably little is known about the genotypes responsible for resistance or susceptibility and even less of the mechanisms involved. Within the past decade several reports have suggested that resistance to some parasites is associated with the animal's haemoglobin type, sheep with HbA being more resistant as judged by faecal egg counts and venous haematocrits than those with HbB. Since the frequency of these haemoglobin types varies in different breeds it has been tacitly assumed that inter-breed variations in resistance are related to differences in the relative haemoglobin type gene frequencies, but none of these studies has indicated whether such resistance is expressed primarily as a resistance to parasite establishment or resistance to the parasites' specific pathogenic effects.

In view of its potential practical significance it was considered worthwhile to examine the concept of breed and haemoglobin type resistance

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to parasitic infections and their possible relationship to the immunological status of the host under carefully controlled experimental conditions. The work represented in this thesis is essentially a study of the role of genetic factors in resistance to two important gastrointestinal helminth parasites of sheep, i.e. Haemonchus contortus and Ostertagia circumcincta.

The first section deals with the inter-relationship between haemoglobin type and breed and the response of sheep to primary infections with H. contortus. For this purpose worm-free Scottish Blackface and Finn Dorset sheep with different haemoglobin types were each infected with 350 H. contortus larvae/kg bodyweight and their responses monitored and compared by a combination of clinical, radioisotopic and parasitological techniques. The results obtained showed that sheep with HbA developed less severe clinical and pathophysiological disturbances, passed fewer eggs and harboured fewer worms at necropsy than animals with HbB and that Scottish Blackface sheep exhibited similar advantages over Finn Dorsets with the same haemoglobin type. Since variations in the severity of the disease as judged by pathophysiological effects correlated closely with worm numbers it was concluded (a) that genetic resistance operates at the level of parasite establishment which in turn is controlled by the immune response elicited, (b) that although HbA is a useful genetic marker for resistance, the degree of protection with which it is associated is very much influenced by other, and as yet undefined "breed" characteristics, and (c) on the basis of a second experiment demonstrating that sheep of each haemoglobin type were equally susceptible to the establishment and pathogenic effects of H. contortus when heavily infected (1400 larvae/kg), it

would appear that the magnitude of the larval intake is an additional factor involved.

The second and third sections are devoted to an examination of the influence of breed and haemoglobin type on acquired resistance to H. contortus, the former dealing with the acquisition of resistance from primary infections terminated by anthelmintic treatment, the latter with the well-known "self-cure" phenomenon. The results of the third section demonstrated that individuals and breeds with high resistance to primary infections, i.e. animals with HbA and belonging to the Scottish Blackface breed are also more resistant to reinfection than for example HbB and Finn Dorset sheep. In terms of worm establishment this resistance was no greater than that acquired during primary infections possibly due to the interruption of antigenic stimulation caused by anthelmintic treatment, but all reinfected animals were nonetheless able to seriously impair the parasites' biotic and pathogenic potential.

Attempts to induce "self-cure" of H. contortus by exposing infected sheep to reinfection or rapidly growing parasite-free grass showed that the reaction could occur under both conditions. This suggests that the phenomenon may be both immunological and non-immunological, the former in all likelihood being a manifestation of a hypersensitivity reaction in the abomasal mucosa, the latter due to the presence of an anthelmintic or anaphylactoid-type factor in the grass. However, whether produced by larvae or grass the reaction was more closely associated with the breed than with the haemoglobin type of the animals concerned, being observed in the majority of the Scottish Blackface sheep of each haemoglobin type

but in only a minority of the Finn Dorsets and Suffolks; even if unable to expel their existing worm populations most sheep were able to impair the reproductive and haematophagic activities of parasites derived from subsequent infections.

The experiment reported in the fourth section was designed to ascertain whether the response of sheep to non-haematophagic parasites is also related to their haemoglobin type. For this purpose HbA and HbB Scottish Blackface sheep were individually infected with 100,000 Ostertagia circumcincta larvae; resistance to the subsequent disease was compared by biochemical and radioisotopic methods and resistance to worm establishment by measurement of worm counts 16 days after infection. In terms of disease no clear difference was observed between the groups, but the presence of smaller numbers of adult worms and more inhibited larvae in HbA than in HbB sheep was suggestive of a better immune response on the part of the former.

The final section examines the genetic control of antibody production to non-parasitic antigens. No difference was observed between HbA and HbB type sheep, or between Scottish Blackfaces and Finn Dorsets in respect of immune elimination of horse gamma globulin, but on the basis of a significantly better response to human serum albumin, and suggestive evidence of a better response to rabbit red cells in HbA than in HbB Scottish Blackface sheep, it seemed reasonable to conclude that the advantages exhibited by the former in relation to parasitic infections were associated with a superior immunological competence.

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GENERAL INTRODUCTION

The continuously growing demand for animal protein in recent decades has led to a rapid expansion and intensification of livestock production, particularly in tropical and sub-tropical regions of the world. Unfortunately, the potential of many development programmes is limited by the existence of helminthic and protozoal diseases which cause countless deaths and incalculable insidious losses to the industry, and which paradoxically assume increasing significance under modern systems of management. It is of some importance, therefore, that these diseases be eliminated or at least controlled, and at the present time, short of rearing animals parasite-free and folding them over uncontaminated pasture, there are essentially two methods of achieving this aim, i.e. "chemical" and "immunological". The former, which involves rearing animals under the protection of anthelmintics, molluscicides or insecticides, necessitates high standards of husbandry and veterinary supervision, and although widely and successfully practised is still often unreliable or impracticable in many developing countries because of insufficient epidemiological information and technical and financial resources.

Immunoprophylaxis utilises the host's capacity to resist or limit parasitic invasion and is currently achieved by vaccination with irradiated larvae or in some instances, artificial infection terminated by drugs. Such procedures have resulted in commercial vaccines against

parasitic bronchitis of cattle and sheep[1,2] and hookworm in dogs[3], while a number of others, e.g. avian coccidiosis[4,5] and syngamiasis[6], bovine theileriosis[7] and ovine schistosomiasis[8], have given promising results in the laboratory. In spite of these advances effective large-scale immunisation against the great majority of parasites remains a distant goal which even if theoretically feasible could prove an uneconomic and unrealistic proposition for all but the most advanced nations. For the majority, which includes those within the vast and often fertile continents of Africa, Asia and South America an alternative short-term, economically more viable and technically less demanding approach is clearly indicated.

One possibility is the development of resistant indigenous stock or improved exotic breeds. Admittedly there is little in the literature to inspire confidence that breeding for the quality of disease resistance will ever in itself be an economic proposition, but a number of observations point to the possibility that such an approach, combined with the more extensive and judicious application of control procedures might be exploited on a greater scale than at present. In the first place there is now an increasing awareness that certain individuals, strains and breeds thrive better in parasite-infested localities than others, and that even in the most carefully controlled experimental infections wide variations in the establishment and pathogenic effects of parasites are the rule rather than the exception. One particularly striking illustration of

this phenomenon is the high resistance exhibited by the dwarf N'dama and Muturu cattle of West Africa over Zebus to natural and experimental trypanosomiasis[9-13], but numerous other studies, some of which are reviewed in more detail later, suggest that similar differences prevail in many host-parasite systems, e.g. bovine and ovine trichostrongylosis[14-37]; bovine theileriosis[38] and babesiosis[39,40]; avian coccidiosis[41,42] and ascaridiasis[43]; rodent malaria[44] and nippostrongylosis[45]; murine trypanosomiasis[46] and trichuriasis[47,48]. Many of these differences could of course be attributed to variation in age, grazing habit, acquired resistance and even infection technique, but nevertheless the overall impression gained is that resistance to many parasites is determined to some extent by the host's genetic constitution, and this has in fact been verified experimentally in a number of host-parasite systems. For example, Ross and co-workers[21] demonstrated heritable resistance to haemonchosis in Nigerian cattle; Whitlock and Scrivner successfully bred sheep with high resistance to Haemonchus contortus[18] and Ostertagia circumcincta[28]; and Ackert, Sucharit and MacDonald, and Wakelin, chickens, rats and mice resistant respectively to Ascaridia lineata[49], Brugia pahangi[50] and Trichuris muris[48]. Impressive differences in susceptibility to Plasmodium berghei have also been obtained by inbreeding mice with high and low resistance[51]. These findings add further weight to the premise that breeding for disease resistance could form a useful adjunct to prophylaxis and thereby facilitate livestock

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production in many areas.

The logical and necessary first step towards achieving this aim is the identification of those genotypes responsible for increased resistance and an understanding of the mechanism by which they operate. Some progress has already been made in both areas, most notably the recognition that the prevalence and severity of a number of diseases caused by haemoprotozoan and haematophagic helminth parasites may be related to genetic variations in red cell biochemistry[23,25,30,33,34,36,37,52-62], and by recent advances demonstrating genetic control of immune responses.[63-65].

Of all the inherited erythrocyte factors implicated in resistance to parasites, haemoglobin type is currently considered the most important. The relevance of this character was first brought to light by Allison's classical studies linking resistance to human malaria with sickle cell haemoglobin[52-54], but since then a number of reports, based largely on results of field surveys have provided circumstantial evidence of analogous relationships in parasitic diseases of livestock. The absence of haemoglobin B for example has been associated with increased tolerance to bovine trypanosomiasis[56], and foetal haemoglobin (HbF) with the low susceptibility of the young bovine to anaplasmosis[62], but by far the most compelling evidence for the involvement of haemoglobin polymorphism in host-parasite interactions concerns the response of sheep to Haemonchus contortus.

Two different haemoglobins, A and B are found in the majority of normal adult sheep[66] and whilst their breed distribution varies[67], their frequency is genetically determined by two alleles which produce three phenotypes, A, AB and B[66,68]. Several authors have produced evidence which suggests that haemoglobin A-type sheep are more resistant to H. contortus than animals with haemoglobin B (HbB). This hypothesis originated from Evans et al.,[23], who found smaller worm burdens and lower faecal egg counts in HbA than in HbAB sheep following experimental infections. Subsequently, Evans and Whitlock[25], reported a significant correlation between haemoglobin type and venous haematocrit in grazing sheep and on this evidence suggested that the presence of HbA might mitigate the effects of natural Haemonchus challenge and exposure to cold. However, it is only within the past few years that haemoglobin type has been more directly implicated in individual and breed resistance to this parasite. Jilek and Bradley[30] observed that HbA Florida Native sheep were less susceptible than HbAB and HbB types as judged by egg and worm counts and haemoglobin levels, and in addition presented evidence which indicated that the higher survival rate of this breed compared with imported Rambouillets under the tropical/sub-tropical conditions prevailing in Florida was a reflection of the significantly higher gene frequency for HbA in the latter (0.56 and 0.19 respectively). Since then work carried out in Kenya[36,37], has confirmed the advantage conferred by HbA to Merinos in

a Haemonchus-endemic area, and indicated that the poor performance of this breed relative to the indigenous Masai and imported Dorpers is related to a higher frequency of HbA alleles amongst these latter breeds.

Collectively the above findings suggest that haemoglobin polymorphism has an important bearing on a number of host-parasite relationships, but as yet remarkably little is known about the mechanisms involved. Certainly with regard to the Haemonchus situation all the indications from the field suggest that the phenomenon is a reflection of variations in worm establishment. This may be the case, but at the moment supportive evidence is based almost entirely on egg counts and haematological indices, i.e. on indirect parameters of infection and not on direct measurements of worm load. Indeed the few attempts made to explain resistance to Haemonchus in terms of worm establishment have either been unconvincing or disappointing. For example, the oft-quoted studies of Evans and his colleagues from which the whole concept of haemoglobin-type resistance to Haemonchus originated[23] certainly revealed smaller parasite numbers in HbA as compared with HbAB sheep, but these differences were not accompanied by differences in the haematological status of the two groups, neither were they significant, nor in this author's opinion a reliable basis for comparison, being obtained from animals which died as a result of infection and which in all likelihood lost a large proportion of their originally established populations[69,70]. Furthermore, in

the only other report comparing the response of animals with different haemoglobin types to standardised infections of H. contortus[34], egg counts and worm loads were actually somewhat higher in HbA than in HbAB or HbB sheep and perhaps significantly the only real advantage exhibited by the former was an ability to maintain relatively higher venous haematocrits.

The apparent disparity between field and experimental observations implies that the better performance of animals with HbA at grass arises not so much from an inherent capacity to resist initial worm establishment - although this may yet prove to be important, but rather (or additionally) from a greater ability to limit the persistence of infection, resist reinfection and/or withstand the stresses of haemorrhage and vagaries of climate. With regard to the first possibility, it is significant that Allonby and Urquhart[37], noted more frequent and effective "self-cure" in HbA Merinos grazing infected pasture, suggesting a genetic link with immunological competence, but it remains to be established whether this also encompasses resistance to reinfection.

One point which is however firmly established, is that HbA does confer a number of cardiovascular properties which, in theory, could be advantageous in Haemonchus-endemic areas. Firstly, the affinity of HbA for oxygen is 30-50% greater than HbB[71,72], and hence in animals with HbA, oxygen uptake from the lungs is facilitated and arterial oxygen content and percentage saturation elevated. Secondly, HbA type sheep have a greater capacity to increase their cardiac output in

response to the anoxic stress imposed by phlebotomy[73,74]. Thirdly, when severely anaemic, sheep which carry the gene for A haemoglobin produce haemoglobin C[75-77], a β-chain variant with an improved oxygen-carrying capacity[78,79]. Finally, there is a suggestion that sheep with HbA are heavier and maintain higher haematocrits, haemoglobin levels and blood volumes than their HbB counterparts[25,37,74,80].

From the foregoing account it is clear that the relationship between haemoglobin polymorphism and resistance to Haemonchus is extremely complex, and that despite extensive investigation many aspects remain largely unresolved and unstudied. The problem is essentially one of determining whether the advantages displayed by individuals with a particular haemoglobin type arise (a) from a superior "innate" resistance to parasite establishment or to the effects of such establishment, or both, i.e. are manifestations of non-specific factors rendering these animals physiologically unsuitable for parasite development and/or better equipped to withstand the subsequent pathophysiological effects; or (b) from the acquisition of a more effective and specific "acquired" resistance involving the immune system; it is also possible, of course, that "innate" and "acquired" factors act in concert thereby increasing their overall effectiveness. At the present time the relative importance of physiological as opposed to immunological factors is either unknown or clouded by deficiencies in experimental design and technique. Indeed one of the major defects has been the reliance placed on field

observations and more particularly faecal egg counts and blood composition. Contrary to the views expressed by many authors, differences in such parameters are not necessarily consistent with parasite numbers[37,70,81], and in a field situation where larval challenge is often continuous and extremely variable are probably a better indicator of acquired than innate resistance; furthermore, even with a knowledge of worm burdens, natural infections are insufficiently precise for detailed analysis of the mechanisms involved.

The same applies to the phenomenon of breed resistance. If indeed such intra-breed variations in resistance to Haemonchus exist, it would seem reasonable to ascertain whether these are related to differences in the relative gene frequencies of the various haemoglobin types or reflections of other, and as yet unknown, breed characteristics.

The above considerations immediately raise two further questions. Firstly, is haemoglobin type a reliable genetic marker for resistance or susceptibility to non-haematophagic parasites?; and secondly, are animals belonging to certain breeds or haemoglobin types generally immunologically more responsive than others? Surprisingly neither possibility has received much attention although the importance of "breed" and "strain" components in resistance to Ostertagia circumcincta[26-28], and Trichostrongylus axei[31], and in the immune response to complex multideterminant antigens[63-65], are well documented.

Clearly, there is a need to appraise, and in many respects re-appraise the whole concept of haemoglobin type and breed resistance to parasitic infections and their possible relationship to immune responsiveness under carefully controlled experimental conditions and utilising techniques which provide reliable and detailed comparative information concerning the physiological and immunological status of the animals concerned. This in effect is the aim of the work described in this thesis.

In the first section the responses of parasite-free Scottish Blackface and Finn Dorset sheep with different haemoglobin types to single infections with H. contortus were monitored and compared by a combination of clinical, radioisotopic and parasitological techniques with a view to determining whether any differences recorded derived from variations in resistance to the establishment and/or pathogenic effects of this parasite. The second and third sections are devoted to an examination of the possible influence of breed and haemoglobin type on the "acquired" component of resistance to H. contortus, the former dealing with the acquisition of resistance from primary infections terminated by anthelmintic treatment, the latter with the well-known "self-cure" reaction. The fourth section examines the influence of haemoglobin type on the response of Scottish Blackface sheep to single infections with Ostertagia circumcincta, and the fifth, the relative capacities of Scottish Blackface and Finn Dorset sheep belonging to various haemoglobin types to produce antibodies

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against a number of non-parasitic antigens.

Each of the separate aspects studied is prefaced by a review of the relevant literature and details of the observations not included in the text may be found in the appendices.

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GENERAL MATERIALS AND METHODS

REARING AND MAINTENANCE OF EXPERIMENTAL ANIMALS

The sheep used were Scottish Blackface, Finn Dorset and Suffolk wethers; those of the former two breeds were 7-10 months old while the Suffolks were 2 years old. The Blackface and Suffolk lambs were born outdoors, removed from their mothers when one week old and transferred to concrete pens with straw bedding. Whole milk was fed during the following four weeks and lamb weaner pellets (British Oil and Cake Mills Ltd., Renfrew, Scotland) introduced from two weeks of age; after weaning at four weeks the diet consisted of a pelleted concentrate (½ lb per 15 lb liveweight daily) and ad lib hay and water. The lambs were castrated, docked and inoculated with a combined clostridial sheep vaccine when eight weeks old, a booster injection being given four weeks later.

The Finn Dorset lambs were obtained from the Animal Breeding Research Organisation, West Linton, Scotland. These animals were reared and maintained essentially as described above except that lambing was indoors and weaning carried out at eight weeks of age.

HAEMOGLOBIN TYPING

Two different haemoglobin types, A and B are found in the majority of normal adult sheep, each being genetically determined by two alleles (A and B) which produce three phenotypes, i.e. AA, AB and BB. These haemoglobins consist of two α and two β globin polypeptide chains. Haemoglobin A and HbB have the same α (termed I_α) but β chains

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(β^A and β^B) whose primary structures differ in seven positions[1-6]. Homozygous and heterozygous animals are most easily identified by electrophoresis of haemolysed erythrocytes at pH 8.5-9.0 when HbA migrates faster towards the anode than HbB and HbAB intermediate between the two (Figure 1).

A third haemoglobin, HbC is found in minute quantities in the blood of normal sheep carrying the β^A structural gene (i.e. phenotypes AA or AB) and in dramatically increased amounts under conditions of anaemic stress. This haemoglobin, which has the same α chains as HbA and HbB but β chains (β^C) differing in the number and sequence of their amino acid residues (141 compared with 145 residues with 17 differences in sequence), is easily recognised since it has an electrophoretic mobility slower than that of HbB[6-11].

For the studies described in this thesis haemoglobin typing was performed on cellulose acetate since in the experience of the author and others, this medium is more easily handled and gives a resolution similar to that obtained by the more conventional starch-gel procedure[12-14]. Cellulose acetate is also suitable for scanning in a recording densitometer thereby permitting evaluation of the relative proportions of the various haemoglobin types, and of particular relevance to these studies, the likely production of HbC in HbA and HbAB animals made anaemic by infection with H. contortus (Figure 2).

Figure 1: Separation of Ovine Haemoglobin Types by Electrophoresis on Cellulose Acetate.

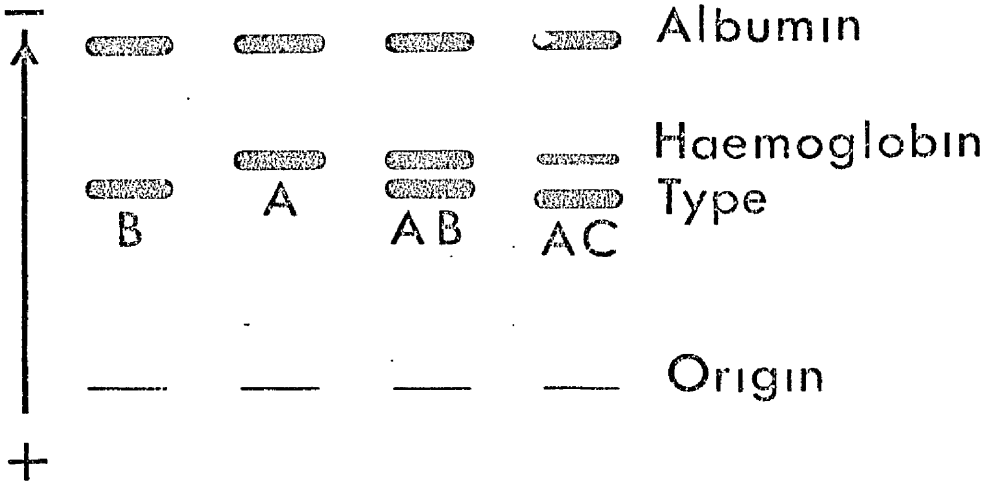
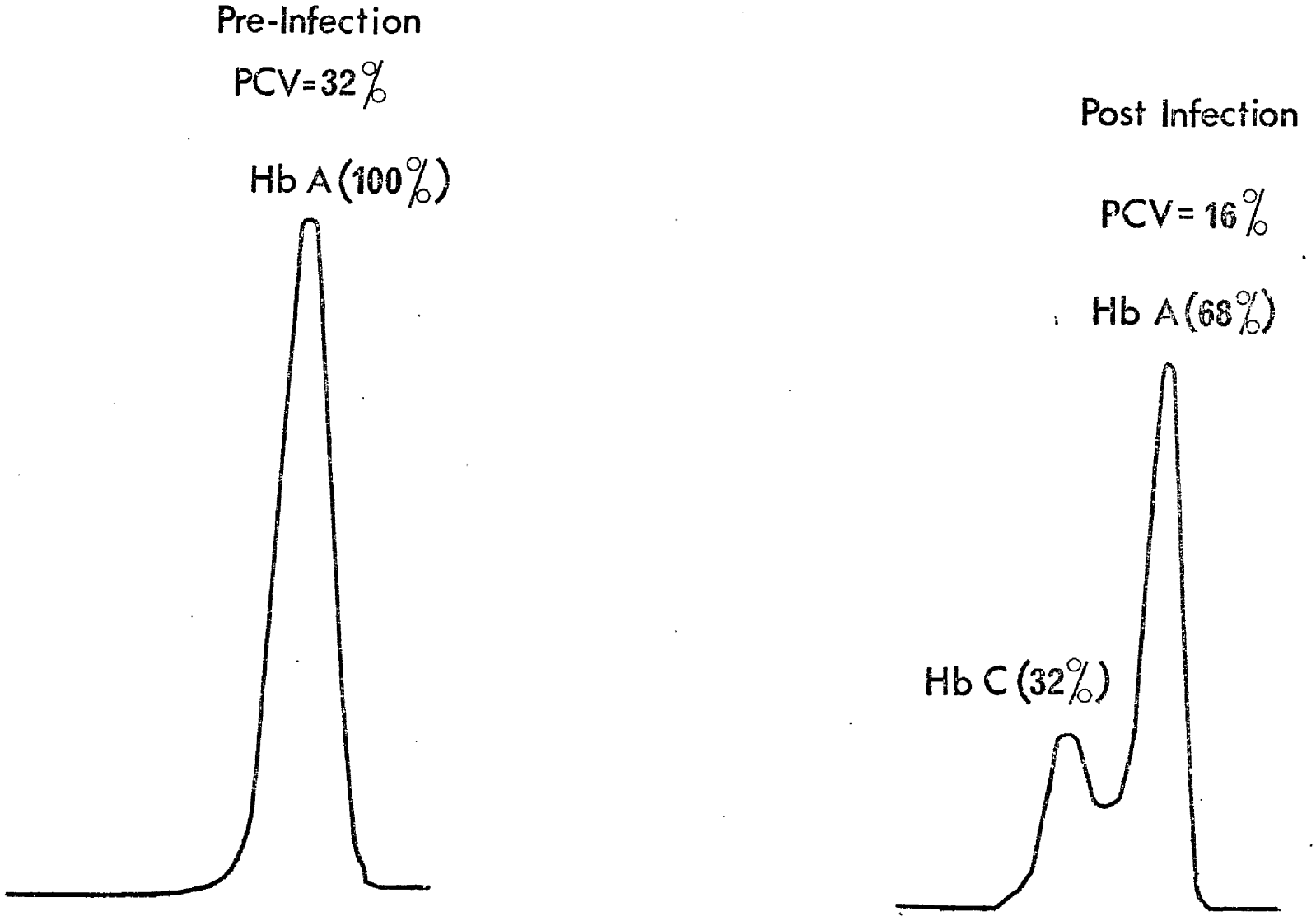


Figure 2: Evaluation of Haemoglobin Types on an Automatic Scanning Densitometer.



Cellulose acetate strips ("Celagram", Shandon Instruments, Camberley, England) were saturated with trisborate buffer (pH 9), lightly blotted to remove excess buffer and laid across the supports of an electrophoresis tank ("Multi-Microband Electrophoresis Kit", Shandon Instruments) containing barbitone buffer (pH 8.5).

Haemolysed blood samples (prepared by addition of an equal volume of distilled water) were applied to the strip about 2.5 cm from the cathode end using an applicator plate. A constant voltage of 150 v was applied for 40 minutes from the power pack. The strips were then removed, placed in a tray containing a 5% aqueous solution of trichloroacetic acid (TCA) for 5 minutes to "fix" the proteins, and subsequently developed by staining for 5 minutes with 0.2% Ponceau S (G.T. Gurr Ltd., London, England) in 3% aqueous TCA. Finally, the strips were washed three times in 5% aqueous acetic acid to remove background coloration and evaluated automatically using a Kipp and Zonnen micro-densitometer DD2; the relative amounts of each haemoglobin were expressed as a percentage of the total.

PARASITOLOGICAL TECHNIQUES

Culture and harvesting of infective *Haemonchus contortus* larvae

A pure strain of *H. contortus*, obtained from the Wellcome Veterinary Research Station, Frant, England, was

passaged through sheep reared and maintained under worm-free conditions. Faeces were collected daily using a faecal bag and cultured by placing approximately 250 g amounts in 500 ml honey jars which were incubated at 22°C for 14 days. Infective third stage larvae were recovered by the method described by Roberts and O'Sullivan[15]. The jars were filled with luke-warm tap water and allowed to stand for several hours in diffuse light. The fluid was then sieved (60 meshes/inch) to remove the coarse debris and the suspension filtered through a double layer of gauze milk filter (Clover No. 9, Johnson and Johnson, Slough, England) using a Buchner funnel fitted to a side-arm flask evacuated by means of a vacuum pump. The filters were then placed, larval side up, on a fine sieve (mesh No. 10) in a Baermann apparatus and left for 2-3 hours to allow larval migration from the filters to the warm water. After settling to the narrow part of the funnel the larvae were drawn off and stored at 6°C.

Preparation and administration of larval inoculum

The concentration of the larval suspension was determined by examining at least ten 0.025 ml aliquots containing a total of not less than 1,000 larvae. The suspension was mixed thoroughly and agitated while sampling. Doses for inoculation were measured by pipetting the calculated volumes of the original larval suspension into universal bottles, a check being made to ensure that the desired number of larvae ± 10 per cent was present.

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The larvae were administered orally to each animal using a 20 ml syringe which was then subsequently flushed twice with water.

Faecal egg counts

Faecal samples collected either directly from the rectum or from collection bags fitted to the animals were examined by a modified McMaster technique[16]. In this method 3 g faeces were mixed with 42 ml water and passed through a sieve (60 meshes /inch); two 15 ml samples of the filtrate were centrifuged in flat bottomed test tubes for 2 minutes at about 1500 g and the supernatant poured off. The sediment of one of these tubes was re-suspended in saturated salt (NaCl) solution and the test tube inverted several times; both chambers (volume 0.15 ml) of a McMaster Worm Egg Counting Slide (Hawksley & Sons, London, England) were then filled with the suspension using a pasteur pipette. The number of eggs in both chambers was multiplied by 50 to give the number of eggs per gram of faeces.

If the sample proved negative for H. contortus eggs using the above method, the second test tube containing sediment was filled with saturated salt solution and thoroughly mixed. More saturated salt solution was then added until the meniscus was above the rim of the tube. The sample was allowed to stand for a few minutes before using a platinum loop to remove the upper layer of the

fluid and smear it on a clean glass slide. A microscopical examination then confirmed the absence of Haemonchus eggs or their presence in small numbers.

Necropsy procedure

All sheep to be necropsied were starved for 24 hours prior to slaughter. A captive bolt pistol was used to shoot the sheep after which it was bled out. The abdomen was opened and the abomasum and omasum separated from the rest of the intestinal tract, care being taken to ensure that the contents remained intact. After removal of the omasum, the abomasum was opened along the greater curvature and washed under slow running water. The abomasal washings and contents were diluted to 2 litres in a graduated polythene bucket and after thorough mixing, two samples, each of 200 ml were taken in a graduated scoop for subsequent microscopic enumeration of worm population; 10 ml of 40% formalin was added as preservative to each sample.

The entire abomasal mucosa was then scraped off with a sharp knife, chopped finely with a cleaver and 200 g lots placed in separate Kilner jars. The jars were then filled with a pepsin-hydrochloric acid (HCl) mixture and incubated for 6 hours at 42°C; the digests were then formalinised, diluted to 2 litres and two samples each of 200 ml removed for worm counting. The pepsin-HCl mixture was similar to that described by Herlich[17]; 10 g of 1:2500 pepsin powder (British Drug Houses, Poole, England)

was dissolved in 600 ml of water and acidified with 30 ml concentrated HCl.

Worm counting and measurement

The 200 ml samples of abomasal washings and digests collected at necropsy were treated as follows: 2-3 ml 45% iodine solution (containing 720 g potassium iodide and 450 g iodine crystals/litre distilled water), were added to each sample with thorough mixing using a 5 ml pipette broken at the 4 ml mark (to obtain a wide bore). 4 ml aliquots were withdrawn and pipetted into petri-dishes. 2-3 ml 5% sodium thiosulphate solution were added to clear the background while allowing the parasites to retain the colour of the iodine thus enabling them to be more easily observed for counting[18]. At least ten 4 ml aliquots from each 200 ml sample were examined under a dissection microscope, and the number of parasites found multiplied by the appropriate dilution factor (50) to give the number of worms in the original 2 litres. This counting technique gives an estimate which varies within ± 10% of the mean[19].

Measurements of H. contortus worms were made by mounting individual parasites on slides which were then viewed on the screen of a projection microscope (Projectina Co.Ltd., Heerbrugg, Switzerland) at a magnification of 10x objective. The resulting image was traced on paper and the length of the tracing determined using an opsometer and the results divided by the magnification to give a measure of worm size.

BLOOD ANALYSES

Collection and storage of samples

Blood samples were collected from a jugular vein into evacuated 5 ml glass tubes (Vacutainer, Becton and Dickinson, New Jersey, USA). Tubes containing 100 International Units heparin as anti-coagulant were used for all haematological examinations. For serum analyses, blood samples were left standing overnight at room temperature in inverted unheparinised tubes and the serum recovered transferred by means of a pasteur pipette into plastic vials which were immediately frozen and stored at $-5^{o}C$.

Packed cell volumes (PCV)

Packed cell volume percentages were determined by the microhaematocrit method. Capillary tubes containing the blood sample were sealed at one end by heat or plasticine and centrifuged for 5 minutes in a microhaematocrit centrifuge (Hawksley & Sons Ltd., London, England); the percentage PCV was determined from the scale on a Hawksley Microhaematocrit Reader.

Haemoglobin concentration (Hb)

Blood haemoglobin was estimated by the cyanmethaemoglobin method of Van Kampen and Zijlstra[20]. 0.02 ml well mixed blood were added to 5 ml dilute potassium ferricyanide solution. Haemoglobin was thus oxidised to haemiglobin which in turn was converted by treatment with cyanide to the stable cyanmethaemoglobin. This compound was measured

colorimetrically at 542 mμ, and the concentration of haemoglobin (g/100 ml) determined with the aid of standard cyanmethaemoglobin solution (Roche Diagnostica, Roche Products Ltd., London, England).

Red cell count (RBC)

Total red cell counts ($x10^6$/cu.mm) were determined by an electronic particle counter (Coulter Model "D", Coulter Industrial Sales Co., Elmhurst, Illinois, USA).

Mean corpuscular volume (MCV) and Haemoglobin concentration (MCHC)

These indices were calculated as follows:

$$\text{MCV } (\mu^3) = \frac{\text{PCV x 10}}{\text{RBC}}$$

$$\text{MCHC } (\%) = \frac{\text{Hb x 100}}{\text{PCV}}$$

Serum proteins

Total serum proteins were estimated by a biuret colorimetric technique[21], and albumin by the bromocresol green method described by Rodkey[22]. Serum globulins were calculated as the difference between total protein and albumin concentrations.

Serum iron

Blood samples collected in iron-free tubes were allowed to clot and the serum obtained treated with an anionic detergent (Teepol, in acetate buffer, pH 5.8) to split the Fe^{+++}-transferrin complex and subsequently with sodium dithionite to reduce free Fe^{+++} to Fe^{++}. Addition of bathophenanthroline disulphonate (7.5 mmol/litre)

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produced a complex, the colour intensity of which was read in a spectrophotometer at 546 mμ. Serum iron concentration was calculated by reference to the colour intensity of a standard solution treated as above.

RADIOISOTOPIC TECHNIQUES

Labelling of red cells with ^{51}Cr

The successful labelling of red cells with ^{51}Cr depends upon the fact that anionic hexavalent chromate penetrates into the cell, is reduced to cationic trivalent chromium (Cr^{3+}), which becomes bound to the globin moiety of haemoglobin[23,24]. Hence the procedure in general consists of incubating a volume of the animal's blood or red cells *in vitro* with a suitable amount of radiochromium as hexavalent chromate (e.g. Na_2CrO_4). Since excessive amounts of chromium can cause damage to the cells it is recommended that the specific activity of the ^{51}Cr should be such that less than 2 μg of chromium is added per ml of packed red cells, equivalent to approximately 0.7 μg/ml whole blood[25].

Procedure

Blood (equivalent to about 7 ml packed red cells) was obtained by jugular puncture and collected in universal bottles containing heparin as anticoagulant. After centrifugation for 10 minutes at 2,000 rpm and removal of plasma the cells were suspended in isotonic saline and gently mixed. A measured volume of isotonic saline containing 1 mCi ^{51}Cr (specific activity 250 μCi/μg Cr) as sodium

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chromate (Radiochemical Centre, Amersham, England) was added with gentle mixing. The labelled cell suspension was incubated at 37°C for 30 minutes with frequent gentle mixing and then centrifuged at 1,500 rpm for 10 minutes. The supernatant was removed and discarded, the cells washed in isotonic saline until free of unbound ^{51}Cr, and finally reconstituted with the retained plasma for injection. In all cases, each sheep received its own erythrocytes and plasma.

Labelling of plasma and red cells with ^{59}Fe

These were labelled *in vivo* by intravenous injection of ferric citrate (Radiochemical Centre, Amersham, England, specific activity 15 μCi/μg) corresponding to a radioactivity of 70 μCi.

Labelling of albumin with ^{125}I

Trace-labelling of proteins with radioidine was carried out by the iodine monochloride method of McFarlane[26]. This method depends upon treating the protein in slightly alkaline solution with iodine monochloride to which has been added the radioactive iodine as carrier-free iodide (obtained from the Radiochemical Centre, Amersham, England, as thiosulphate-free $Na^{125}I$) and results in substitution of ^{125}I in the tyrosine residues to give mono and di-iodo tyrosine groupings (Figure 3). The introduction of less than 1 atom of iodine per molecule of albumin ensures that no marked change occurs in its physiochemical and immunological properties[27].

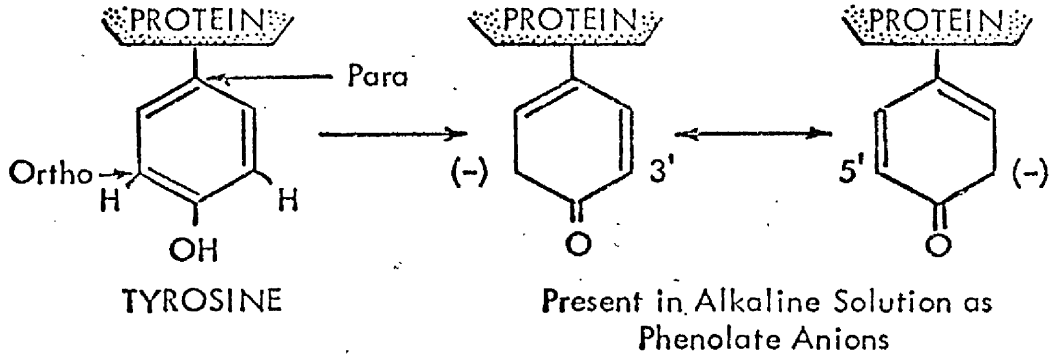
Materials

Albumin

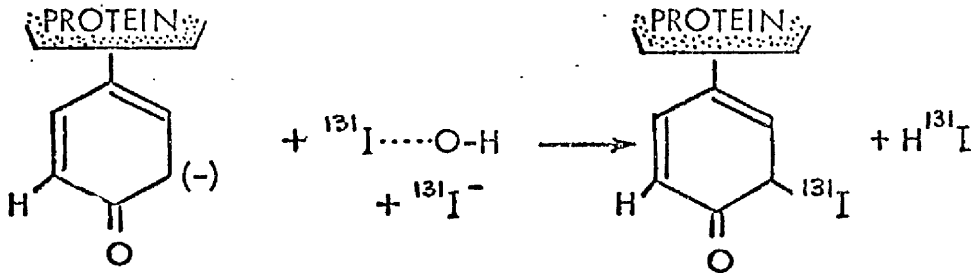
Commercial sheep albumin (Cohn Fraction V, Pentex, Incorp.,

FIGURE 3.

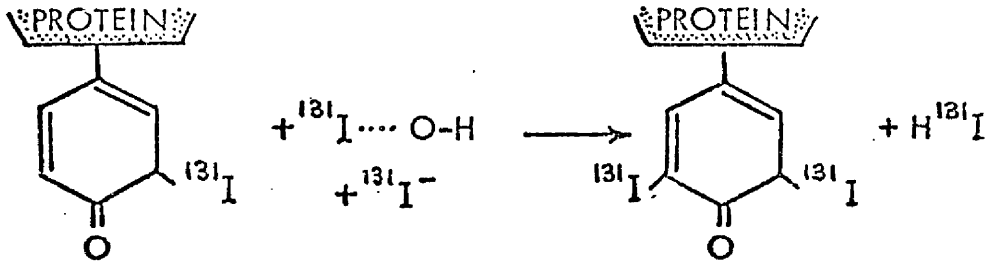
MECHANISM OF IODINE SUBSTITUTION ON TYROSINE



(A) Formation of 3' or 5' MONO-IODOTYROSINE



(B) Followed immediately by substitution of I for H on remaining ortho position forming 3', 5' DI-IODOTYROSINE



Kankakee, Illinois, USA) was used in all experiments.

Iodine monochloride

A solution containing 0.42 mg I/ml as iodine monochloride (ICl) in M NaCl and approximately 0.01 N with respect to HCl was used. This was prepared by dissolving 5.00 g potassium iodide (KI) and 3.22 g potassium iodate (KIO_3) in 37.5 ml distilled water. To this was added 37.5 ml concentrated HCl and 5 ml carbon tetrachloride (CCl_4) and the mixture shaken vigorously; 0.1 M KI was then added dropwise until a faint pink colour appeared in the CCl_4. This stock solution, which contains approximately 147 mg I/ml as ICl was diluted 1:350 with isotonic saline to provide a solution containing 0.42 mg I/ml.

Glycine buffers

Two glycine buffers were used for the labelling process; one, buffer A (pH 8.5) was used for conversion of ICl to hypoiodite (IOH), while the other (buffer B, pH 9.0) was employed for solution of the protein at an alkaline pH.

Buffer A: 45 ml M-glycine in 0.25 M NaCl + N NaOH to pH 8.50.

Buffer B: 40 ml M-glycine in 0.25 M NaCl + N NaOH to pH 9.00.

Iodination procedure

A 2% solution of sheep albumin was prepared by dissolving 600 mg freeze-dried protein in 30 ml isotonic

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saline and buffered by addition of 15 ml glycine buffer B. 10 mCi of carrier-free radioiodine was added to 2.5 ml of a freshly prepared solution of ICl containing 0.42 mg I/ml; the iodine monochloride solution was then converted to hypoiodite by addition of 10 ml glycine buffer A and immediately mixed with the protein solution. The labelled preparation was transferred to a dialysis sac containing 2 g "carrier" protein (bovine serum albumin). Carrier protein was added to reduce the specific activity of the labelled albumin to less than 5 µCi/mg, thereby reducing the possibility of radiation decomposition[27,28]. The labelled protein was dialysed for 48 hours at 5°C against two 20-litre changes of isotonic saline to remove unbound iodide and finally centrifuged for 30 minutes at 1,500 rpm prior to injection.

Labelled protein prepared as described contains 0.9 atoms/molecule assuming 100% incorporation.

Injection of radioisotopes

All radioactive materials were injected into a jugular vein through a jugular catheter, (Portex Plastics Ltd., Hythe, England), and the catheter flushed out with isotonic saline before being withdrawn.

Radioactivity measurements

One ml samples of blood and plasma were pipetted into counting bottles and made up to a volume of 15 ml with 0.02 N NaOH. The volume of each daily urine collection was measured and a 15 ml aliquot taken for radioassay. Each

24-hour faecal collection was weighed and mixed thoroughly and random 15 g samples packed in counting tubes. Radioactivity measurements were carried out using an automatic well-type gamma scintillation spectrometer (Nuclear Chicago, High Wycombe, England). Standard solutions of all labelled preparations were assayed at regular intervals and corrections for radioactive decay based on the activities of these solutions. In most of the experiments reported in this thesis radioactivity due to three isotopes ^{59}Fe, ^{51}Cr and ^{125}I was measured simultaneously. Separation of isotopic mixtures was achieved by the application of "overlap" factors calculated from the relative count rates of standard solutions of these isotopes at each photopeak.

STATISTICAL METHODS

Statistical methods employed were those described by Bishop[29]. Half-life values quoted in the text were calculated by regression analysis and unless otherwise stated, correlation coefficients of radioactivity against time were very highly significant ($r > -0.95$). Standard errors (SE) and p values (student's t-test) are quoted. A p value equal to or less than 0.05 is regarded as being statistically significant.

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SECTION 1

THE INFLUENCE OF HAEMOGLOBIN TYPE AND BREED ON THE RESPONSE OF SHEEP TO PRIMARY INFECTIONS WITH *Haemonchus contortus*

INTRODUCTION

In the introduction to this thesis the possible existence and basis of genetically-determined resistance to H. contortus in sheep were critically evaluated. Field observations strongly suggested that infection with this parasite was influenced by the animal's haemoglobin type and breed, but attempts to corroborate this experimentally and provide a rational explanation were generally unsuccessful or unconvincing. It was concluded that this discrepancy could only be resolved on the strength of more detailed and carefully controlled investigations into the nature and relative importance of genetic and acquired factors in the response to infection with Haemonchus. In attempting to unravel some of the complexities of the problem, it seemed appropriate to ascertain at the outset whether the response of worm-free sheep to single standardised doses of infective larvae was in fact haemoglobin type and breed related, and if so, to determine to what extent this was a reflection of differences in resistance to worm establishment and/or the subsequent disease. The experiments described in this section were therefore designed to examine these aspects.

Infection with H. contortus is characterised by profound and often fatal anaemia, hypoalbuminaemia, weight loss and the presence of large numbers of worm eggs in the faeces[1-8]; initially the anaemia is normocytic and normochromic, but heavy or long-standing chronic infections are usually

accompanied by macrocytosis and hypochromia, low serum iron levels and depleted liver iron stores[9-12].

Not unnaturally most of the published work on genetic resistance to this parasite has utilised differences in venous haematocrit (PCV) and faecal egg counts as the standard criteria of worm establishment and disease resistance, yet even a cursory glance as these data reveals a trend which is far from encouraging. For example, Evans et al.,[13] found no difference in PCV between HbA and HbAB Merinos, but on 70% of the occasions when egg counts were determined the former had values which were generally 10-15% lower than the latter; Jilek and Bradley's[14] "high" and "low" resistance Florida Native ewes exhibited average PCV values of 29% and 26% respectively, but showed no real difference in egg counts; the PCV of the HbA Florida Native sheep used by Radhakrishnan et al.,[15] fell from 35% to 27% and from 33% to 27% in similarly infected HbB sheep, and egg counts stabilised at around 3,200 and 1,700 per gm respectively; and finally, Allonby and Urquhart[16] recorded mean PCV's of about 24% and 19%, and 24% and 21% respectively in HbA and HbB Merino ewes and lambs, but surprisingly no difference in egg counts between any of these groups except during periods of self-cure[17].

Regardless of their possible significance, differences in PCV and egg counts are neither the reliable indicators of infection that some authors might believe, nor are they

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axiomatic with resistance to the disease. Any statement to the contrary inherently assumes that a given worm burden produces the same degree of anaemia and egg count irrespective of haemoglobin type or breed, or expressed in another manner, that each worm sucks the same amount of blood and produces the same number of eggs, and that all sheep have a uniform capacity to withstand haemorrhage. This, of course, is completely unrealistic - firstly, because the amount of blood consumed and number of eggs produced by each Hamonchus are extremely variable, ranging between 0.02 and 0.07 ml per day and between 7×10^6 and 15×10^6 per female worm per day respectively, depending upon the stage, size and total number of parasites present[8,11,12,18,19]; and secondly, because the ability to withstand or counteract haemorrhage is a function of the host's blood volume and ability to replace among others, red cells and plasma proteins. Since resistance to this parasite can operate at the level of retardation or inhibition of development with resultant suppression of egg production[20,21] and presumably also blood loss, it is quite possible that egg counts remain depressed and PCV's elevated in association with a relatively high establishment; likewise animals with higher volumes and synthetic capacities will obviously become less anaemic (and hypoproteinaemic) following a given blood loss than those less well endowed. In other words, the PCV and egg count presented by an infected animal are neither directly not indeed sufficiently closely related to worm burden to allow accurate assessment of resistance to worm establishment; clearly this is only possible by direct measurement.

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To quantify resistance to the effects of worm establishment naturally requires a detailed knowledge of the effects themselves. Again it cannot be over-emphasised that this simply cannot be obtained from standard clinical tests such as PCV or serum albumin determinations, for the obvious reason that these indices are merely secondary manifestations of more deep-rooted and complex functional disturbances. Disease resistance can therefore be properly assessed only when these primary disturbances have themselves been measured. Since the major consequence of infection with H. contortus is loss of whole blood and its constituent red cells and plasma proteins into the abomasum, any conclusion regarding the parasites' pathogenic effects must be based on accurate measurements of these losses and their damage to the haematological and plasma protein status of the animal as a whole. Hence in addition to changes in red cell and protein concentration, it is necessary to describe the disease in terms of its effects on the total amounts and rates of degradation and synthesis of these constituents, i.e. in terms of their pool sizes and turnover. This type of dynamic information can only be obtained through the application of radioisotopic techniques.

Radioisotopes have already been used extensively in investigations of the anaemias and plasma protein changes associated with a number of helminthic parasites of sheep,

including H. contortus[10,12,18,22-26], but surprisingly the methodology developed for these studies has never yet been extended to cover the possible existence of genetic variability in resistance to haemonchosis. In the absence of the information which these techniques can so readily provide, it therefore remains to be established whether haemoglobin type and breed-related differences in PCV and serum albumin levels do in fact reflect fundamental differences in disease resistance per se, and if so whether this resistance derives from parasite or host factors, e.g. differences in the blood-sucking properties of individual worms or inherent variations in blood volume, red cell and protein synthetic capacities and haemoglobin C production.

From the foregoing account it is clear that the whole concept of resistance to Haemonchus and/or haemonchosis can only be proven when the development, establishment and pathophysiological effects of the parasite have been measured simultaneously and compared in animals with different Hb type and breed characteristics. Equally obvious is that to have any real significance, this information must derive from studies utilising radioisotopic as well as the more conventional haematological, biochemical and parasitological techniques. In the experiments reported in this section, the development and pathogenesis of single standardised H. contortus infections were monitored and compared in previously worm-free Scottish Blackface and Finn Dorset sheep (a Finnish Landrace and Dorset cross) of

similar age and known haemoglobin type. Resistance to the development and establishment of the parasite was assessed on the basis of egg output and worm recovery, and resistance to the attendant disease from concurrent measurements of changes in blood composition and red cell and albumin turnover.

MATERIALS AND METHODS

Experimental animals and design

Two experiments were carried out using a total of 33 Scottish Blackface and Finn Dorset wethers which had been reared and maintained parasite-free from birth. In the first, 10 Scottish Blackface (comprising 4 HbA and 4 HbAB and 2 HbB) and 8 Finn Dorset (4 HbAB and 4 HbB) sheep which were approximately 7 months old were each injected with ^{51}Cr-labelled erythrocytes, ^{59}Fe-citrate and ^{125}I-albumin and 3 days later infected with 350 third-stage *H. contortus* larvae per kg bodyweight; measurements of red cell and albumin turnover were made during the following 28 days at which point the animals were given a second injection of labelled materials; 4 days later the animals were necropsied and their worm burdens determined.

The second experiment was of similar design, but restricted to sheep of the Scottish Blackface breed. 15 worm-free wethers, aged about 9 months were divided into 3 equal groups on the basis of haemoglobin type. Each was injected with labelled materials and measurements of red cell and albumin kinetics made over the following 4 weeks; each was then given a second injection of radioisotopes, infected with 1400 infective *H. contortus* larvae per kg bodyweight and the kinetic measurements continued for a further 24 days when the animals were necropsied and their worm burdens determined.

The sheep were confined in standard metabolism cages throughout, and in addition to hay and water which were fed ad lib, were dosed orally each day with 10 ml 0.75% KI to block the thyroid and thereby ensure rapid excretion of ^{125}I. The sheep were weighed and blood collected for haematological and biochemical analyses twice weekly; faecal egg outputs were determined daily.

Haemoglobin typing

The haemoglobin type of each sheep was determined before infection and at regular intervals thereafter by electrophoresis of blood on cellulose acetate strips.

Haematological and biochemical analyses

Packed cell volume percentages (PCV), blood haemoglobin concentrations (Hb) and red cell counts (RBC) were measured and the mean corpuscular volumes (MCV) and haemoglobin concentrations (MCHC) of the red cells calculated as described earlier. Total serum proteins and serum albumin, globulin and iron concentrations were all measured by the techniques outlined previously.

Parasitological techniques

Culturing of larvae, faecal egg counts and determination of worm burdens at necropsy were performed as described earlier.

Labelling procedures

^{125}I-labelled albumin

Labelled albumin was prepared by trace-labelling a buffered solution containing 600 mg ovine albumin with

10 mCi Na ^{125}I. 2 g bovine albumin was added as "carrier" and the preparation dialysed for 2 days against 40 litres of saline. Solutions containing about 200 μCi ^{125}I were administered on each occasion.

Labelling of red cells with ^{51}Cr

Heparinised blood was treated with ^{51}Cr as sodium chromate and incubated at 37°C for 30 minutes. The labelled cells were then washed 3 times with 0.9% NaCl and reconstituted with plasma for injection. At each injection every sheep received a suspension of its own labelled cells containing approximately 700 μCi ^{51}Cr.

Plasma and red cell ^{59}Fe labelling

^{59}Ferric citrate (specific activity 7 μCi/μg) corresponding to a radioactivity of about 50 μCi was injected on each occasion.

Injection and sampling procedures

Labelled materials were injected together from separate syringes via a 3-way tap and jugular catheter. The first blood sample (5 ml) was collected from the opposite vein 15 minutes later, a further 6 samples at regular intervals over the following 3 days and subsequent samples daily. 1.0 ml aliquots of blood and plasma were diluted to 15 ml with dilute NaOH for radioactivity determination. For measurements of plasma iron turnover rates, 1.0 ml samples of plasma collected 15, 30, 60, 90, 120 and 180 minutes after injections of radioiron were similarly prepared. The total urine and faeces excreted during each 24 hr

were collected and representative samples (15 ml and 15g packed to a volume of 15 ml respectively) prepared for counting. Standard solutions of all labelled materials were prepared by diluting aliquots of each labelled preparation injected.

Radioactivity measurements

These were made using an automatic gamma scintillation counter, separation of isotopic mixtures being achieved by gamma-ray spectrometry and use of the standard solutions of each isotope; corrections for radioactive decay were also based on the activities of these standards.

Calculations and expression of results

As mentioned earlier in the text the most significant clinical features of ovine haemonchosis are anaemia and hypoalbuminaemia. To obtain detailed information on the aetiology of these changes and their relationship to the parasites' development within the host requires an appreciation of (a) the concentrations of red cells and albumin in the peripheral blood, obtained by the standard haematological and biochemical methods described earlier, (b) the total amounts of these constituents in the body and their rates of breakdown and synthesis, values which are best measured by the isotopic techniques outlined below, and (c) the inter-relationship between the above parameters and the more conventional indices of parasite activity and development referred to earlier, i.e. faecal egg counts and worm load.

During the work described in this thesis surveillance of parasite activity relied heavily on isotopic methods. The following is a brief account of the theory behind the use of these techniques and of the mathematical analyses performed; further details are given in the references cited.

Measurements based on ^{51}Cr-labelled red cells

Following intravenous injection of red cells labelled with ^{51}Cr and measurement of the radioactivities in blood, urine, faeces and standards, a number of important indices pertaining to the haematological status of the recipient were obtained.

Blood and circulating red cell volumes

These were estimated by application of the "dilution principle". Following injection of the labelled cells, 15 minutes were allowed for complete mixing within the circulation after which the first blood sample was withdrawn. The radioactivity of a carefully measured 1 ml aliquot of this sample was divided into the injected activity to obtain the blood volume (BV). Using the PCV of the 15 minute sample, the radioactivity per ml of packed red cells was calculated from the following equation:

$$\text{counts/min/ml red cells} = \frac{\text{counts/min/ml blood} \times 100}{\text{PCV}}$$

and divided into the injected activity to obtain the circulating red cell volume (RCV). To enable comparison of animals of different weight, all volumes were expressed on a bodyweight basis.

It should be stressed that while this technique provides an accurate index of RCV, figures obtained for BV underestimate true blood volume. This is because of a fundamental error in assuming that the peripheral venous PCV is representative of the haematocrit of the body as a whole, when in fact because the proportion of erythrocytes in the capillary bed is lower than in venous blood, venous PCV overestimates total body PCV by about 10%. The problem can be circumvented by estimating the animal's plasma and red cell volumes simultaneously, e.g. by using ^{125}I-labelled albumin and ^{51}Cr-labelled erythrocytes. In the work reported in this thesis, measurements of RCV and BV were generally based on this latter procedure, but in some instances indirect calculations were made, based on measurements of plasma volume by dilution of labelled albumin (see later for details). Such estimates, although less accurate than those based on the double isotope technique, are nonetheless perfectly adequate for the comparative studies envisaged.

Red cell survival (Apparent red cell half-life or $t_{\frac{1}{2}}$)

The ideal tracer for measuring red cell survival should have a number of properties, the most obvious of which being that it should be capable of attaching to the erythrocytes *in vitro* or *in vivo*, should remain firmly attached throughout the life of the cell, and should not be reutilised by or reincorporated into new red cells following removal of the originally-labelled cells from the circulation; finally, and most important of all, it should not damage the cell.

No label has yet been found which fulfils completely all these conditions, but erythrocytes labelled with ^{51}Cr provide indices of red cell survival which although by no means quantitative for reasons which are described below, are nevertheless very useful for comparative purposes. The value normally used to describe the persistence of ^{51}Cr-labelled cells in the circulation is the "apparent red cell half-life" or $t_{\frac{1}{2}}$. In the present studies this was obtained by converting the radioactivity of each blood sample to activity per ml of red cells and expressing this as a percentage of the equilibrium 15 minute post-injection value. A semi-logarithmic plot of red cell radioactivity against time was then made which as illustrated in Figure 1 normally consists of two phases, i.e. an early phase during which the red cell activity falls rapidly, followed by a period when activity declines exponentially with time. It was from the slope of the best straight line fitted from the circulating radioactivities during this latter period that the $t_{\frac{1}{2}}$ was calculated as the time taken for the activity to fall by 50%.

Since the normal red cell lifespan in sheep is about 120 days[27], it is obvious that ^{51}Cr red cell $t_{\frac{1}{2}}$ values (normally about 15 days) are a gross underestimate of true red cell survival. The reason for this is that the disappearance of labelled cells from the circulation reflects two main processes - loss of red cells by senescence or breakdown, and elution of isotope from intact cells[28,29].

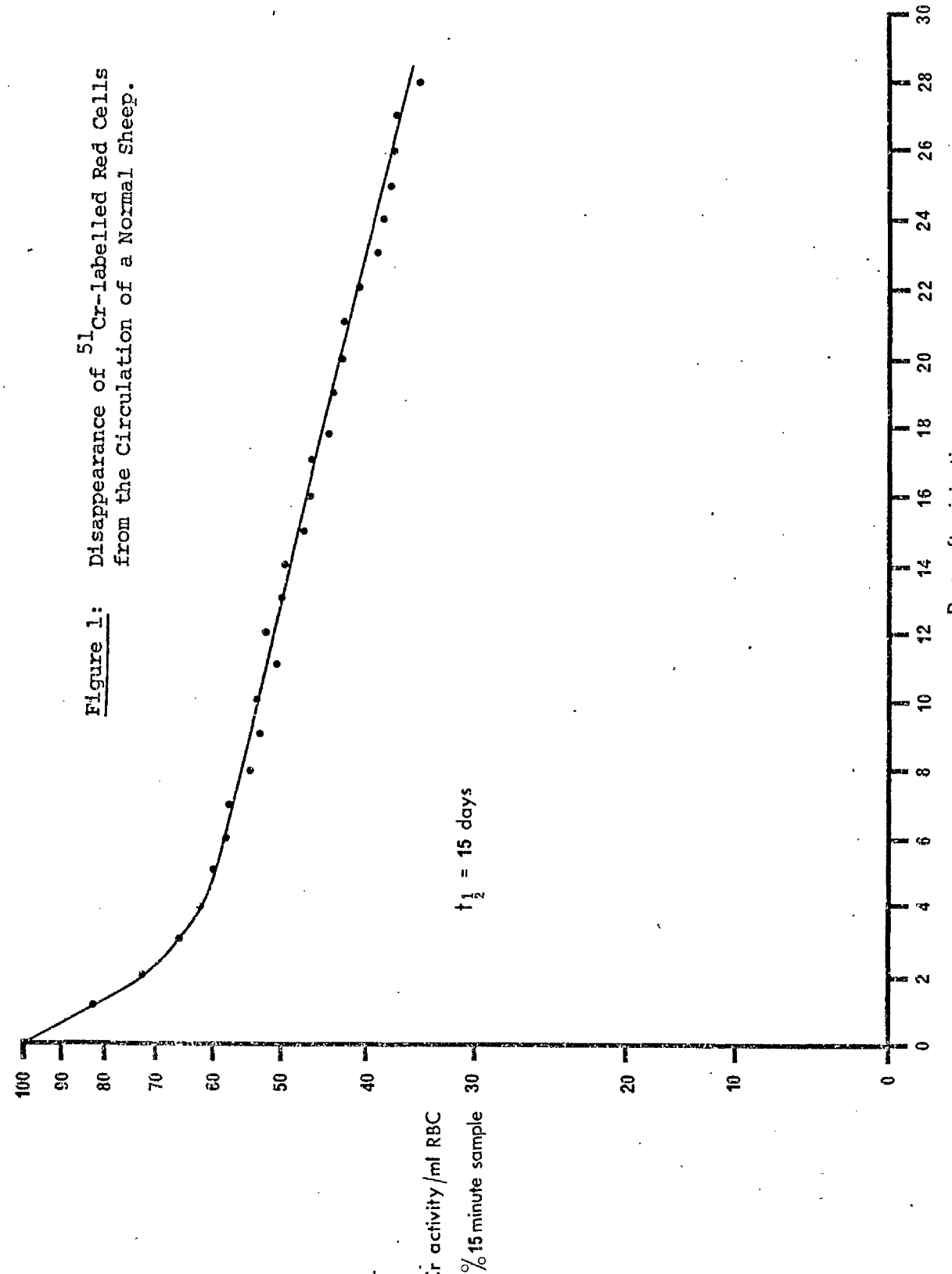

Figure 1: Disappearance of ^{51}Cr-labelled Red Cells from the Circulation of a Normal Sheep.

From the shape of the curve shown in Figure 1, it is apparent that although very much more marked during the first few days following injection of labelled cells (over this period more than 60% of the injected activity may be lost), the process of elution continues for as long as any labelled cells remain in the circulation; hence the prefix "apparent" when referring to red cell half-life values measured with ^{51}Cr.

Gastrointestinal haemorrhage and iron loss

Perhaps the greatest use of ^{51}Cr-labelled red cells is in estimating gastrointestinal haemorrhage. While this can be done with considerable accuracy in most species because there is minimal reabsorption of isotope when labelled cells are given orally or injected directly into the alimentary tract[30,31], in sheep it appears that a small proportion of the ^{51}Cr (on average 12%) is absorbed into the blood leading to some underestimation of haemorrhage[18].

In the present studies abomasal bleeding was estimated as faecal "clearances" of whole blood and red cells, obtained by dividing the total daily faecal radioactivity by the activities per ml of blood and red cells respectively taken at the beginning of each faecal collection period. These figures represent the amounts of blood and red cells (expressed as ml per day) which would have had to pass into the gut to account for the radioactivity in the faeces, but for a number of reasons they cannot be regarded as quantitative. Firstly, as mentioned above there may be some absorption of label from the gut leading to non-quantitative excretion of isotope in

the faeces and hence underestimation of blood loss. Secondly, a small amount of ^{51}Cr derived from labelled erythrocytes broken down within the circulation passes into the alimentary tract via the bile, tending in theory to cause a slight overestimation of faecal "clearance". In practice the contribution made by this process to faecal radioactivity is negligible after the phase of rapid elution of isotope from the cells, i.e. the initial 72 hours after injection and hence may be obviated (as in these studies) by making no measurements of "clearance" during this period. Thirdly, because the interval betwen passage of labelled cells into the gut and excretion of isotope in the faeces is unknown, circulating blood and red cell radioactivities cannot be related to faecal radioactivity with a great deal of confidence. Nevertheless, in spite of these potential sources of inaccuracy, faecal "clearances" based on the ^{51}Cr-red cell technique do provide very valid estimates of gastrointestinal blood loss and as such are useful as indices of the activities of blood-sucking parasites.

A further advantage of the technique is that in addition to blood loss, daily estimates may be made of the amounts of iron lost into the gut as haemoglobin. In the experiments reported here these were calculated using the formula of Roche and his colleagues[31]:-

$$\text{Iron lost into gut (mg/day)} = \frac{\text{Hb (g\%)} \times 3.34 \times {}^{51}\text{Cr "clearance" (ml)}}{100}$$

where 3.34 equals mg iron/gm haemoglobin.

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Measurements based on ^{59}Fe

Although much useful information on red cell turnover is obtained from the use of ^{51}Cr-labelled red cells, in many respects this is rather one-sided since it relates mainly to rates and routes of red cell breakdown. In order to obtain a better overall understanding of the functional state of the erythron, it is important, especially in situations where excessive amounts of blood pass into the gut, to be able to monitor the animal's capacity to synthesise red cells and reutilise some of their more valuable constituents, e.g. iron. Red cell production may be assessed indirectly from ^{51}Cr faecal "clearances" and changes in total red cell mass, but direct measurements are possible only by using a label which is incorporated directly into haemoglobin. Since the introduction of radioiron of high specific activity measurement of plasma iron turnover has become a widely accepted index of haemoglobin synthesis and hence red cell production. A further advantage of this isotope is that measurements of faecal radioactivity provide estimates of faecal iron excretion and hence of haemoglobin iron reabsorption when combined with the ^{51}Cr-red cell technique described earlier. A brief description of these methods is given below; further details are provided by Pollcove and Mortimer[32].

Plasma iron turnover

Iron exchange between the various tissues of the body is achieved by a transport mechanism in which the iron is

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attached to the β_1-globulin transferrin. Since two-thirds of the iron normally present in the body is in haemoglobin, and since the turnover of this pigment is much greater than that of the iron storage compounds ferritin and haemosiderin which make up more than 95% of the remaining iron, it follows that most of the metal leaving the plasma at any one time is directed to the bone marrow for incorporation into haemoglobin. Hence with a knowledge of the rate of clearance of a tracer amount of ^{59}Fe from the plasma and of the plasma iron concentration, the amount of iron turned over through the plasma may be measured.

From the argument presented above such rates should theoretically reflect iron utilised for haemoglobin synthesis and hence erythropoiesis, but for two reasons they are in fact an overestimate. In the first place a small proportion of the iron (and therefore ^{59}Fe) cleared from the plasma is carried to iron stores and second, of the iron taken to the bone marrow about 25% is not incorporated into erythroblasts but instead becomes fixed to a labile iron pool from which it is subsequently released back into the plasma. Plasma iron turnover rates should therefore not be considered as absolute measurements of haemoglobin synthesis but rather as approximate indices of erythroid marrow activity.

In the work reported in this thesis, plasma iron turnover rates were measured following intravenous injection of ^{59}Fe as ferric citrate. Blood samples were removed at regular intervals during the subsequent 3 hours and standard aliquots

of plasma assayed for radioactivity. Radioiron plasma concentrations were expressed as percentages of the 15 minute post-injection value and plotted on semi-logarithmic paper as a function of time. Since the ^{59}Fe concentration declined exponentially during the period of observation the fraction of plasma iron removed per unit of time (k) was calculated in accordance with first order kinetics, i.e.

$$k = \frac{0.693}{t\frac{1}{2}}$$

where 0.693 equals the natural logarithm of 2 and $t\frac{1}{2}$ the time taken for the plasma activity to fall by 50%

The plasma iron turnover rate (PITR) was then calculated from the modified formula of Huff and his associates[33]:

$$\text{PITR (mg/day)} = \frac{\text{Serum iron (mg/ml)} \times \text{Vp (ml)} \times 0.693 \times 1440}{t\frac{1}{2}\text{ (min)}}$$

where Vp equals the plasma volume, measured as described later by dilution of radioiodinated albumin, and 1440 the number of minutes per day.

To allow comparisons between individuals this rate was expressed as mg/day/kg bodyweight.

A simpler standard of reference, permitting comparison between individuals, relates PITR to 100 ml whole blood. This was calculated from the equation of Bothwell et al.,[34]

$$\text{PITR (mg/day/100 ml blood)} = \frac{\text{Serum iron (mg\%)} \times 0.693 \times 1440}{t\frac{1}{2}\text{ (min)}} \times \frac{100-\text{PCV}}{100}$$

Faecal iron excretion and haemoglobin iron reabsorption

Following its removal from the plasma, ^{59}Fe is incorporated into haemoglobin and subsequently re-enters the circulation as

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red cells. By measuring the radioactivity of standard aliquots (normally 1 ml) of blood samples removed daily after injection, the rate of iron incorporation into red cells may be monitored and to some extent quantified by expressing the total circulating red cell activity (obtained by multiplying the maximum count rate/ml packed red cells by the total red cell volume) as a percentage of the dose injected. The figure obtained, which is known as the red cell iron utilisation index is often used to assess erythropoiesis under "steady-state" conditions, but in situations where rapid changes in red cell mass might be expected, e.g. in H. contortus infections it is impossible to measure with any degree of accuracy and has therefore not been included in this thesis.

Nevertheless by monitoring ^{59}Fe blood and red cell radioactivities and relating these to daily faecal radioactivities, "clearance" figures similar to these described earlier for ^{51}Cr may be calculated. Since iron lost into the alimentary tract as haemoglobin is potentially available for re-utilisation, any ^{59}Fe radioactivity which appears in the faeces must reflect iron actually lost from the body by this route. Therefore, from determination of blood loss based on the daily ^{59}Fe faecal activity, together with a knowledge of the blood haemoglobin level at the beginning of each faecal collection prior, an estimate may be made of faecal iron excretion using the equation of Roche and his colleagues[31]

$$\text{Faecal iron excretion (mg/day)} = \frac{\text{Hb (g\%)} \times 3.34 \times {}^{59}\text{Fe blood "clearance" (ml)}}{100}$$

where 3.34 is the amount of iron in 1 g haemoglobin.

Since faecal iron excretion and intestinal iron losses were both measured in these studies, haemoglobin iron reabsorption could be estimated from the following equation:

Haemoglobin iron reabsorbed (mg/day) = Iron lost into gut (mg/day)- Faecal iron excretion (mg/day).

Measurements of albumin distribution and catabolism

Like all proteins in the body albumin is constantly broken down and synthesised and in order to understand the cause of any changes in serum albumin concentration occurring in animals infected with H. contortus it is necessary to study albumin metabolism. Albumin trace-labelled with radioiodine by the method described earlier is particularly suitable for this purpose because (a) it is free from significant denaturation and therefore behaves metabolically like the animal's own unlabelled molecules[35]; (b) there is no reutilisation of the label once the protein has been degraded[36,37], and (c) provided the thyroid is "blocked" by prior administration of stable iodide the isotope from degraded protein is excreted quantitatively. The main route of excretion is the kidneys and although passage of labelled iodide also occurs into the gastrointestinal tract, faecal excretion is normally negligible since iodide is absorbed in the stomach and small intestine[38].

In the studies reported here measurements of the various parameters of albumin metabolism were based largely on the mathematical model illustrated in Figure 2. This "two-compartment" system assumes that there is one common extravascular albumin pool (EA) which communicates with

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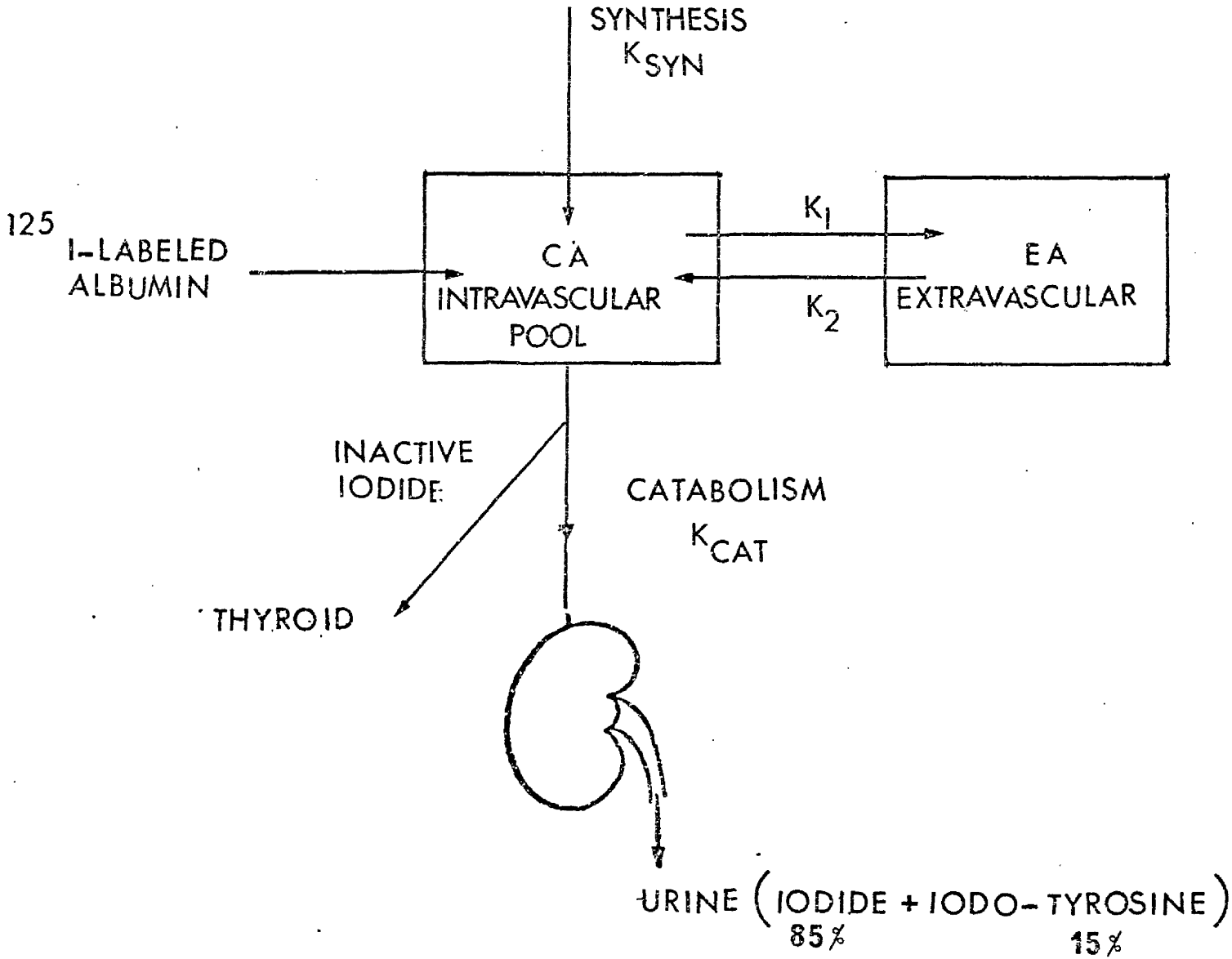
Figure 2: Distribution and Metabolism of ^{125}I-Labelled Albumin.



intravascular albumin (CA) through pores in the capillary wall, and that protein transfer from one pool to the other takes place in either direction, k_1 reflecting outward movement and k_2 the return via lymphatic flow. It must be stressed that since there are at least two main groups of extravascular pools each of which equilibrates with plasma at different rates, k_2 reflects the average return rate of protein from all the extravascular compartments[39].

Three further assumptions are inherent in the model. First, that albumin synthesis (k syn) is "intravascular". There is no doubt about the validity of this - albumin is synthesised exclusively in the liver[40], and upon release from the parenchymal cells immediately enters the venous blood or hepatic lymph. Second, that albumin catabolism (k cat) is also "intravascular". Again this appears to be valid because although a large number of organs catabolise the protein, e.g. the liver[41], kidney[42] and gastrointestinal tract[43], only "intravascular" catabolism fulfils the requirement of the constant day to day degradation established for this protein[44,45]. Finally, in common with all others this model assumes that the animal is in a "steady-state", i.e. that k syn and k cat are equal and that CA and EA remain constant. In work with parasitised animals this is clearly not valid; even so a great deal of useful information - particularly of a comparative nature may still be obtained.

Following intravenous injection of labelled preparations, and analysis of plasma, urine and faeces for radioactivity measurements of albumin pools and catabolic rate were made

after first separating whole body radioactivity into its intra- and extravascular components (Figure 3). This was achieved by expressing the count rate of each plasma sample as a percentage of the equilibrium 15 minute post-injection sample and plotting the values on a semi-logarithmic scale against time (Q_P). Activity retained in the body at the end of each day was calculated by subtracting from the injected dose the cumulative activity excreted in both urine and faeces, expressed as a percentage of the injected activity and plotted on semi-logarithmic paper against time (Q_R). Extravascular activity (Q_E) was then obtained indirectly as the difference between Q_R and Q_P at the end of each daily collection period.

Plasma volume (Vp)

This was calculated from the radioactivity of the plasma sample taken 15 minutes after injection of labelled albumin and application of the "dilution principle".

Intravascular albumin (CA)

The total amount of albumin present within the bloodstream was determined from Vp and the serum albumin concentration of a blood sample removed immediately prior to injection.

CA (g) = Vp (ml) x Serum albumin (g/ml)

Total body albumin (TA)

Two procedures were employed - the "extrapolation" method[46] and the "equlibrium-time" method[44].

In the extrapolation procedure the linear point of the plasma activity curve (Q_P) was extrapolated to the

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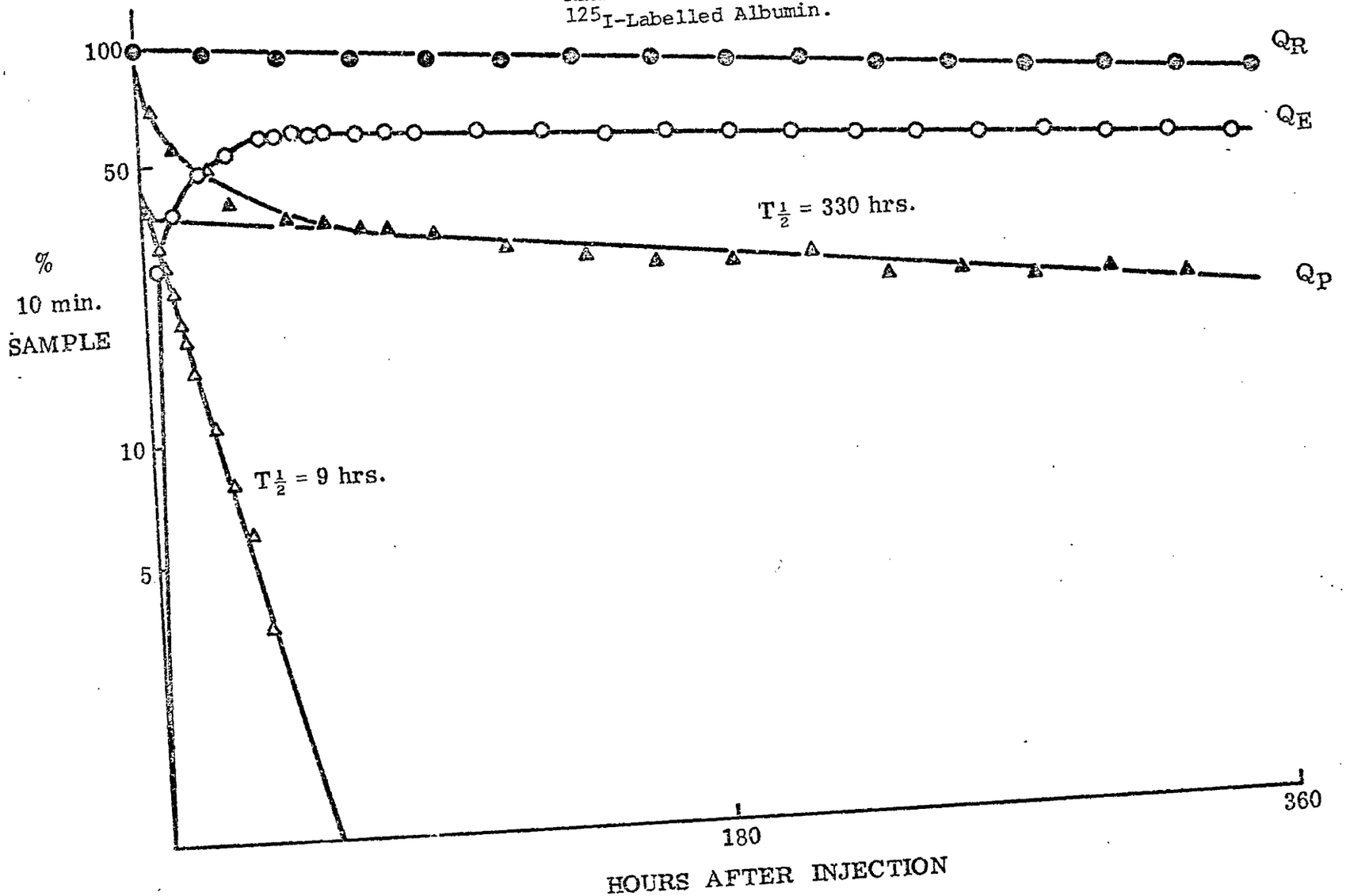
Figure 3: Whole Body, Plasma and Extravascular Radioactivities following injection of ^{125}I-Labelled Albumin.



ordinate, the intercept (C_1) noted and TA calculated from:

$$TA\ (g) = \frac{CA}{C_1}$$

Calculations based on the latter method were made by noting the values of Q_E and Q_P at the equilibrium time, (i.e. when Q_E was maximal), and substituting these into the equation:

$$TA\ (g) = \frac{CA\ (Q_P + Q_E)}{Q_P}$$

It should be noted that the assumptions inherent in these calculations differ. The extrapolation method assumes that distribution of labelled albumin between CA and EA is complete after the initial rapid decline in Q_P and therefore that C_1 indicates the fraction of TA present in the plasma had equilibration of labelled albumin between CA and EA been instantaneous; it further assumes that albumin synthesis and catabolism take place both intra- and extravascularly. In fact, albumin distribution between CA and EA is a continuous process and synthesis and catabolism are purely intravascular. Therefore, activity/mg albumin (specific activity) is always lower in the plasma, and as a result of net transfer of high specific activity protein from EA into CA the slope of the plasma activity curve is reduced, C_1 underestimated, and TA overestimated. The equilibrium-time method assumes intravascular catabolism and immediate excretion of radioiodide following degradation of the protein. It is, therefore, theoretically more acceptable than the extrapolation procedure but is technically more

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troublesome, requiring quantitative collections of urine and faeces and estimation of the equilibrium time from a phase in the Q_E curve where selection of the time of maximal radioactivity is difficult. For these reasons, and since no significant differences were found between values calculated by the two methods, figures quoted in the text relating to TA (and hence EA - see below) are the average of the values obtained by both procedures.

Extravascular albumin (EA)

The size of the extravascular pool was calculated as the difference between TA and CA, and like all others was expressed on a bodyweight basis:

$$EA\ (g) = TA - CA$$

Albumin catabolism

Albumin catabolism was assessed in two ways. First, on the basis of the "apparent half-life" ($t_{\frac{1}{2}}$) of the linear part of the plasma activity curve. While for reasons mentioned earlier such values underestimate the true rate of protein catabolism, they do provide good qualitative indices and are therefore most useful in comparative studies of the type described here.

The other method employed was based on analysis of excreted radioactivity. This method, which was first introduced by Campbell et al.,[44] assumes that protein catabolism occurs intravascularly and therefore that any radioactivity excreted must represent the degradation of a certain fraction of the labelled albumin present within the bloodstream. This fraction, known as F(CA) or K, was

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calculated daily by dividing the total activities excreted in the urine (U) and faeces (F) by the total activity present in the plasma, obtained by multiplying the radioactivity/ml plasma at the beginning of each collection period by Vp.

$$F(CA) = \frac{\text{Total excreted activity}}{\text{Total plasma activity}} = \frac{U + F}{\text{Activity/ml plasma} \times Vp}$$

Fractional catabolic rates were then converted to absolute amounts using the equation:

Mass of albumin catabolised (g/day) = CA x F(CA)

and finally related to bodyweight.

Estimation of gastrointestinal plasma leak

In view of the likelihood that most of the changes in albumin catabolism would be associated with loss of plasma into the abomasum, faecal "clearances" of plasma, similar to those described earlier for blood and red cells using ^{51}Cr and ^{59}Fe, were calculated by dividing the total radioactivity in each 24-hour collection of faeces by the activity/ml of plasma taken at the beginning of the collection period. These values underestimate the extent of any plasma loss because of radioiodide absorption from the gut, but are still of considerable qualitative significance.

RESULTS

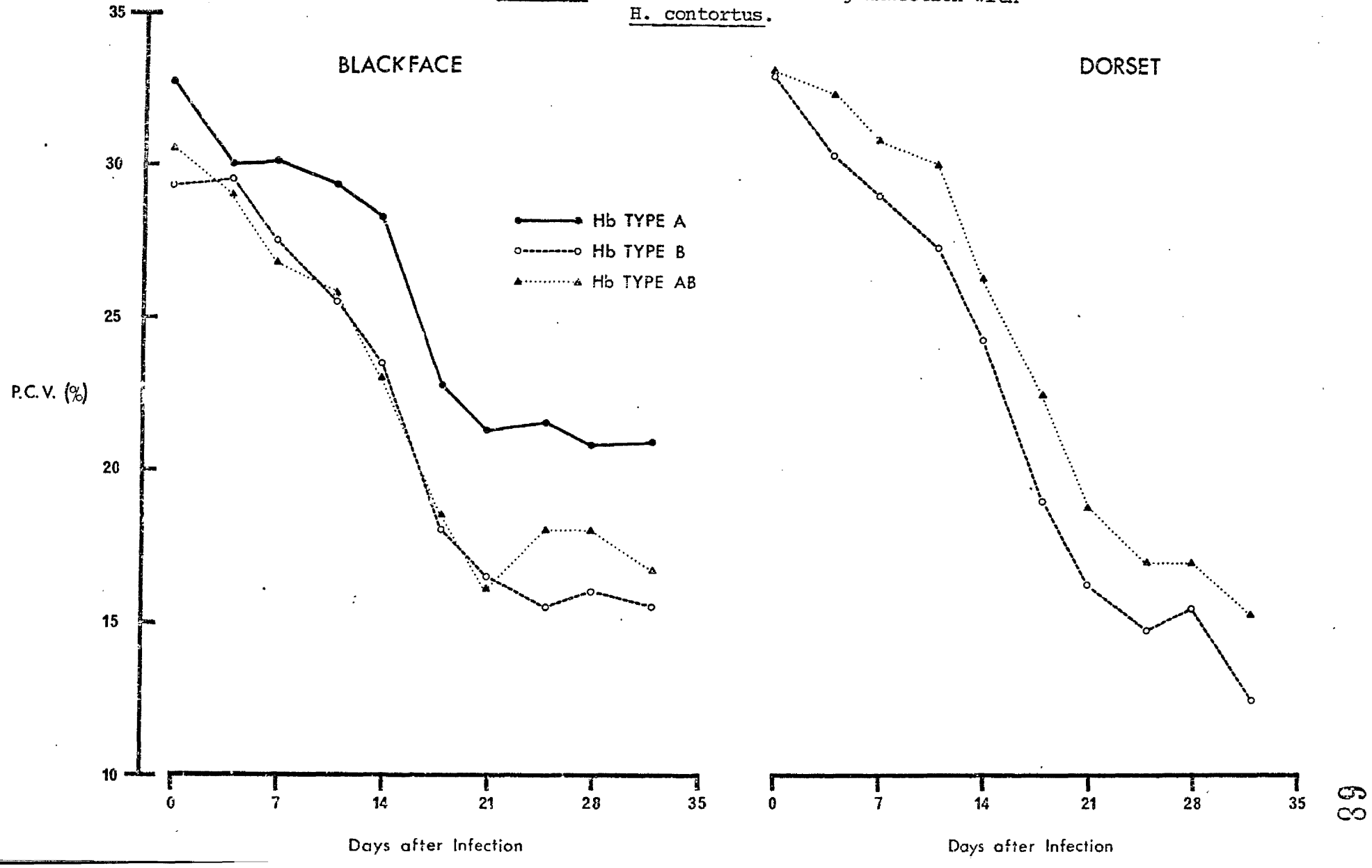
Experiment 1

Scottish Blackface and Finn Dorset wethers were grouped according to breed and haemoglobin type, infected with 350 H. contortus larvae/kg bodyweight and necropsied 32 days later. Haematological and biochemical estimations together with measurements of red cell and albumin kinetics and faecal worm egg output were made throughout the course of the investigation. For ease of presentation only average figures are included in the illustrations, but since group sizes were small and individual variations often large, detailed information regarding the response of individual sheep is given in Appendix 1.

Haematological Observations

A number of fairly consistent features emerged with regard to the haematological status of the sheep both prior to and following infection (Figure 4). Even before infection Scottish Blackface sheep of Hb type A had significantly higher PCV's than their HbB counterparts ($P < 0.02$), with HbAB types intermediate between the two. This relationship was not apparent in the Dorsets, HbAB and HbB group mean values being identical but slightly higher than the corresponding figures for the Blackfaces.

Figure 4: PCV Values Following Infection with H. contortus.

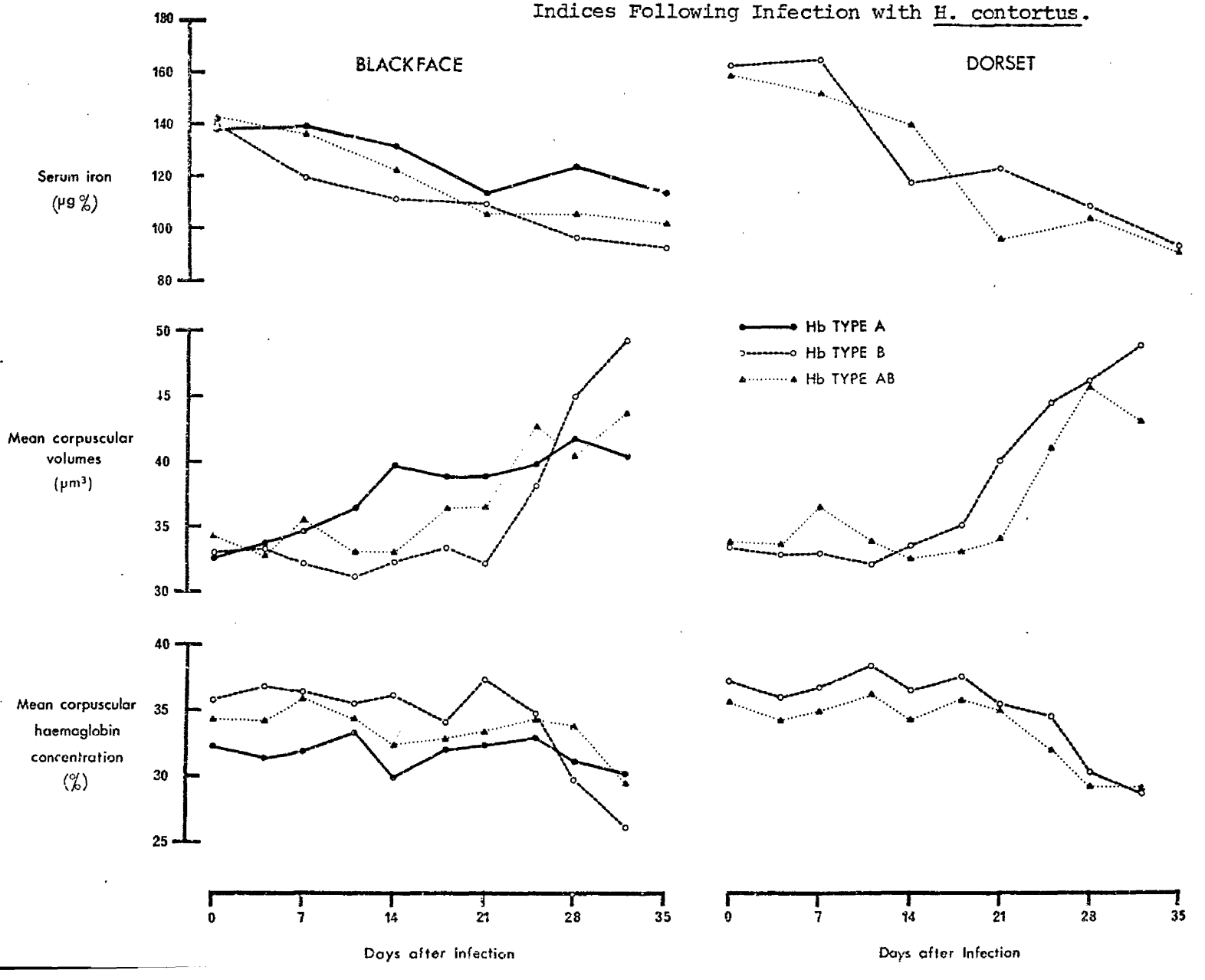


During the course of infection all animals developed severe anaemia, but within each breed this was most pronounced in HbB types. For example, between the 8th and 24th days, which was the period of most marked haematological change, PCV's of HbB Blackfaces fell by 46% to a mean level of 16%, whereas those of the HbA sheep were relatively well maintained at 21%, representing a drop of 28%. The response of HbAB Blackfaces was similar to that of their HbB counterparts, but group differences within this breed were significant only between HbA and HbB ($p < 0.05$). Haemoglobin B-type Dorsets suffered reductions in PCV virtually identical to those of comparable Blackfaces (i.e. about 50%) and significantly greater than HbAB sheep of the same breed ($p < 0.05$).

With the exception of the HbB Dorsets, whose PCV's deteriorated further between day 24 and necropsy, most sheep maintained their values at the low levels referred to above throughout the remainder of the study; at necropsy the only statistically significant breed difference was that HbB Blackfaces had higher haematocrits than similar animals of the Finn Dorset breed ($p < 0.05$).

The trends observed with regard to total red cell count and haemoglobin concentration were in general similar to those described for PCV, but a number of differences emerged which were reflected in alterations in both erythrocyte size (MCV) and haemoglobin content (MCHC). Both indices remained steady during the early

Figure 5: Serum Iron Concentrations and Red Cell Indices Following Infection with H. contortus.



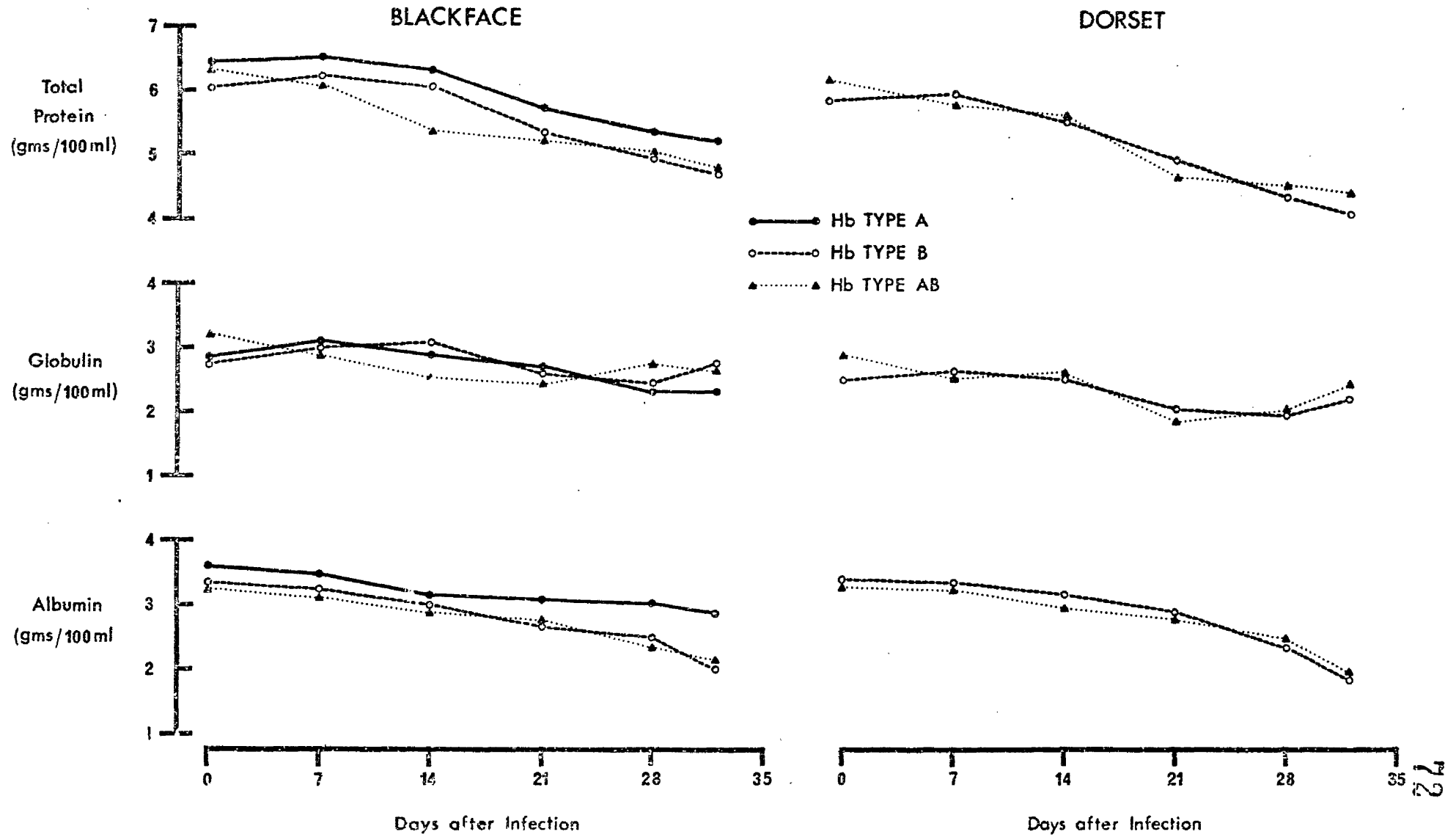
stages of the disease, but from about the 18th day onward macrocytosis and hypochromia became progressively more dominant features of the anaemia, particularly in the HbB sheep of each breed (Figure 5). Although no significant breed difference was noted in either index, MCV's were significantly higher at necropsy in the HbB Blackfaces than in both HbAB ($p < 0.05$) and HbA ($p < 0.01$) sheep of the same breed; MCHC's were also significantly lower in HbB than HbA Blackfaces at this time ($p < 0.01$).

Biochemical Observations

Serum iron concentrations were similar in all animals at the outset (ranging between 130 µg% and 160 µg%) and changed little during the first two weeks of infection (Figure 5). Thereafter values tended to decline and while there were no significant group differences at the termination of the study (all falling within the range 90 µg%-130 µg%), the extent of the deterioration experienced by each was breed and haemoglobin type-related in that HbB and HbAB Dorsets suffered relatively greater reductions (50% and 24% respectively), and the least dramatic decline (18%) was associated with the HbA Blackfaces.

The serum protein changes recorded during the study are illustrated in Figure 6. Prior to infection no consistent inter-relationships were apparent between these values and the breed or haemoglobin type of the animals concerned, except that there was some indication that total protein and albumin concentrations were higher in Blackface

Figure 6: Serum Protein Concentrations Following Infection with H. contortus.



sheep and within each breed, with each substitution of an A for a B allele.

The most marked disturbances attributable to infection occurred from about the 2nd week after larval administration, and were characterised by progressive reductions in total protein and albumin levels. At necropsy the lowest values for both components were associated with HbB Dorsets and the highest with HbA Blackfaces. No significant breed differences were recorded but a number of group differences were apparent, particularly in the Blackfaces where HbA types had a significant advantage at necropsy over their HbB and HbAB counterparts with respect to both albumin ($p < 0.01$) and total protein concentrations ($p < 0.05$).

Serum globulins remained fairly static throughout with the result that albumin:globulin ratios generally declined; the few group differences recorded were minimal.

Production of Haemoglobin C

Electrophoretic and subsequent densitometric analyses of the haemoglobin samples collected at weekly intervals during the experiment revealed a decrease in the percentage of HbA and a corresponding increase in the percentage of HbC around the third and fourth weeks after infection (Figure 7), i.e. about the time PCV and Hb levels fell below 20% and 7% respectively. These changes occurred only in sheep which carried the gene for HbA while no replacement of HbB was observed even in severely anaemic HbAB or HbB types. Although the proportion of HbA replaced by HbC in AB type

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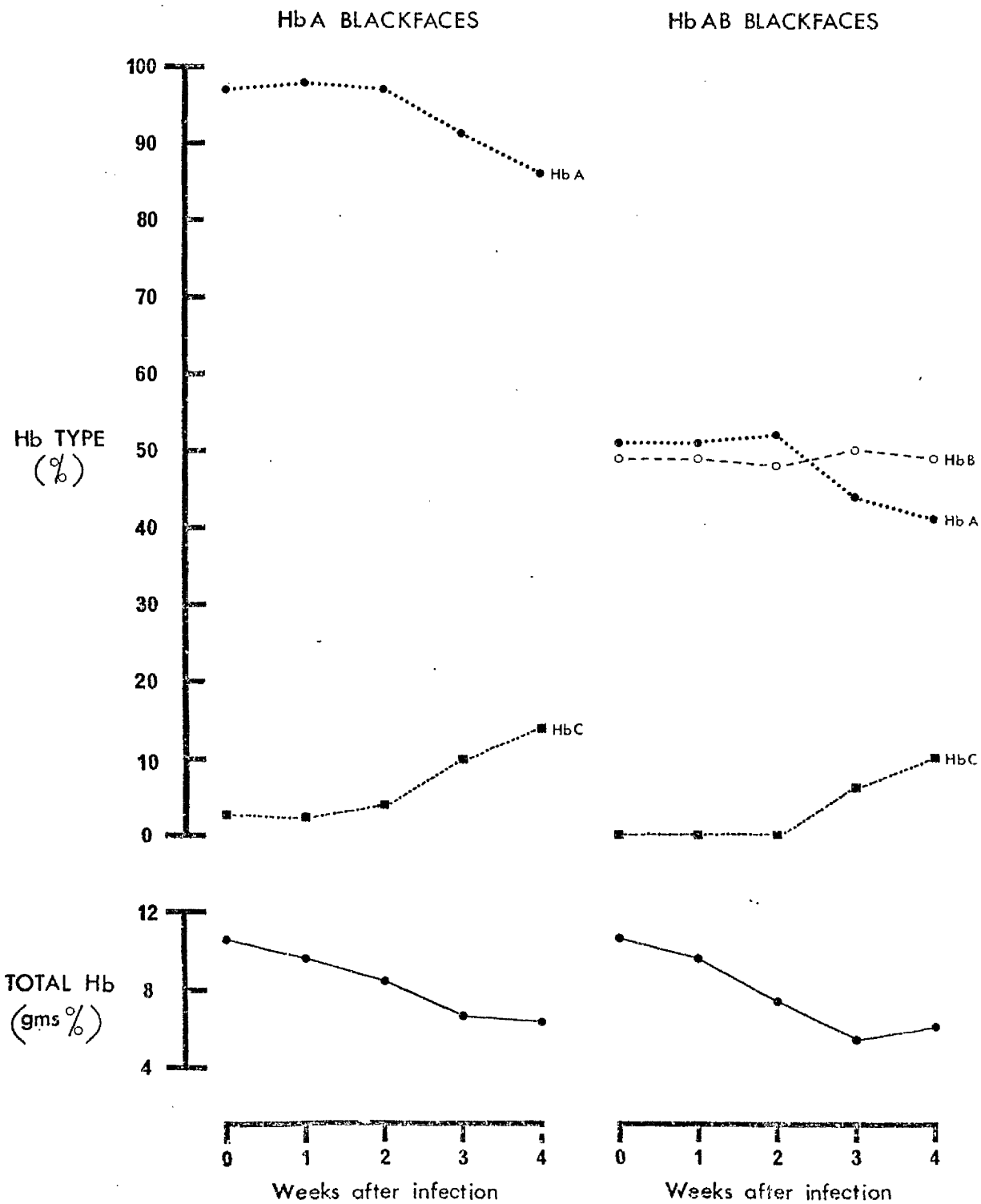
Figure 7: Production of HbC Following Infection with H. contortus.



sheep (about 22% on day 28) was greater than in HbA animals (14%), the concentration in the latter was greater (0.85 g% and 0.67 g% respectively). Within each group, HbC production was enchanced to the greatest extent in the most anaemic sheep but breed and haemoglobin type differences in the ultimate concentration attained were minimal.

Parasitological data

The mean faecal worm egg output of each group is depicted in Figure 8 from which it can be observed that the general pattern was similar in all groups, i.e. eggs were first detected around the 15th-17th days after administration of larvae and increased dramatically in numbers over the following week to reach a level which was maintained thereafter. Egg output was clearly highest in HbB Dorsets (31 million per day) and lowest in HbA Blackfaces (11 million per day) with other groups intermediate between these figures, but because of the considerable variability experienced the only significant group difference was the lower rate of production in HbA over HbB Blackface ($p < 0.05$).

The number of infective larvae given to each sheep, the number and size of the worms recovered, and the percentage of the administered dose of larvae established as adults are shown in Table 1. These figures demonstrate an obvious interaction between worm recovery, breed and haemoglobin type. Fewer worms were recovered from Blackface than from Dorset sheep of comparable haemoglobin type and within both breeds, from animals with an allele for HbA. Small group sizes and

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Figure 8: Faecal Egg Output Following Infection with H. contortus.

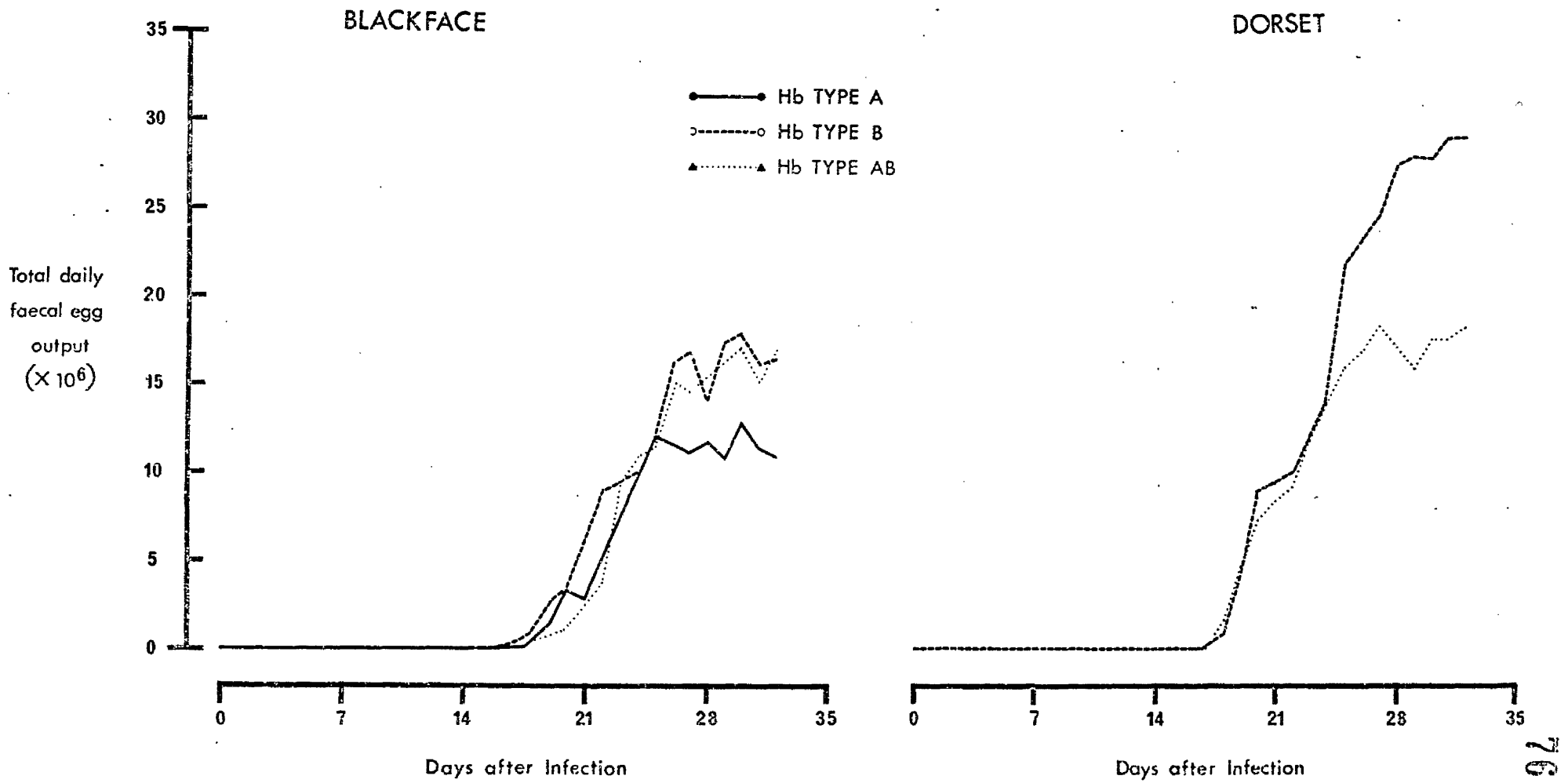

TABLE 1.

Worm Counts at Necropsy of Sheep Infected with 350 *H. contortus* Larvae/kg.

	Sheep No.	Larval Dose	Worms Recovered	Group Mean worm lengths (mean) male	± S.E. female	% Recovery
Blackface HbA	34	9100	900			9.9
	51	8700	950			10.9
	60	9700	1350			13.9
	71	8300	1600			19.3
Mean		8950	1200	13.0	19.6	13.5
S.E.		299	167	1.1	1.4	2.1
Blackface HbAB	48	9400	2200			23.4
	54	7500	1600			21.3
	79	7500	1300			17.3
	93	6900	1150			16.7
Mean		7825	1563	12.9	18.8	19.7
S.E.		544	232	1.4	1.5	1.6
Blackface HbB	53	7900	1650			20.9
	85	7400	1950			26.3
Mean		7650	1800	13.8	17.2	23.6
S.E.		250	150	2.2	1.8	2.7
Dorset HbAB	473	9000	1550			17.2
	485	9600	2100			21.9
	514	9200	2100			22.8
	520	8300	2350			28.3
Mean		9025	2025	13.8	20.7	22.6
S.E.		272	169	1.2	1.9	2.3
Dorset HbB	480	10200	4400			43.1
	489	8300	2450			27.5
	501	7600	2700			35.5
	541	9100	3000			33.0
Mean		8950	3138	14.7	20.8	34.8
S.E.		533	436	2.3	4.6	3.2

individual variation precluded significance in many instances, but within the Dorset breed, worm burdens were lower in the HbAB group ($p < 0.02$), HbA Blackfaces had significantly fewer worms than HbB sheep of the same breed ($P < 0.05$) which in turn harboured fewer worms than the HbB Dorsets ($p < 0.05$).

A further feature illustrated is that despite variation in worm load, the mean lengths of the worm recovered from each of the experimental groups were remarkably similar (these measurements were based on 100 worms taken at random from the combined worm burdens of each group).

Pathophysiological data

Blood volumes

The results of the blood volume measurements made prior to infection and repeated 28 days later are given in Tables 16 and 17 (Appendix 1).

From the pre-infection data it is apparent that within the Blackface breed, sheep of Hb type A held a distinct advantage over each of the other haemoglobin types with respect to the size of all blood compartments. Circulating red cell volumes were approximately 25% greater in this group than in HbAB ($p < 0.02$) and HbB ($p < 0.01$) types and essentially similar differences were recorded in plasma and blood volumes. No such variation was apparent in the Dorsets, but animals of this breed had higher volumes than Blackfaces of the same haemoglobin type ($p < 0.05$-< 0.01). Undoubtedly, the major reason for these differences was the variation in liveweight of the animals concerned - HbA

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Blackfaces were clearly heavier than others of the same breed and Dorsets had a weight advantage over Blackfaces of comparable haemoglobin type; moreover when compartment volumes were related to liveweight all the above mentioned intra-breed and several inter-breed differences were nullified, although in all parameters HbAB Dorsets retained their superiority over HbAB Blackfaces ($p < 0.05$-< 0.02).

The two outstanding features revealed by the results at 28 days post-infection were, firstly the low red cell volumes of all animals compared with pre-infection values, and secondly the clear indication that the depletion suffered by individual sheep was both breed and haemoglobin type related, with Dorsets of each type experiencing more dramatic reductions than comparable Blackfaces (i.e. 60% compared with 50%) and HbA types of the latter breed being least affected of all (i.e. 40% reduction). Considering the initial red cell volume of each group it is evident that the deficit incurred by Dorset over Blackface sheep was even greater in absolute than in percentage terms (mean 330 ml compared with 190 ml respectively).

Since plasma volumes were generally unaffected by the disease, contractions in blood and red cell volumes were usually closely related.

Red cell survival

Red cell survival was assessed on the basis of the circulating half-life ($t_{\frac{1}{2}}$) of ^{51}Cr-labelled erythrocytes

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during two stages of the investigation, i.e. over the first week following larval administration - to provide an index of survival in the absence of excessive abomasal bleeding, and again between the 3rd and 4th weeks to assess the relative effects of the adult worm population of each group. No significant group variations were noted over the early stages of the infection with $t_{\frac{1}{2}}$ values generally falling within the normally accepted range for sheep (320-450 hrs), but a number of major differences were apparent during the post-patent period of observation. At this stage red cell survival was lower ($p < 0.05$-< 0.01) in all groups, $t_{\frac{1}{2}}$'s ranging between 98 hrs in HbB Dorsets and 199 hrs in HbA Blackfaces, but the only significant difference recorded between comparable groups was the longer survival of labelled cells in the circulation of HbA than in HbAB Blackfaces ($p < 0.02$).

Gastrointestinal blood and red cell losses

Gastrointestinal blood and red cell losses were estimated during the first 28 days of infection by relating the daily faecal ^{51}Cr radioactivity to that present in the blood and red cells respectively. Figure 9 which illustrates the average losses recorded, gives an insight into the pattern and extent of bleeding suffered by each group.

Excessive harmorrhage into the gut was first detected in all animals between the 8th and 10th days, increased rapidly in severity over the following 10-12 days, reaching a peak which was generally maintained thereafter. However, considerable

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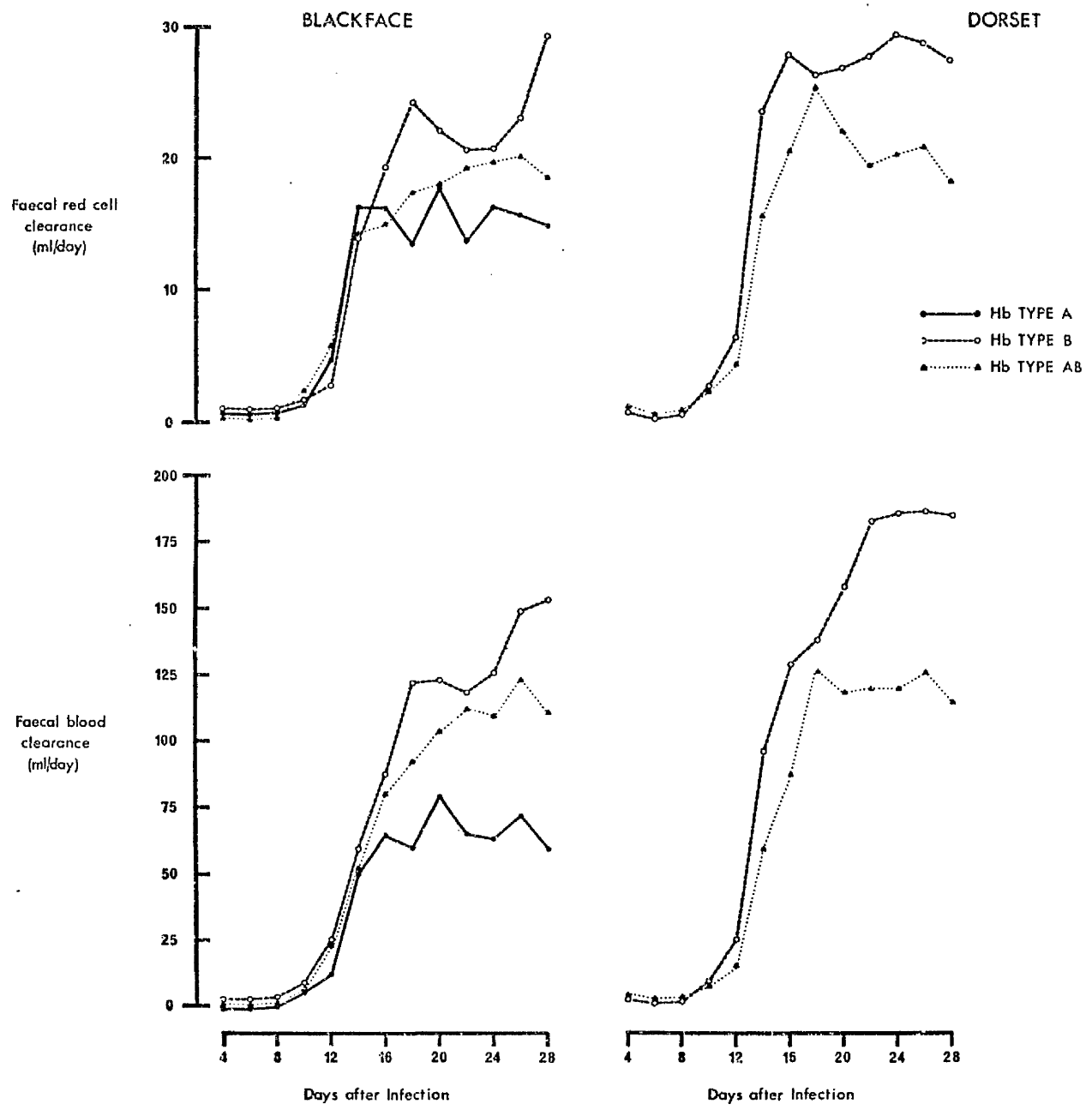
Figure 9: The Onset and Development of Abomasal Haemorrhage after Infection with H. contortus.



variability existed between groups and also between individuals within each group in the severity of this haemorrhage. Of the Blackface sheep, HbA types lost less blood via the gut than HbB sheep ($p < 0.05$), and in the Dorsets haemorrhage was less severe in HbAB than in HbB animals ($p < 0.05$); however, differences between groups of the same haemoglobin type were not significant - even in the case of HbB sheep where losses of both blood and red cells were about 50% higher in the Dorset breed.

Two further point merit emphasis with regard to these results. Firstly, the cumulative red cell loss suffered by each animal was considerably in excess of the total red cell deficit. This was particularly apparent in the Dorsets which on average lost approximately 400 ml red cells between the 8th and 28th days, but was also noticeable in the Blackfaces which lost about 300 ml over the same period. The fact that reductions in red cell volume amounted, as mentioned earlier, to 330 ml and 190 ml respectively suggests in an indirect way that erythropoiesis was accelerated in all groups, but especially those under most anaemic stress, namely the Dorsets. Secondly, although group variations in blood loss were clearly most pronounced after patency they were already quite apparent by about the 12th day after infection. The relevance of this observation is discussed later.

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TABLE 2.

Inter-relationship between Egg Output, Blood Loss and Worm Burden in Scottish Blackface Sheep.

	Sheep No.	Average daily egg output ($X10^6$)	Average daily blood loss (ml)	Average eggs/ female worm ($X10^3$)	Average blood loss/worm (ml)	Average eggs/ml blood ($X10^3$)
Haemoglobin A	34	4.9	38.8	10.9	0.043	126
	51	7.3	56.2	15.4	0.059	130
	60	12.8	86.5	19.0	0.064	148
	71	9.9	93.1	12.4	0.058	106
Mean		8.7	68.7	14.4	0.056	128
S.E.		1.7	12.8	1.8	0.004	9
Haemoglobin AB	48	13.8	157.2	12.5	0.071	88
	54	14.2	105.8	17.8	0.066	134
	79	*	*	*	0.064	*
	93	8.8	73.8	15.3	0.069	112
Mean		12.3	113.9	15.2	0.068	111
S.E.		1.7	23.0	1.5	0.002	13
Haemoglobin B	53	14.2	104.6	17.2	0.063	136
	85	13.4	163.7	13.7	0.084	82
Mean		13.8	134.2	15.5	0.074	109
S.E.		0.4	30.0	1.8	0.011	27

* Died on day 23

TABLE 3.

Inter-relationship between Egg Output, Blood Loss and Worm Burden in Finn Dorset Sheep.

	Sheep No.	Average daily egg output($X10^6$)	Average daily blood loss (ml)	Average eggs/ female worm($X10^3$)	Average blood loss/worm (ml)	Average eggs/ml blood ($X10^3$)
Haemoglobin AB	473	11.1	83.7	14.3	0.054	133
	485	13.4	121.5	12.5	0.058	110
	514	14.4	132.6	13.7	0.063	109
	520	18.6	143.9	15.8	0.061	129
Mean		14.4	120.4	14.1	0.059	120
S.E.		1.6	13.1	0.7	0.002	6
Haemoglobin B	480	17.2	219.3	7.8	0.050	78
	489	10.6	124.5	8.6	0.051	85
	501	26.0	190.2	19.3	0.047	137
	541	21.0	186.1	14.0	0.062	113
Mean		18.7	180.0	12.4	0.053	103
S.E.		3.3	19.9	2.7	0.003	14

Inter-relationships between haemorrhage, egg output and worm burden

Although excessive haemorrhage clearly preceded the appearance of eggs in the faeces, blood loss and egg output were obviously inter-related during the latter stages of the experiment and in general correlated well with worm burdens (Tables 2 and 3). Nonetheless there was a great deal of variation between individuals in the haematophagic and reproductive capacities of their parasite populations, worms of some sheep not only causing greater haemorrhage (0.08 ml blood/worm/day) than those of others (0.04 ml blood/worm/day), but also producing more eggs per unit of blood consumed (i.e. 148,000 eggs/ml blood compared with 78,000 eggs/ml blood). It should be emphasised however that there was no indication that the metabolic activity of individual parasites as assessed by either index was in any way influenced by the host's breed or haemoglobin type.

Gastrointestinal loss and reabsorption of haemoglobin iron

The amounts of iron lost into the gut, excreted in the faeces and reabsorbed, were estimated daily between the 6th and 28th days of infection (earlier measurements were precluded by the time lag between injection of ^{59}Fe and its appearance in amounts sufficient to allow accurate determination of red cell radioactivity). Table 4 allows comparison of the average values recorded prior to the onset of significant haemorrhage (i.e. between the 6th and 10th days after infection) with those obtained between the 21st and 28th days when all

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animals were anaemic. The main point to emerge from the early data, was that all iron which passed into the gut was lost from the body - indeed the average enteric iron loss was less than that excreted in the faeces. The reason for this apparent anomaly is that faecal iron is derived both from senescent red cell and desquamation of intestinal iron-containing cells; hence calculation of gut iron loss from ^{51}Cr faecal activity underestimates true iron loss but the error involved is small especially in situations dominated by excessive gastrointestinal haemorrhage.

The figures obtained at 3-4 weeks after infection are important for two reasons. First, they demonstrate the magnitude of the iron loss experienced by individual sheep at the height of the infection - these are clearly related to the severity of the haemorrhage, and as discussed earlier to the breed and haemoglobin type of the animal concerned; and secondly, they show that in only a few instances was there any evidence of significant iron reutilisation. Interestingly, this capacity, which was clearly of limited value when considered against the background of the amounts of iron passed into the gut, was best demonstrated by the most anaemic groups, i.e. the Dorsets and HbB Blackfaces.

The failure of sheep infected with Haemonchus to reutilise iron passed into the gut as haemoglobin must inevitably cause shifts in iron distribution between circulation and stores. The extent of such redistribution

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TABLE 4.

Gastrointestinal Loss and Reabsorption of Haemoglobin Iron.

	Sheep No.	6-10 days after infection: Fe lost into gut (mg/day)	6-10 days after infection: Fe excreted in faeces (mg/day)	6-10 days after infection: Fe re-absorbed (mg/day)	21-28 days after infection: Fe lost into gut (mg/day)	21-28 days after infection: Fe excreted in faeces (mg/day)	21-28 days after infection: Fe re-absorbed (mg/day)
	34	0.9	3.0	-	10.1	10.7	-
Blackface	51	1.1	3.8	-	13.7	14.6	-
HbA	60	1.4	9.6	-	20.6	22.1	-
	71	1.2	10.0	-	20.3	20.0	0.3
Mean		1.2	6.6	-	16.2	16.9	0.1
S.E.		0.1	1.9	-	2.6	2.6	0.1
	48	1.6	8.9	-	29.6	31.4	-
Blackface	54	0.9	2.9	-	22.2	22.0	0.2
HbAB	79	1.0	5.2	-	*	*	*
	93	1.5	11.5	-	15.5	11.5	4.0
Mean		1.3	7.1	-	22.4	21.6	1.4
S.E.		0.2	1.9	-	4.1	5.8	1.3
Blackface	53	1.3	6.9	-	21.0	17.3	3.7
HbB	85	1.1	4.6	-	30.0	25.6	4.4
Mean		1.2	5.8	-	25.5	21.5	4.1
S.E.		0.1	1.2	-	4.5	4.2	0.4
	473	1.2	7.9	-	16.0	16.7	-
Dorset	485	1.7	6.7	-	23.3	20.7	2.6
HbAB	514	1.5	5.3	-	28.5	22.0	6.5
	520	2.1	7.2	-	28.5	29.3	-
Mean		1.6	6.8	-	24.1	22.2	2.3
S.E.		0.2	0.6	-	3.0	2.6	1.5
	480	1.8	6.2	-	38.9	39.4	-
Dorset	489	1.7	7.6	-	27.9	29.3	-
HbB	501	1.8	6.8	-	33.3	32.8	0.5
	541	1.9	7.6	-	35.0	29.7	5.3
Mean		1.8	7.1	-	33.8	32.8	1.5
S.E.		0.1	0.3	-	2.3	2.3	1.3

* Died on day 23

may be ascertained from changes in blood volume and haemoglobin concentration and is best illustrated by the two extremes of the present experiment, i.e. the HbB Dorsets and HbA Blackfaces. Before infection the former had a circulating iron pool of approximately 600 mg and reserves totalling about 350 mg (this assumes iron stores of 15 mg/kg). Over the following 4 weeks, 600 mg were excreted in the faeces and 150 mg remained in the circulation as haemoglobin, most of which presumably originated from storage compounds - even allowing for a compensatory increase in dietary iron absorption. Since these sheep were losing in excess of 30 mg/day it is evident that stores were rapidly approaching the stage of complete exhaustion. By virtue of their smaller size Blackface sheep were potentially more vulnerable with 540 mg in the circulation and stores containing about 350 mg, but because of their smaller worm load, faecal losses totalled only 300 mg and 250 mg was still retained in the blood after 28 days. Clearly, the iron stores of these sheep were still relatively healthy and well able to provide the additional amounts necessary to keep pace with losses of 16 mg/day.

Plasma iron turnover

The results of the ferrokinetic measurements made immediately prior to infection and again 28 days later are given in Tables 5 and 6. The former revealed no major differences between animals of any group; all parameters falling within the normally accepted values

for sheep; plasma iron turnover rates were somewhat higher in the Dorsets when calculated in absolute terms, but when related to bodyweight were remarkably similar in all groups.

On repeating the measurements a number of distinctive features emerged, which although common to all sheep, differed in degree according to breed and haemoglobin type. In the first place, and as mentioned earlier, serum iron levels were depressed relative to pre-infection values. Secondly, there was a marked increase in the amount of iron turned over through the plasma of all groups, but particularly in the HbB Dorsets which exhibited a three- to fourfold increase in the interval between injections, and significantly higher rates 28 days after infection than HbAB sheep of the same breed ($p < 0.05$). Accelerated erythropoiesis was also notable in the Blackfaces where the most marked rise was recorded in the HbB group and the least in animals of haemoglobin type A; differences between these groups were highly significant ($p < 0.02$). Undoubtedly, the major factor responsible for these changes was the greatly accelerated plasma ^{59}Fe clearance at 4 weeks post-infection; at this stage average rates of iron clearance were between two (in HbA Blackfaces) and six times (in HbB Dorsets) greater than the corresponding pre-infection figures.

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TABLE 5.

Ferrokinetic Indices of Blackface Sheep Before and 28 Days After Infection with *H. contortus*

	Sheep No.	Before infection: Plasma ^{59}Fe $t_{½}$ (min)	Before infection: Plasma iron turnover rate mg/day	Before infection: Plasma iron turnover rate mg/kg/day	Before infection: Plasma iron turnover rate mg/kg/100 ml blood	After infection: Plasma ^{59}Fe $t_{½}$ (min)	After infection: Plasma iron turnover rate mg/day	After infection: Plasma iron turnover rate mg/kg/day	After infection: Plasma iron turnover rate mg/kg/100 ml blood
Haemoglobin A	34	157	8.3	0.31	0.58	70	16.5	0.77	1.26
	51	135	10.3	0.41	0.70	46	20.0	0.82	1.79
	60	147	11.2	0.40	0.75	86	18.8	0.67	1.47
	71	145	7.8	0.33	0.52	55	14.9	0.60	1.40
Mean		146	9.4	0.36	0.64	64	17.6	0.72	1.48
S.E.		45	0.8	0.03	0.05	9	1.1	0.05	0.11
Haemoglobin AB	48	160	8.1	0.31	0.52	42	16.3	0.61	1.33
	54	145	7.0	0.33	0.49	31	32.2	1.43	2.58
	79	145	8.4	0.43	0.61	*	*	*	*
	93	159	8.2	0.46	0.59	28	27.0	1.48	2.27
Mean		152	7.9	0.38	0.55	34	25.2	1.17	2.06
S.E.		4	0.3	0.04	0.03	4	4.7	0.28	0.37
Haemoglobin B	53	165	6.4	0.28	0.45	43	31.0	1.39	2.30
	85	138	7.6	0.36	0.67	25	24.8	1.14	2.06
Mean		152	7.0	0.32	0.56	34	27.9	1.27	2.18
S.E.		14	0.6	0.04	0.11	9	3.1	0.13	0.12

* Died day 23

TABLE 6.

Ferrokinetic Indices of Finn Dorset Sheep Before and 28 Days After Infection with H. contortus.

	Sheep No.	Before infection: Plasma ^{59}Fe $t_{\frac{1}{2}}$ (min)	Plasma iron turnover rate: mg/day	mg/kg/day	mg/kg/100 ml blood	After infection: Plasma ^{59}Fe $t_{\frac{1}{2}}$ (min)	Plasma iron turnover rate: mg/day	mg/kg/day	mg/kg/100 ml blood
Haemoglobin AB	473	170	11.5	0.45	0.83	45	23.7	0.95	1.92
	485	175	9.9	0.36	0.63	39	18.7	0.75	1.56
	514	145	8.9	0.38	0.61	30	30.6	1.35	2.84
	520	178	10.2	0.38	0.64	37	34.2	1.30	2.09
Mean		167	10.1	0.39	0.68	38	26.8	1.09	2.10
S.E.		8	0.5	0.02	0.05	3	3.5	0.14	0.27
Haemoglobin B	480	190	9.2	0.36	0.63	27	27.4	0.99	2.60
	489	137	13.4	0.53	0.91	33	35.4	1.41	3.17
	501	155	12.0	0.41	0.77	28	41.5	1.36	3.00
	541	153	10.6	0.48	0.72	29	39.3	1.73	3.84
Mean		159	11.3	0.45	0.76	29	35.9	1.37	3.15
S.E.		11	0.9	0.04	0.06	1	3.1	0.15	0.26

Albumin pools and turnover

Albumin pool sizes were measured before and 28 days after infection and catabolic rate in the intervening period using ^{125}I-labelled albumin. A number of important features are revealed by the albumin pool figures (see Tables 24 and 25, Appendix 1). Firstly, and in common with the observations on PCV and circulating red cell volume referred to earlier, the albumin status of normal HbA Blackfaces, as measured by the amounts of protein present both intra- and extravascularly, was superior to that of similar animals belonging to the HbAB and HbB genotype ($p < 0.05$), which in turn had smaller pools than the corresponding Dorsets ($p < 0.05$), but as before these differences were merely reflections of group variation in bodyweight. Secondly, comparing the pre- and post-infection data, it is obvious that while all animals experienced significant albumin depletion during the course of infection, the extent of this varied, being most pronounced in the HbB Dorsets (26 gm) and least in HbA Blackfaces (16 gm) with other groups suffering losses intermediate between these extremes. Analysis of the data obtained 4 weeks after infection revealed that Dorsets failed to retain their superior albumin status over Blackfaces, but that within this latter breed, the HbA group still enjoyed an advantage over their HbAB and HbB partners ($p < 0.05$ and $P < 0.02$ respectively). Finally, although both pools contributed to the depletion of body albumin, by far the major loss occurred from extravascular

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sites; this is clearly indicated by the reduced EA:CA ratios 4 weeks after infection.

From the measurements of albumin degradation (Figure 10) it is apparent that animals of all groups experienced progressive hypercatabolism from about the 12th day after infection, and that the ultimate magnitude of such elevations was both breed and haemoglobin type related, Dorsets attaining noticeably higher fractional catabolic rates than Blackfaces of the same haemoglobin type ($p < 0.05 - < 0.01$) and in the latter, animals of haemoglobin type A exhibiting somewhat lower rates than their HbB counterparts. That the underlying cause of the increased albumin breakdown was excessive loss of plasma into the gut is indicated by the close relationship in all groups between patterns of albumin catabolism and faecal "clearance" of radioiodide.

These features were accompanied by high absolute rates of degradation and marked reductions in the "apparent half-life" of labelled albumin; all these changes were closely inter-related and correlated with worm burden (Tables 28 and 29, Appendix 1).

It is not possible to measure albumin synthesis using radioiodinated molecules, but a rough indication of relative rates may be obtained from pool sizes and catabolic rates. For example, HbB Dorsets which, as mentioned earlier, lost about 34 gm albumin during the first four weeks of infection, catabolised 6 gm per day during the latter two

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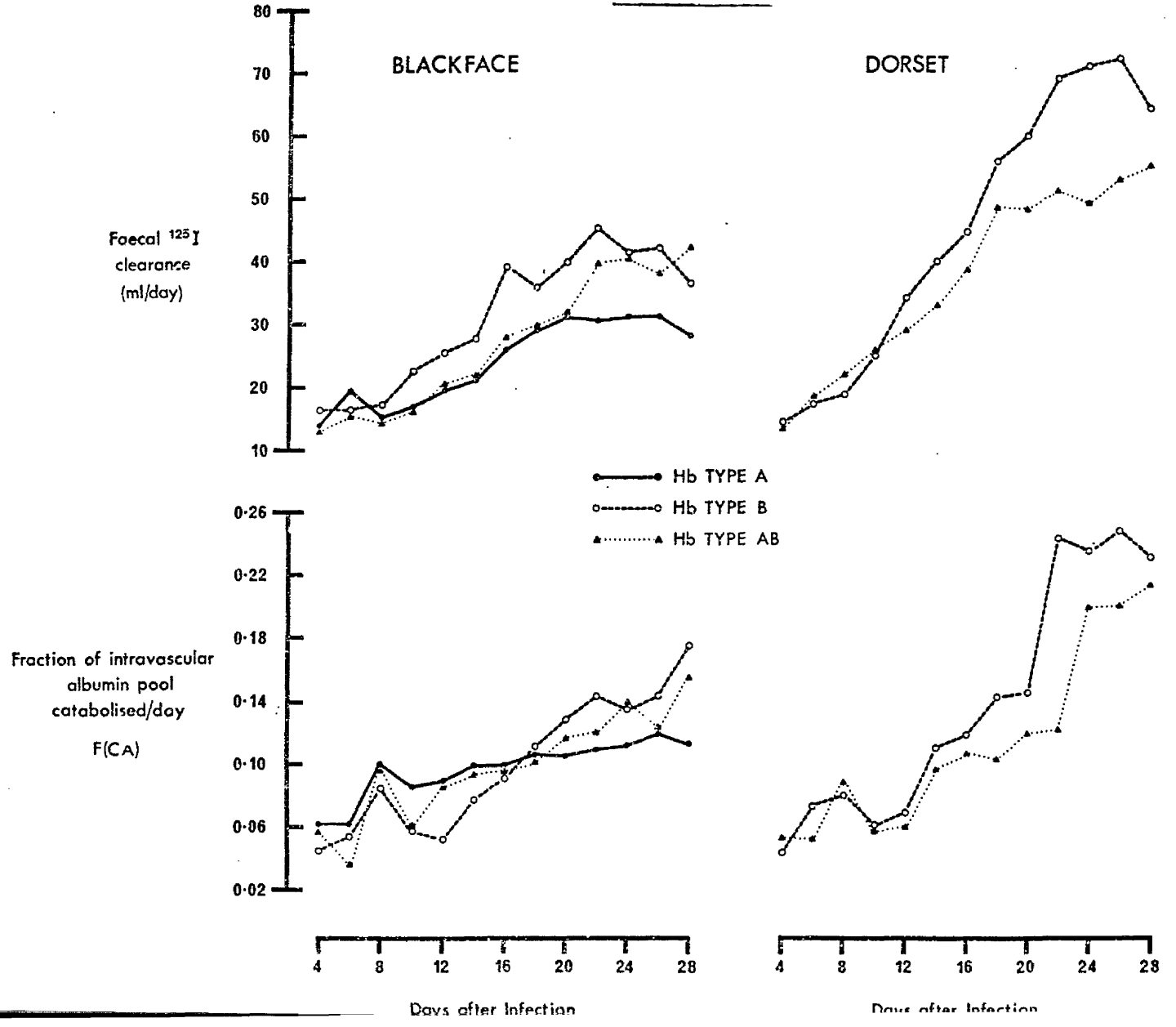
Figure 10: Albumin Degradation Following Infection with H. contortus.



weeks, i.e. a total of 84 gm. Assuming the major depletion occurred over this period, it follows that about 50 gm were synthesised. By contrast, HbA Blackfaces lost 13 gm catabolised 42 gm, and hence presumably synthesised 29 gm. These figures suggest that albumin synthesis, like erythropoiesis, was accelerated to the greatest extent in animals under the most severe catabolic stress.

Experiment 2

The results of the first experiment show that when infected with moderate numbers of H. contortus larvae, HbA type sheep are more resistant to worm establishment than similarly infected HbB type sheep. The aim of this experiment was to determine whether a similar situation exists under conditions of heavy larval intake. For this purpose Scottish Blackface sheep were divided into three equal groups on the basis of haemoglobin type, infected with 1400 H. contortus larvae/kg bodyweight and necropsied 24 days later. As in the previous experiment the response of all animals was monitored by a combination of clinical and radioisotopic methods, but to obtain more detailed background information on normal animals of each haemoglobin type red cell and albumin kinetics were studied for an additional period of 28 days prior to infection. To simplify description of data, average values are presented in the illustrations and full details of the changes recorded in individual animals are given in Appendix 1.

Haematological and biochemical observations

The average values recorded for PCV, MCV, MCHC and serum iron are illustrated in Figures 11 and 12. Contrary to the findings of the earlier experiment there was no indication that the haematological indices of normal HbA type sheep were superior to those of HbAB or HbB animals, nor was there any evidence that the course of the anaemia which developed during the subsequent Haemonchus infection was in any way

Figure 11: PCV Values Before and After Infection with 1400 *H. contortus* Larvae/Kg Bodyweight.

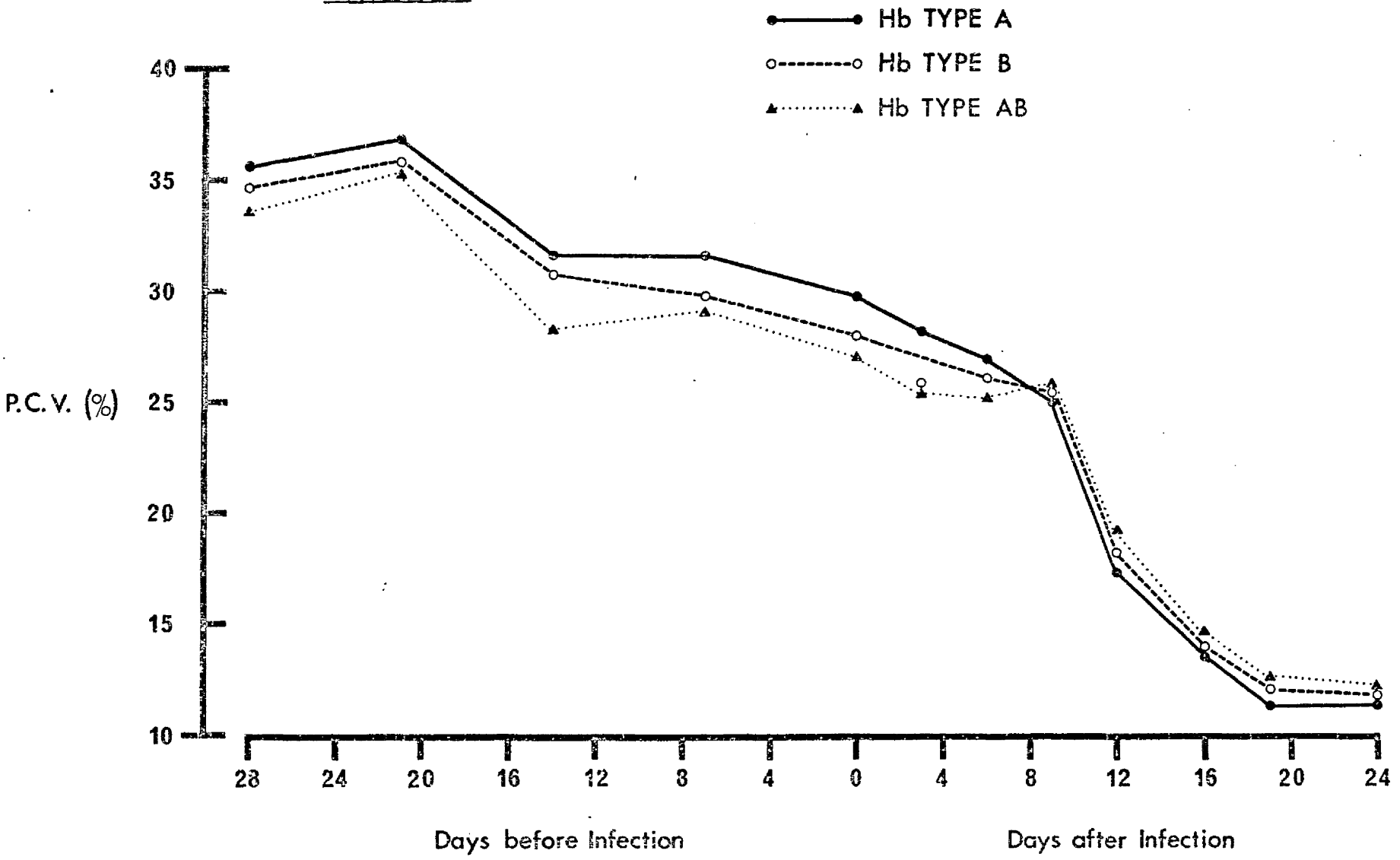
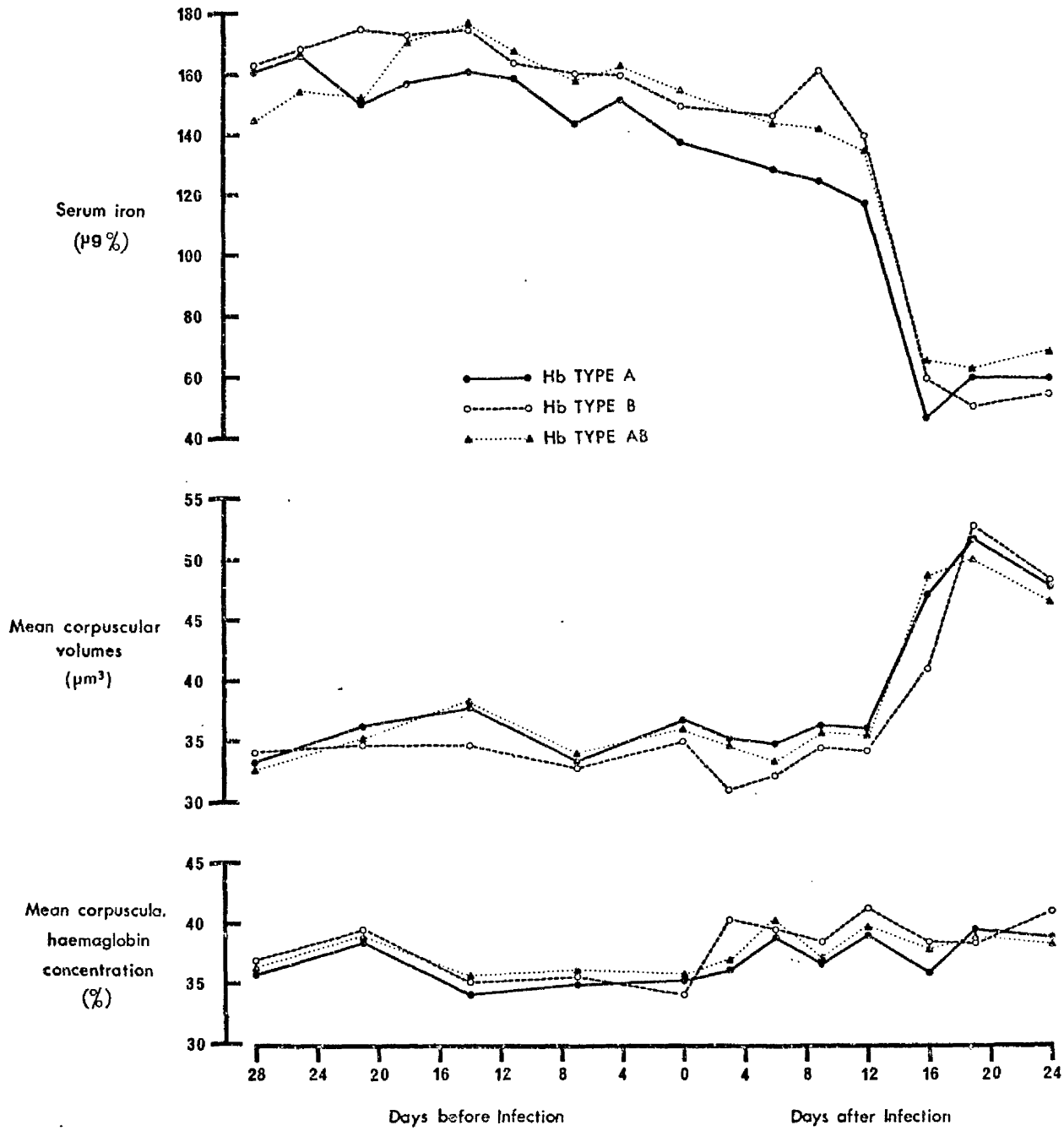


Figure 12: Serum Iron Concentrations, MCV and MCHC Values Before and After Infection with 1400 H. contortus Larvae/Kg Bodyweight.



influenced by this character. Indeed, throughout the investigation, animals of each haemoglobin type responded in an almost identical manner, exhibiting minor reductions in PCV during the pre-infection period and dramatic reductions from the 9th day thereafter which were accompanied terminally by marked macrocytosis.

Total protein, albumin and globulin concentrations were similar and well maintained in all groups until 2 weeks after infection when each deteriorated progressively to levels unrelated to haemoglobin type (Figure 13).

HbC was first detected in small amounts about 16 days after infection in sheep with HbA and HbAB, and thereafter in progressively increasing amounts until the termination of the experiment. By this stage the percentage of HbA in the former had decreased from 98% to 74% and HbC increased from 2% to 26%, representing an ultimate concentration of 1.2 g%. In HbAB sheep the rise in HbC levels was less dramatic and by necropsy this component contributed approximately 11% or 0.6 g% of the total blood haemoglobin ($p < 0.05$).

Parasitological data

The number of infective larvae given to each sheep together with the number and sex ratio of the worms recovered from the abomasal washings and digests are given in Table 7. It is apparent that approximately the same number of larvae became established in all groups (about 35% of the dose

Figure 13: Serum Protein Values Before and After Infection with 1400 H. contortus Larvae/Kg Bodyweight.

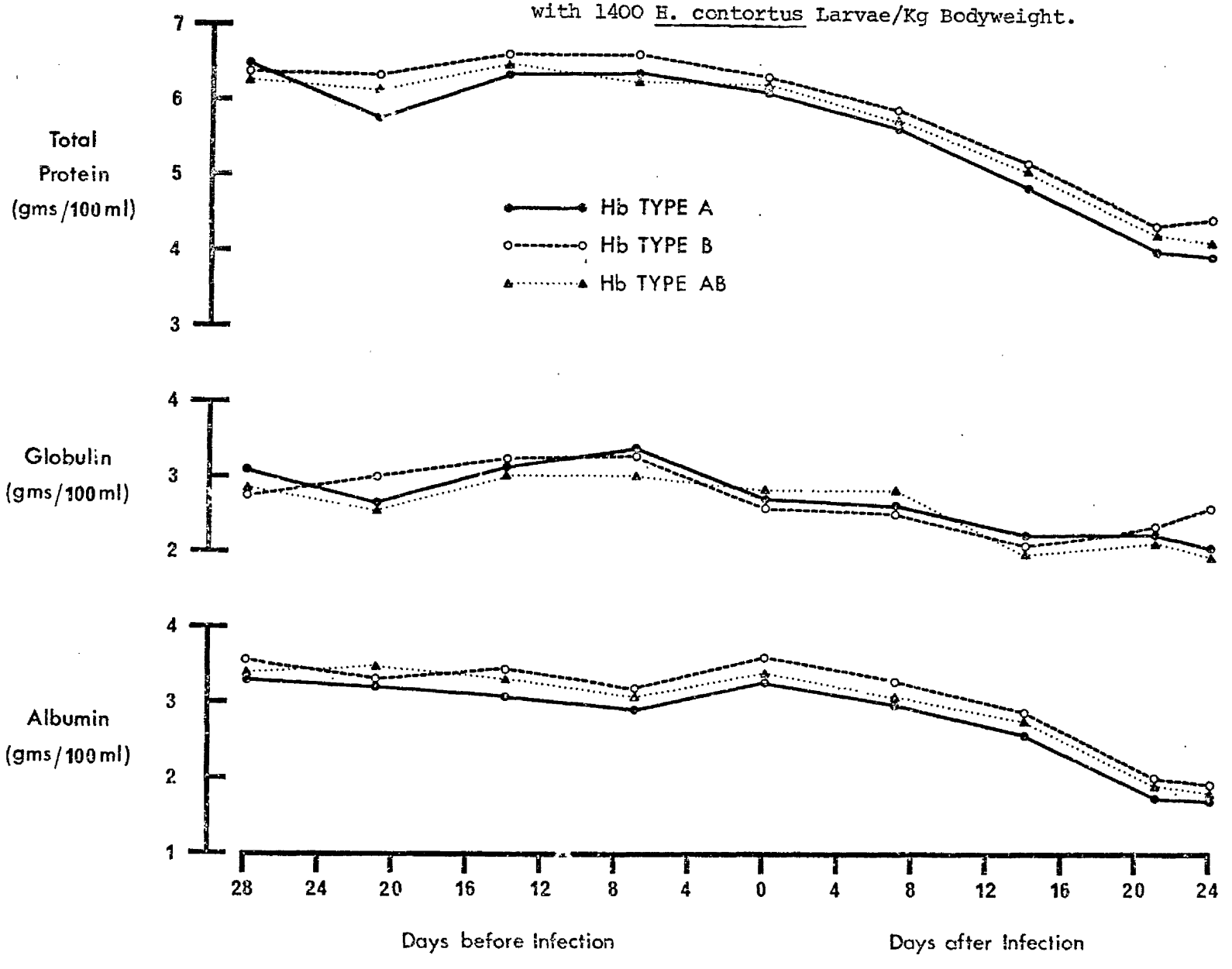


TABLE 7.
Worm Counts at Necropsy.

	Sheep No.	Dose of Larvae	Total worm recovery	% Recovery	% 4th Stage	Male:Female
	966	47600	8080	17.0	0.6	1:1
	977	44700	8420	18.8	-	1:1
Blackface HbA	990	53400	16680	31.2	0.3	5:3
	988	43200	20440	47.3	0.7	1:1
	987	53100	29650	55.8	-	4:5
Mean		48400	16654	34.0	0.3	1.1:1
S.E.		2103	4028	7.7	0.2	
	978	42600	6770	15.9	4.4	3:2
	970	43200	14120	32.7	-	5:4
Blackface HbAB	984	43500	18760	43.1	0.3	1:1
	973	40800	19290	47.3	-	1:1
	979	42000	21670	51.6	-	3:2
Mean		42420	16122	38.1	0.9	1.3:1
S.E.		480	2639	6.4	0.9	
	3	42300	4930	11.7	-	3:4
	6	48000	11020	23.0	-	1:1
Blackface HbB	2	43200	12570	29.1	-	1:1
	1	48600	19910	41.0	-	1:1
	8	42600	23930	56.2	-	4:3
Mean		44940	14472	32.2	-	1:1
S.E.		1382	3359	7.6	-	

administered) and that only a small percentage of the total worm burden was inhibited at the 4th stage.

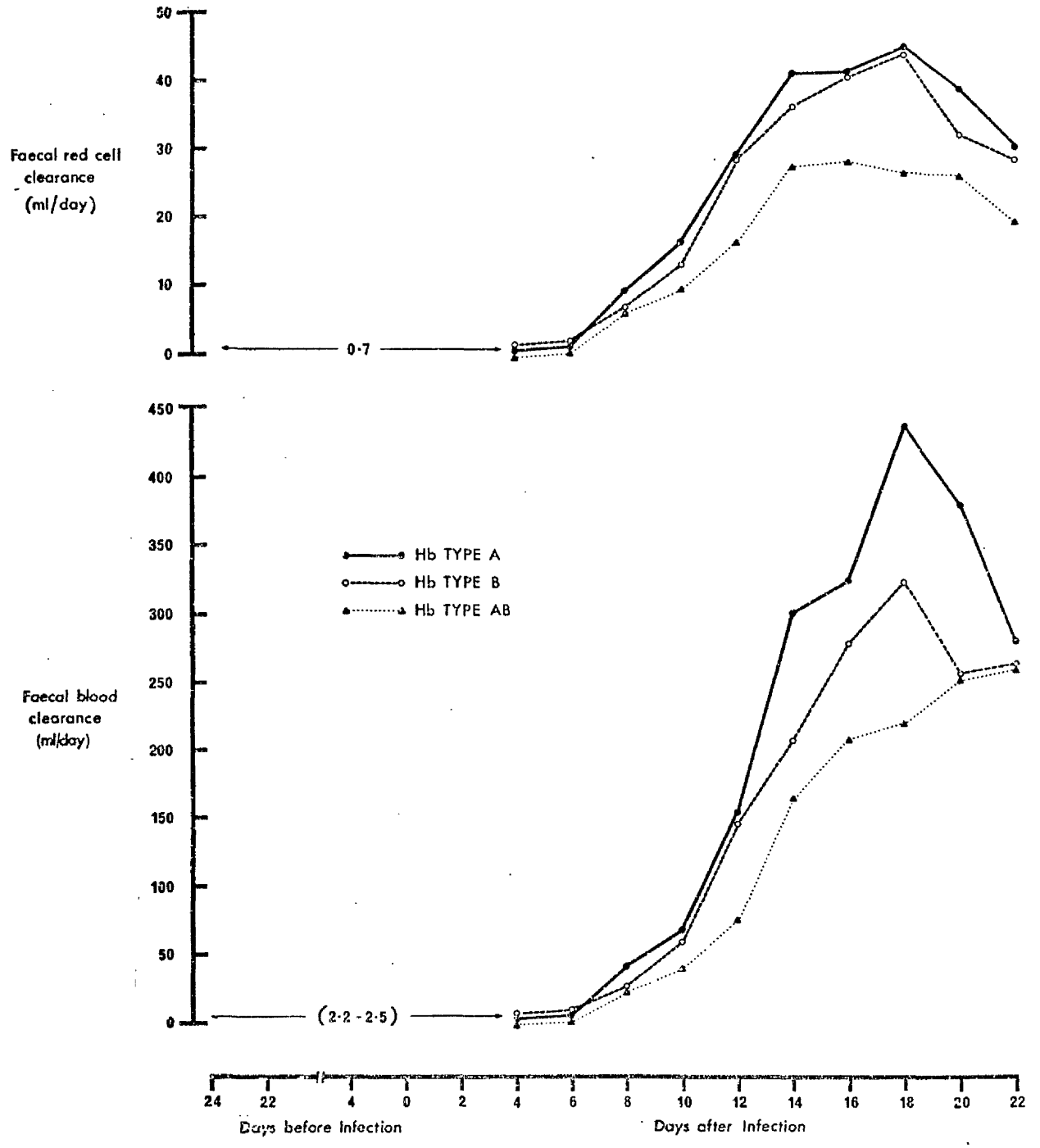
Pathophysiological data

Blood volumes and red cell kinetics

Blood volumes were estimated at 28 days and again immediately before infection. On each occasion these measurements yielded values compatible with bodyweight and PCV and have therefore been combined to provide the relevant background information and a basis for comparison with the data obtained prior to necropsy on the 24th day of infection (Table 40, Appendix 1). Animals of each haemoglobin type suffered precipitous but essentially similar reductions in circulating red cell mass during the course of infection which despite some compensatory expansion of plasma volume resulted in noticeable reductions in blood volume. It is also relevant to stress that any advantage held initially by these sheep over their predecessors in respect of blood compartment size was rapidly eroded; indeed, by day 24 the average erythrocyte depletion recorded in this experiment was about 400 ml or 70%, reductions which were clearly compatible with the heavier worm burdens of the animals concerned.

The information provided in Figure 14 gives a detailed insight into the underlying cause of the anaemia which developed in these sheep. It hardly needs to be stated that excessive haemorrhage into the gut was the

Figure 14: Gastrointestinal Losses of Whole Blood and Red Cells Before and After Infection with 1400 H. contortus Larvae/Kg Bodyweight.



principal aetiological factor; this is clearly shown by the massive increase in faecal blood and red cell "clearances" recorded for animals of all groups during the latter two weeks of the study, by the dramatic shortening of red cell survival during the same period and by the synchronous development of these changes with the anaemia. As in the previous experiment the severity of haemorrhage and anaemia experienced by individual sheep were closely related and correlated well with worm burden, but one interesting and significant difference which emerged was that in the present study the average blood loss per worm ranged from 0.01-0.03 ml/day, i.e. less than half the figure previously recorded; apparently individual worms in heavily infected sheep remove less blood than worms from animals with light burdens, and this in addition to the shorter duration of the infection explains the surprisingly small PCV difference recorded between the two sets of experimental animals.

Further information on the changes in erythrocyte kinetics which accompanied these more acute infections is given in Table 8. The plasma iron turnover data leaves little doubt that haemoglobin synthesis was stimulated in response to blood loss, and to the same extent in all groups. Equally apparent however, is that by comparison with the increase demonstrated in the earlier study, the response of the present animals was poor since rates of erythropoiesis rarely exceeded twice the pre-infection values and in the

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TABLE 8.

Ferrokinetic Indices Before and 24 Days After Infection.

	Sheep No.	Serum iron (µg %)	Plasma ^{59}Fe $t_{\frac{1}{2}}$ (min)	Plasma iron turnover rate mg/day	mg/kg/day
	966	139	174	11.7	0.32
	977	121	152	9.7	0.30
Blackface HbA	987	136	141	16.4	0.42
	988	146	150	13.2	0.42
	990	149	154	13.4	0.35
Mean		138	154	12.9	0.36
S.E.		5	5	1.1	0.03
	970	156	156	12.4	0.40
	973	144	149	11.6	0.39
Blackface HbAB	978	143	216	9.0	0.30
	979	185	209	11.4	0.34
	984	147	149	13.8	0.41
Mean		155	176	11.6	0.37
S.E.		8	15	0.8	0.02
	1	159	186	11.3	0.34
	2	142	100	20.6	0.64
Blackface HbB	3	165	132	16.5	0.54
	6	126	161	11.1	0.32
	8	158	157	12.4	0.38
Mean		150	147	14.4	0.44
S.E.		7	15	2.0	0.06
	966	52	54	16.2	0.45
	977	67	47	20.1	0.67
Blackface HbA	987	*	*	*	*
	988	67	53	20.2	0.68
	990	55	46	18.2	0.51
Mean		60	50	18.7	0.58
S.E.		3	2	0.9	0.50
	970	52	41	16.6	0.54
	973	82	41	28.3	0.96
Blackface HbAB	978	79	50	20.6	0.69
	979	79	54	22.7	0.72
	984	52	55	15.2	0.51
Mean		69	48	20.7	0.68
S.E.		7	3	2.3	0.80
	1	45	62	12.6	0.38
	2	79	65	18.0	0.62
Blackface HbB	3	37	41	12.1	0.40
	6	49	59	13.1	0.38
	8	64	43	21.1	0.71
Mean		55	54	15.4	0.50
S.E.		8	5	1.8	0.07

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TABLE 9.

Gastrointestinal Loss and Reabsorption of Haemoglobin Iron.

	Sheep No.	Before Infection: Fe lost into gut (mg/day)	Before Infection: Fe excreted in faeces (mg/day)	Before Infection: Fe reabsorbed (mg/day)	16-24 days After Infection: Fe lost into gut (mg/day)	16-24 days After Infection: Fe excreted in faeces (mg/day)	16-24 days After Infection: Fe reabsorbed (mg/day)
	966	0.7	1.0	-	65.0	64.0	1.0
	977	0.7	1.1	-	44.6	46.6	-
Blackface HbA	987	1.0	1.3	-	70.2	68.0	2.2
	988	0.8	0.9	-	65.3	62.3	3.0
	990	1.0	1.3	-	30.0	33.3	-
Mean		0.8	1.1	-	55.0	54.8	1.2
S.E.		0.1	0.1	-	7.6	6.5	0.6
	970	0.8	0.8	-	29.5	28.3	1.2
	973	0.6	1.1	-	58.2	57.0	1.2
Blackface HbAB	978	1.4	1.4	-	27.8	25.0	2.8
	979	0.8	1.0	-	50.7	52.5	-
	984	0.8	1.0	-	33.0	36.4	-
Mean		0.9	1.0	-	39.8	39.8	1.0
S.E.		0.1	0.1	-	6.1	6.4	0.5
	1	0.6	0.9	-	46.5	45.0	1.5
Blackface HbB	2	1.0	1.6	-	58.0	57.0	1.0
	6	0.9	0.7	-	29.2	34.2	-
	8	0.7	0.9	-	66.2	60.0	6.2
Mean		0.8	1.0	-	50.0	49.1	2.2
S.E.		0.1	0.2	-	8.0	5.9	1.4

majority of cases were only marginally greater or even less than these. It is of course possible and indeed likely that the data at 24 days post-infection underestimated the erythropoietic capacity of these sheep at an earlier stage of the disease, but the fact remains that despite a 400% increase in plasma clearance, the total amount of iron carried to the marrow was limited terminally by the low serum iron levels.

Iron losses into the gut and via the faeces before infection and between the 16th and 24th days thereafter are given in Table 9; these provide a dramatic illustration of the magnitude of iron depletion resulting from haemorrhage and a clear basis for the accompanying deficiency. Before infection the total iron reserves of these sheep were about 1500 mg, of which approximately 800 mg was haemoglobin and 500 mg as storage compounds (these figures may be calculated from the initial blood volume and haemoglobin concentration and the assumption of 15 mg/kg storage iron). During the initial 15 days of infection daily faecal iron losses were 20-25 mg, and by the 24th day the average animal had lost 800 mg iron or its total complement of haemoglobin. The depletion of storage iron caused by such losses, calculated from terminal blood volumes and haemoglobin levels was 300 mg - a considerable loss by any standard, and when considered against the unlabile nature of many iron stores certainly sufficient to impair the erythropoietic response to haemorrhage. Finally, these findings once again demonstrate

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the inability of sheep to reabsorb anything but small amounts of iron passed into the gut as haemoglobin, even when severely anaemic.

Albumin pools and catabolic rate

The results of the albumin pool measurements made before infection and 24 days later are shown in Table 45 of the Appendix, while the figures for albumin catabolism and faecal "clearance" of radioiodide during the intervening period are presented in Figure 15. The former revealed that the mass of albumin present in each compartment was approximately the same in all groups before infection, and that a significant ($p < 0.02$) but equal reduction (about 35%) in the intravascular pool occurred during the subsequent 24 days. Unfortunately, extravascular albumin was not determined following infection, but in view of the results from the previous experiment it is certain that this too was depleted, and probably to a greater extent than the intravascular pool.

The data in Figure 15 provide a further spectacular illustration of the damaging effects of heavy Haemonchus infections in sheep. The fraction of the intravascular albumin pool degraded each day which had fluctuated around 7% during the entire pre-infection period increased progressively from the 8th day thereafter, reaching a peak about 10 days later of 30-40%. The fact that this massive hypercatabolism was paralleled by an equally dramatic elevation in plasma "clearance" demonstrates

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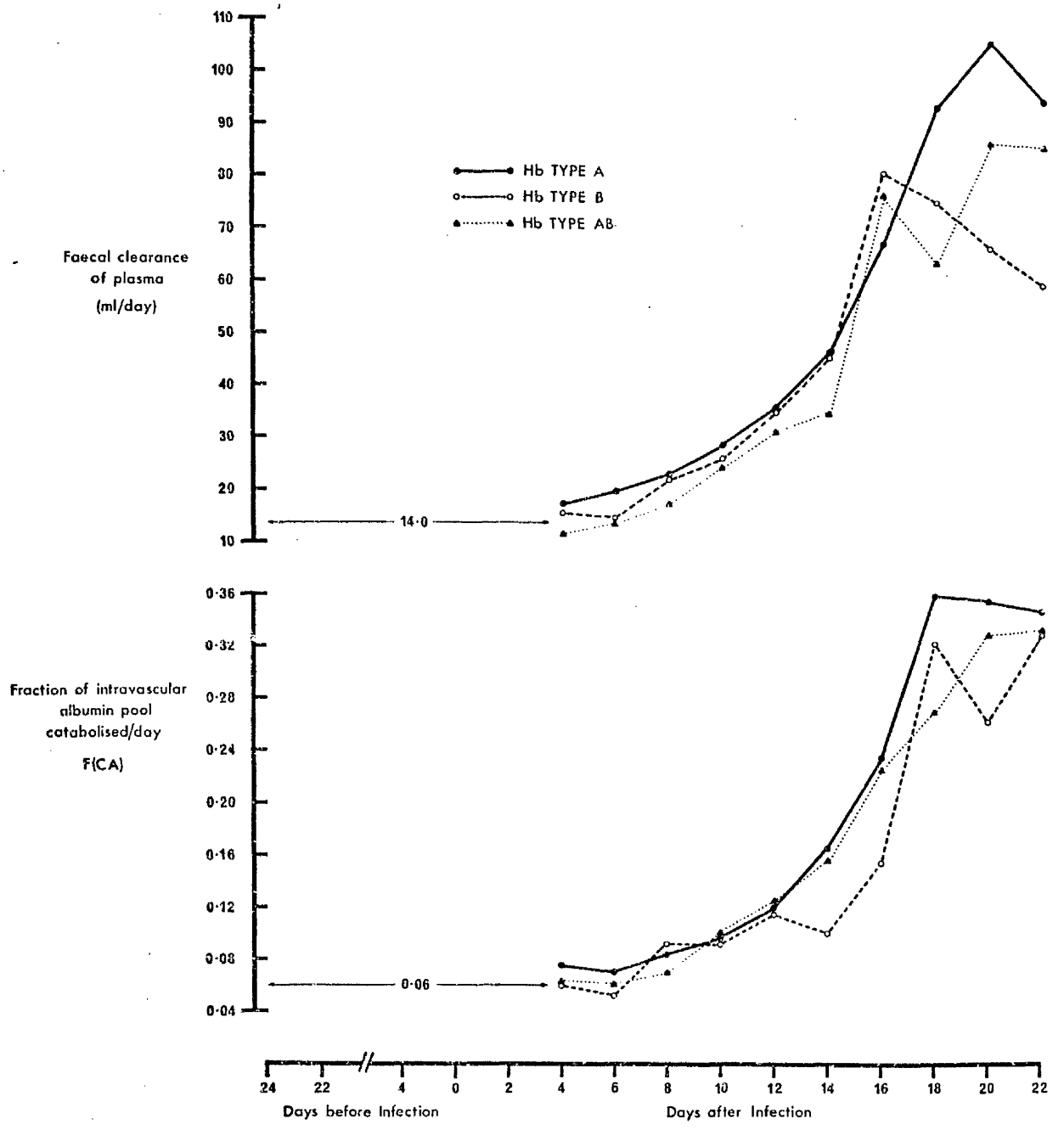
yet again the fundamental importance of excessive enteric plasma leak in the aetiology of the hypoalbuminaemia which accompanies this disease.

Figure 15: Albumin Catabolism Before and After Infection with 1400 H. contortus Larvae/Kg Bodyweight.



DISCUSSION

The principal aims of the work represented in this section were to examine the possible existence and basis of breed and haemoglobin-type related differences in the resistance or susceptibility of sheep to primary infections with H. contortus. Apart from the limitations imposed by small group sizes which admittedly are substantial, the logical conclusion to be drawn from the experimental results is that such differences do in fact exist, provided the animals concerned are not exposed to massive challenge. This conclusion is based on the lower worm recovery and development of less severe clinical and pathophysiological disturbances in Scottish Blackface than in Finn Dorset sheep given the same relative number of larvae; by the expression of similar advantages in animals of both breeds carrying a gene for HbA; and finally by the demonstration that sheep of each haemoglobin type were equally susceptible to the establishment and pathogenic effects of these parasites when heavily infected.

The question of how such differences arise is complex but a useful start may be made by considering the disease itself and the possibility that disease resistance is a genetically-determined characteristic. In introducing this section the author suggested that the pathogenic effects of Haemonchus, and hence resistance to these effects could be assessed only from a knowledge of the direct functional

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disturbances arising from parasitic activity, and while useful for diagnosis, indirect indices of infection, e.g. the anaemia and hypoalbuminaemia were insufficiently precise for this purpose. The validity of this statement has been completely borne out by what is best described by the very tenuous relationship observed between "cause" and "effect".

Take for instance the anaemia. The results presented here illustrate all the classical features of the development of this condition, which commences with a sharp fall in haematocrit in association with the feeding activities of 4th and 5th stage larvae and young adults, but latterly is characterised by little further deterioration in this index. These features are well known and along with the appearance of macrocytosis, hypochromia and depressed serum iron levels have been reviewed by several authors[10,25,47]. On the basis of PCV measurements one might therefore conclude that the larval and early adult stages of H. contortus are more pathogenic than mature egg-laying parasites when in fact, as the isotopic measurements clearly show, the latter are manifestly the more voracious blood suckers. This paradox is best explained by the "minimum haematocrit deficit" theory first proposed by Ractliffe et al.,[48] and subsequently confirmed experimentally by Dargie and Allonby[12]. The basis of this theory is that the erythropoietic system of sheep is stimulated only when PCV's drop below a certain minimum level (about 20-25%) and even at lower values the full potential

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for red cell synthesis is not attained for a period of 1-2 weeks depending on the severity of the anaemia. The sharp fall in PCV attributable to the larval stages, although caused directly by haemorrhage is therefore in part a reflection of a latency in the host's erythropoietic response, and the more gradual subsequent drop, as a consequence of the attainment of a more closely balanced equilibrium between breakdown (or loss) and synthesis; this point is well illustrated by the ferrokinetic data 28 days after infection. Equally well illustrated by the results of the acute experiment is that the erythropoietic time lag is of greatest importance under conditions of heavy larval intake where fatal amounts of red cells may be lost before the erythroid marrow can expand sufficiently to replace them. Further support for this general hypothesis is given by the observation that blood erythropoietin levels of sheep remain depressed until such time as the animal is severely stressed[49] and also by the work of Charleston[50] and Allonby[17] demonstrating significant anaemia prior to the appearance of changes in the distribution and extent of erythroid tissue in the bone marrow.

A further feature of the anaemia recorded in these experiments was the failure of infected sheep to re-utilise anything but minor quantities of iron lost into the gut as haemoglobin. This confirms earlier reports[12,51], and implies that a major consequence of this disease is progressive depletion of iron stores leading ultimately to frank iron deficiency with resultant impairment of red cell synthesis.

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Such a situation, referred to as "marrow exhaustion" and characterised by very low serum iron levels and reduced plasma iron turnover[10,12] was not recorded in the present studies, although judging by the size and rate of faecal iron excretion in the Dorsets was rapidly being approached by these animals.

The above considerations naturally lead back to the question of the reliability of PCV measurements as indicators of the "effects" and "level" of infection. This is suitably answered by the fact that HbA Blackfaces (Experiment 1) with terminal PCV's of 21% experienced average losses of 65 ml/day and harboured 1,200 worms, while the HbB Dorsets lost 160 ml/day were infected with 3,200 worms but still managed to maintain haematocrits of 14%. Admittedly a PCV difference of 30% is significant but hardly reflects a difference of 150% in the severity of haemorrhage, or for that matter a difference of 170% in worm load. In the same vein, it is perfectly feasible that an animal whose erythropoietic system has become geared to meeting the demands of an increased rate of red cell breakdown, e.g. sheep suffering from the syndrome of "chronic" haemonchosis[8,11,12] may show no significant change in PCV over prolonged periods; while at the other end of the scale sheep whose erythropoietic machinery has reached the stage of exhaustion may become severely anaemic when infected with small numbers of parasites and suffering relatively minor haemorrhage. Hence in many instances a low PCV may arise not so much because the animal is heavily infected but because it has been carrying moderate worm burdens for an extended

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period of time. The same applies to serum albumin levels which obviously fell as a result of the hypercatabolism arising through haemorrhage, but which in many instances were artificially maintained by a combination of protein transfer from extravascular reserves and increased synthesis. This aspect of the disease, which has already been described in detail[11,25], emphasises yet again the pitfalls in using clinical parameters for defining disease severity and hence comparing disease resistance and worm establishment.

On the other hand, if any single fact emerged from these studies it was that variations in the pathophysiological effects arising from infection were closely related to differences in worm burden. This implies that genetic resistance or susceptibility to H. contortus is expressed primarily at the level of worm establishment and that any other considerations, e.g. resistance to the "effects" of worm establishment are of secondary and indeed minor importance. This latter statement may seem incompatible with the observation that some sheep synthesised more red cells and plasma proteins than others (e.g. the Dorsets and HbB types), and also with the existence before infection of genetically related differences in body composition, e.g. weight, PCV, blood volume and metabolic reserves. Admittedly these factors had some effect on the disease, but their importance has to be put in perspective. For instance the observed differences in red cell and protein synthesis were merely the natural consequence of variations in the hypoxic

stress acquired as a result of infection; they should therefore not be considered as evidence that the capacity to withstand haemorrhage is a genetically-determined characteristic.

There is however a further and possibly more compelling reason for rejecting the hypothesis that resistance to the pathogenic effects of this parasite is influenced to any significant extent by bodyweight or weight-related physiological factors - namely, that the advantages derived from even major weight gains are so rapidly eroded by relatively small differences in worm load as to be of limited practical value. There are numerous examples of this in the text, but a comparison of some data from two of the sheep - (Nos. 485 an HbAB Dorset and 54 an HbAB Blackface) provides a useful illustration of the point. Before infection the former held many advantages - it was heavier (27 kg as opposed to 21 kg), had a much higher PCV and total red cell volume (36% and 30%, and 544 ml and 362 ml respectively) and clearly was in a better position to withstand the consequences of infection. Instead, by the end of the experiment, and despite a higher rate of red cell synthesis in the Dorset, the values recorded for these indices were such that the advantage had swung decisively in favour of the smaller Blackface animal (PCV's were 13% and 19%, and red cell volumes 168 ml and 200 ml respectively). The significance of these changes is that they occurred in sheep with distinctly different weight and haematological characteristics, but relatively

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minor differences in worm burden, i.e. 2,100 and 1,600 adult H. contortus. Since a survey of over 100 Scottish Blackface and Finn Dorset sheep failed to reveal a statistically significant correlation between HbA and increased values for either bodyweight or PCV (see other sections), the possibility of the relationship between haemoglobin polymorphism and infections with Haemonchus being expressed at the level of resistance to "disease" as opposed to "establishment" becomes even more remote.

Obviously the only condition under which the physiological status could possibly exert any meaningful effect on disease pathogenesis would be where, to use the term first coined by Evans and Whitlock[52], "all other factors are equal", i.e. where larval intake and susceptibility or resistance to parasite establishment are themselves the constant factors. Such a situation is unlikely to prevail in the field where quite apart from any other consideration, heavier animals in all likelihood consume more larvae in the course of satisfying their greater appetite and metabolic requirements; for this reason the number of larvae administered in the present experiments was adjusted for differences in bodyweight.

This immediately raises speculation as to the role of HbC. The results of these experiments which incidentally are the first to demonstrate the presence

of this haemoglobin in HbA and HbAB sheep made anaemic with H. contortus confirm many features of its synthesis but unfortunately add little to our understanding of its function. It is clear for instance that the stimulus for A-C switching has to be severe, requiring a lowering of the haemoglobin level to about 7% with corresponding reductions in PCV and red cell counts; equally apparent is that the amount of HbC produced and its rate of production are closely related to the severity of the anaemic stress. These features, already well documented by others and reviewed by a number of authors[53-55] suggest that HbC functions purely as an aid to survival and has no direct influence on worm establishment - either in worm-free sheep, as shown here, or incidentally in sheep made anaemic by previous infection (Section 3). This conclusion is supported by the observation that worm activity as judged by blood loss and egg output was already radically altered priot to the appearance of significant amounts of HbC, and subsequently by the demonstration that animals with a high proportion of this haemoglobin are at least as susceptible, if not more susceptible to reinfection. Hence in the final analysis it would appear that the capacity to produce HbC, like the capacity for growth, is at best of limited functional value to a potential host and unlikely to explain the better performance of some breeds and haemoglobin types in Haemonchus endemic areas;

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this must therefore be seen primarily as a consequence of genetically-determined variations in the level of infection such as observed in the present studies.

The establishment and survival of this parasite in a potential host could obviously be regulated by many factors acting at any stage during its development. While it is conceivable that worm establishment in sheep with HbB for instance is favoured by the greater availability of oxygen[56] and methionine[57] for respiratory and metabolic purposes, i.e. that there is in effect a direct haemoglobin-parasite interaction as suggested by Evans et al.,[13], this would seem unlikely and in any event could hardly explain the differences observed between individuals with the same haemoglobin type belonging to different breeds. What is much more likely is that variations in infection levels both within and between breeds arise from variations in the immune response elicited, although admittedly no attempt was made to relate these to specific immunological events. Since the nature of the immune mechanisms which operate against this parasite are as yet unknown[58] it is clearly difficult to equate differences in what is essentially the outcome of a complex series of processes, any or all of which could be under genetic control, with one particular reaction; for this reason it was considered more useful as a first step to examine the development of these mechanisms in terms of their effect on the parasites' feeding and reproductive activities as well as on their ultimate size and number.

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The results of the blood loss measurements shown in Figure 9 suggest that genetic resistance is first expressed on the parasite at some point between the 10th and 12th days of its development, but more effectively over the subsequent 6-8 days. Since the 4th ecdysis of H. contortus occurs around the 10th day of infection[2], it would appear that worm development proceeds normally in all animals to the 4th and to a lesser extent to the 5th larval stage, and that differences in ultimate establishment arise from variations in the effectiveness of expulsion during the attainment of sexual maturity. While it is possible that the expulsion process was preceded by a period of arrested or inhibited development, this was not reflected in the worms recovered at necropsy which apart from 1-2% arrested in the 4th stage were of a size corresponding to adulthood, i.e. males >10 mm and females >14 mm[2,21]. Also noteworthy was that neither the size nor the sex ratio of these worms was dramatically different in any group, neither was there any evidence that breed and haemoglobin-type related differences in total blood loss and egg output derived from variations in the feeding and reproductive activities of individuals within a given population; in fact the average figures obtained here for both activities corresponded closely with those reported by others[7,12,18,59].

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On the basis of these findings it would therefore appear that whatever the nature of the response directed against the parasite (possibly a hypersensitivity reaction induced by antigens released during the 4th moult[60-62]), it left no permanent damage on the surviving worms. Since it is well recognised that the capacity to mount an immunological response is delayed in the presence of excessive amounts of antigen[63], the equal susceptibility of all haemoglobin types to heavy infection may simply reflect the lack of an effective response during the critical period of larval development. At the same time it must be stressed that at all levels of antigenic stimulation the effects observed are relative rather than absolute and that although HbA is a useful genetic marker for resistance to H. contortus, the degree of protection with which it is associated is very much influenced by other, and as yet undefined, "breed" characteristics.

SUMMARY

To test the hypothesis that the response of sheep to primary infections with H. contortus is genetically-determined, Scottish Blackface and Finn Dorset sheep of known haemoglobin type were each infected with 350 third-stage larvae/kg bodyweight. Resistance to the subsequent disease was monitored and compared by haematological, biochemical and radioisotopic methods, and resistance to parasite development and establishment by measurements of egg output and of worm recovery 32 days after infection.

The results obtained demonstrated that sheep with HbA developed less severe clinical and pathophysiological disturbances, passed fewer eggs and harboured fewer worms at necropsy than animals with HbB, and that Scottish Blackfaces exhibited similar advantages over Finn Dorsets with the same haemoglobin type. Since variations in disease severity as judged by pathophysiological effects were broadly in line with parasite numbers it was concluded that genetic resistance operates at the level of parasite establishment which in turn is controlled by the immune response elicited. The mechanism of the immune response was not determined but appeared to operate only against 4th and 5th stage larvae, since no difference was found in the metabolic activities of individual adult worms within any of the experimental groups.

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A subsequent experiment, designed to examine the response of sheep to heavier infection (1400 larvae/kg), failed to reveal any correlation between haemoglobin type and worm establishment. It was suggested that this was a reflection of a delayed immune response caused by exposure to excessive amounts of antigen.

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SECTION 2

THE INFLUENCE OF HAEMOGLOBIN TYPE AND BREED ON THE RESPONSE OF SHEEP TO PRIMARY AND SECONDARY INFECTIONS WITH *Haemonchus contortus*.

INTRODUCTION

The results of the first experiment described in this thesis indicated that resistance to the establishment of H. contortus in previously worm-free sheep given 7,000-10,000 infective larvae was influenced by the animal's breed and haemoglobin type; in general, fewer parasites reached maturity in Scottish Blackface than in Finn Dorset sheep, and within both breeds, in animals with an allele for haemoglobin A. Whilst the true severity of the disease as judged by a variety of clinical parameters was often largely masked by differences in blood compartment sizes and available metabolic reserves prior to infection, and by variations in red cell and protein synthetic capacities thereafter, the underlying pathophysiological disturbances were broadly in line with parasite burden. The implications of these findings are twofold: Firstly, genetic resistance to this parasite is expressed primarily at the level of worm establishment and secondly, barring the unlikely involvement of non-specific physiological factors, this resistance is almost certainly immunological in origin, i.e. a manifestation of specific acquired resistance.

The object of the experiment described in this section was essentially to test the validity of this hypothesis.

Despite the apparent lack of an effective acquired immunity to Haemonchus in the field, numerous attempts have been made to engender this experimentally by prior

administration of larval or worm antigens in one of four forms, i.e. normal and X-irradiated larvae and somatic and metabolic extracts. Although much of this effort has been in vain, the data made available by these investigations collectively provide a useful insight into the developmental aspects and expression of resistance, and are therefore relevant to the present work.

From the literature pertaining to these aspects, it would appear that there are two prerequisites for the induction of an effective resistance to H. contortus. Firstly, the animal must have reached a certain level of immunological competence, and secondly, it must be presented with sufficient amounts of so-called "functional" antigens, i.e. antigens which stimulate immune responses which adversely affect the parasite's biotic potential[1]. In worm-free sheep, immunological competence or responsiveness to Haemonchus is primarily a function of age. This was first clearly demonstrated by Manton et al.,[2] who showed that 2-4 month old Dorset Down lambs given 10,000 normal larvae, either in a single dose or as a trickle infection over 60 days, failed to develop any resistance to subsequent challenge, whereas 10-12 month old animals subjected to a similar regimen were almost solidly immune. Subsequent work using X-irradiated larvae as the source of antigen and other breeds of sheep has largely confirmed the "innate" immunological unresponsiveness

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of the young ovine to H. contortus. For example, Jarrett and Urquhart and their colleagues[3,4], as well as Bitakaramire[5] successfully immunised Scottish Blackface lambs ranging from 7-17 months of age using a schedule of two doses of 10,000 attenuated larvae given at one month intervals, but attempts by Urquhart et al.,[6] to repeat this in younger animals (5-12 weeks old) resulted in almost complete failure. Variations in the technique and schedule of immunisation were to no avail in enhancing the development of resistance in these young lambs. Further albeit indirect evidence of immunological unresponsiveness to H. contortus has been provided by attempts to vaccinate sheep of various breeds with parenteral inoculations of larvae or somatic and metabolic extracts. Using the former, Wilson and Samson[7] failed to protect 2½-5 month old lambs whereas Stoll[8] reported high resistance in sheep ranging between 7 and 12 months of age. Likewise, Silverman[9] obtained some protection (50-60%) with larval extracts in 4-6 month old lambs, but very recent work by Nielson[10] using essentially the same material failed to confirm this in slightly younger animals.

Whilst the general impression gained from the above findings is that sheep become immunologically responsive to Haemonchus at some point between 6 and 12 months of age, the development of this attribute may be seriously retarded and indeed permanently depressed by exposure to infection in early life. This state of "acquired" immunological

unresponsiveness or "tolerance" was brought to light by the studies of Lopez and Urquhart[11] who demonstrated that adult (2 year old) Merino sheep reared under worm-free conditions could be vaccinated against an experimental challenge by prior administration of two doses of X-ray attenuated larvae, but similar animals reared in endemic areas remained susceptible. The implication of this work is clear, i.e. that the apparent absence of an effective immunity in adult sheep arises from infection during the immunologically unresponsive period. Other work however, suggests that acquired immunological unresponsiveness is not confined to animals which have experienced such early antigenic stimulation. For example, Dineen and Wagland[12] showed that the response of 7-8 month old Merino X Border Leicester sheep given six sensitising infections each of 3,000 H. contortus larvae over 12 weeks to a challenge infection of 3,000 larvae was modified by prior removal of the sensitising worm burdens. When challenge was superimposed on the existing infection virtually all of the challenge dose became established, whereas removal of the sensitising burdens (about 6,000 worms) one week before challenge resulted in the establishment of only a small proportion of the challenge infection (437 worms). These findings were attributed to immunological exhaustion produced by prolonged antigenic stimulation. In a subsequent study, this time using animals only 3-4 months old, Wagland and Dineen[13]

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produced less convincing evidence of a greater resistance to challenge at 4 and 8 weeks than at 2 and 16 weeks after removal of sensitising infections, suggesting the existence of an early immunologically "latent" period and a relatively rapid loss of resistance in the absence of infection. On the basis of this and other work carried out in collaboration with Donald and Adams, Dineen[14] stated that anthelmintic treatment interfered with the development and maintenance of resistance to Haemonchus and proposed that the use of these drugs be restricted to individuals showing signs of clinical disease.

From the above considerations it might appear that the existence of immunological unresponsiveness implies permanent susceptibility. While it cannot be disputed that haemonchosis affects animals of all ages, and this is frequently quoted as evidence against the acquisition of resistance in the field[15,16], the fact remains that the vast majority of sheep in endemic areas do survive despite neonatal infection and prolonged antigenic stimulation. Such animals may very well be infected, but nonetheless must also have developed some kind of functional immunity indicating that the unresponsive stage can be broken or at least its effects minimised. In this connection it is significant that Christie and his colleagues[17,18] have shown that 2-month old Scottish Blackface lambs, in common with older (i.e. 7½ month old) animals can develop a very marked resistance to subsequent

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reinfection when given several large doses of infective larvae interspersed with anthelmintic therapy. Likewise, Dineen and co-workers[19] were able to produce a significant degree of immunity in 2-3 month old Merinos by daily dosing with 100 larvae for 30 days, but administration of 3,000 larvae as a single dose proved non-immunogenic. Also relevant in this context were the rather disappointing results obtained by Mulligan et al.,[20] in the course of attempts to vaccinate 7-month old Merino lambs with X-irradiated larvae. Using a schedule of 2,000 attenuated larvae followed 5 weeks later by a second dose of 5,000 larvae similarly treated, these authors succeeded in inducing a solid resistance to subsequent challenge with 10,000 normal larvae in only 6 out of 10 animals - a finding which compares unfavourably with the consistent protection obtained with two doses of 10,000 irradiated larvae in Scottish Blackface lambs of the same age. While these results collectively suggest that the size and pattern of antigenic stimulation have an important bearing on the acquisition of resistance to this parasite, they could equally well be interpreted as evidence for the existence of a fundamental difference in the immunological competence of Scottish Blackface and Merino sheep.

This possibility is supported by a number of other observations. Firstly, by the failure of Lopez and Urquhart[11] to protect 7-month old Merino lambs using the larger irradiated dose schedule proven successful with

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similar animals of the Scottish Blackface breed, and secondly, by the apparent ability of the latter to expel established worm populations gradually at some stage between 3 and 16 weeks following initial infection[21] while in the former such populations are maintained for 9 months or more before there is a significant decline[22].

These findings, together with the work already reviewed in this thesis naturally lead back to the possibility of a genetically-determined resistance to Haemonchus and to two points made earlier in this context i.e. (a) that the field surveys from which the concept originated were based almost exclusively on measurements of egg counts and haematological indices and as such were neither sufficiently accurate nor direct to provide the type of information necessary for a proper definition of any breed, strain or haemoglobin type differences recorded; and (b) that although such differences were in all likelihood manifestations of acquired immunity rather than of some non-specific factor, both possibilities required detailed investigation under carefully controlled experimental conditions. The necessity of the experimental approach has already been completely validated by the previously reported studies dealing with resistance to primary infections; the object of the experiment described in this section of the thesis is essentially to extend this approach to secondary infections with a view to obtaining

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more definitive evidence for the premise that genetic variations in resistance to Haemonchus are immunologically mediated.

For this purpose Scottish Blackface and Finn Dorset sheep of known haemoglobin type were each infected with 350 H. contortus larvae/kg bodyweight, treated with an anthelmintic 5 weeks later, and together with worm-free controls challenged with 10,000 larvae and necropsied after 32 days. Since the sensitised sheep and the animals used in the first experiment of the thesis were infected with the same number and batch of larvae, worm burdens of the latter could be used as an index of parasite establishment from the primary infection. The course of each infection was monitored and compared by clinical, radioisotopic and parasitological methods, particular attention being given to the effects of immune damage on the parasites' development and biotic potential.

Experimental animals and design

Twelve 7-month old Scottish Blackface and Finn Dorset wethers, reared and maintained parasite-free as described earlier, were divided into 4 groups of 3 sheep on the basis of breed and haemoglobin type. Groups 1 and 2 were Scottish Blackface sheep of Hb types A and AB respectively while Groups 3 and 4 were Dorsets whose haemoglobin types were respectively AB and B. All animals received a single oral dose of 350 H. contortus larvae/kg bodyweight and 35 days later this infection was terminated with thiabendazole ("Thibenzole", Merck Sharp & Dohme Ltd., Hoddesdon, England), administered at a rate of 80 mg/kg. Eighteen days later each was challenged with a further 10,000 H. contortus larvae and necropsied after 32 days; 10,000 larvae were also given to 5 worm-free sheep to control the challenge infection. Red cell and albumin kinetics were measured following intravenous injections of ^{51}Cr-labelled erythrocytes, ^{125}I-labelled albumin and ^{59}Fe citrate at regular intervals throughout the study.

During the entire experiment the sheep were kept in metabolism cages and fed hay and water ad lib; each was also dosed orally with 10 ml 0.75% KI daily, beginning 4 days prior to infection.

Blood analyses and parasitological techniques

Blood samples were collected twice weekly for estimations of PCV, Hb and RBC counts, and weekly for measurements of total serum protein, serum albumin,

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globulin and iron concentrations. Faecal worm egg counts were determined daily, and worm burdens at necropsy. The techniques adopted for all these measurements have been described elsewhere.

Radioisotopic methods

Labelling, injection and sampling procedures

The techniques used for labelling sheep albumin with ^{125}I, red cells with ^{51}Cr and transferrin with ^{59}Fe have been described in detail elsewhere. Individual sheep received approximately 250 µCi ^{125}I, 800 µCi ^{51}Cr and 50 µCi ^{59}Fe, on each of 4 occasions, i.e. before and 28 days after infection; at the time of challenge, and finally 3 days before necropsy. Injection and sampling procedures were similar to those described in the previous section, while the methods adopted for radioactivity analyses were outlined in some detail in the General Materials and Methods.

Calculations and expression of results

The data obtained from the radioactivity determinations performed were essentially the same as those described in the previous section. Red cell volumes (RCV), apparent red cell $t_{\frac{1}{2}}$ and gastrointestinal losses of red cells and haemoglobin iron were measured from the ^{51}Cr sample radioactivities, and plasma iron turnover and faecal excretion of iron from ^{59}Fe activity; haemoglobin iron reabsorption was assessed on the basis of the differences recorded between iron losses in the faeces and the gastrointestinal tract.

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Plasma volumes (Vp), albumin pool sizes and catabolic rates were measured from the ^{125}I data.

RESULTS

Groups of Scottish Blackface and Finn Dorset wethers were infected with H. contortus, treated with anthelmintic 5 weeks later, and after an interval of 18 days challenged with a further dose of Haemonchus larvae; 5 worm-free sheep acted as challenge controls. The effect of each infection and treatment was monitored by analytical and radioisotopic methods and individual worm burdens determined at necropsy 32 days after challenge. To simplify the presentation of results, the response of each group is described chronologically and mean values used as the basis for group comparisons; some individual data are included in the tables, the remainder appear in Appendix 2.

Haematological and biochemical observations

The changes observed in PCV, MCV, MCHC and serum iron are illustrated in Figures 1 and 2. The course and type of anaemia which developed following initial infection were similar to those recorded in the earlier experiment utilising the same dose of larvae. Haematocrits which were initially higher in Dorsets than in Blackfaces of comparable haemoglobin type and to a lesser extent in HbA than in HbAB Blackfaces, fell sharply in all groups between the first and third weeks, but thereafter only gradually prior to anthelmintic administration. By this stage the average Dorset PCV had deteriorated by 62% (more so in the HbB group) and that of the Blackfaces by only 39%, placing the advantage decisively in favour of the latter.

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Figure 1. PCV Values Following Infection, Anthelmintic Treatment and Reinfection with H. contortus.

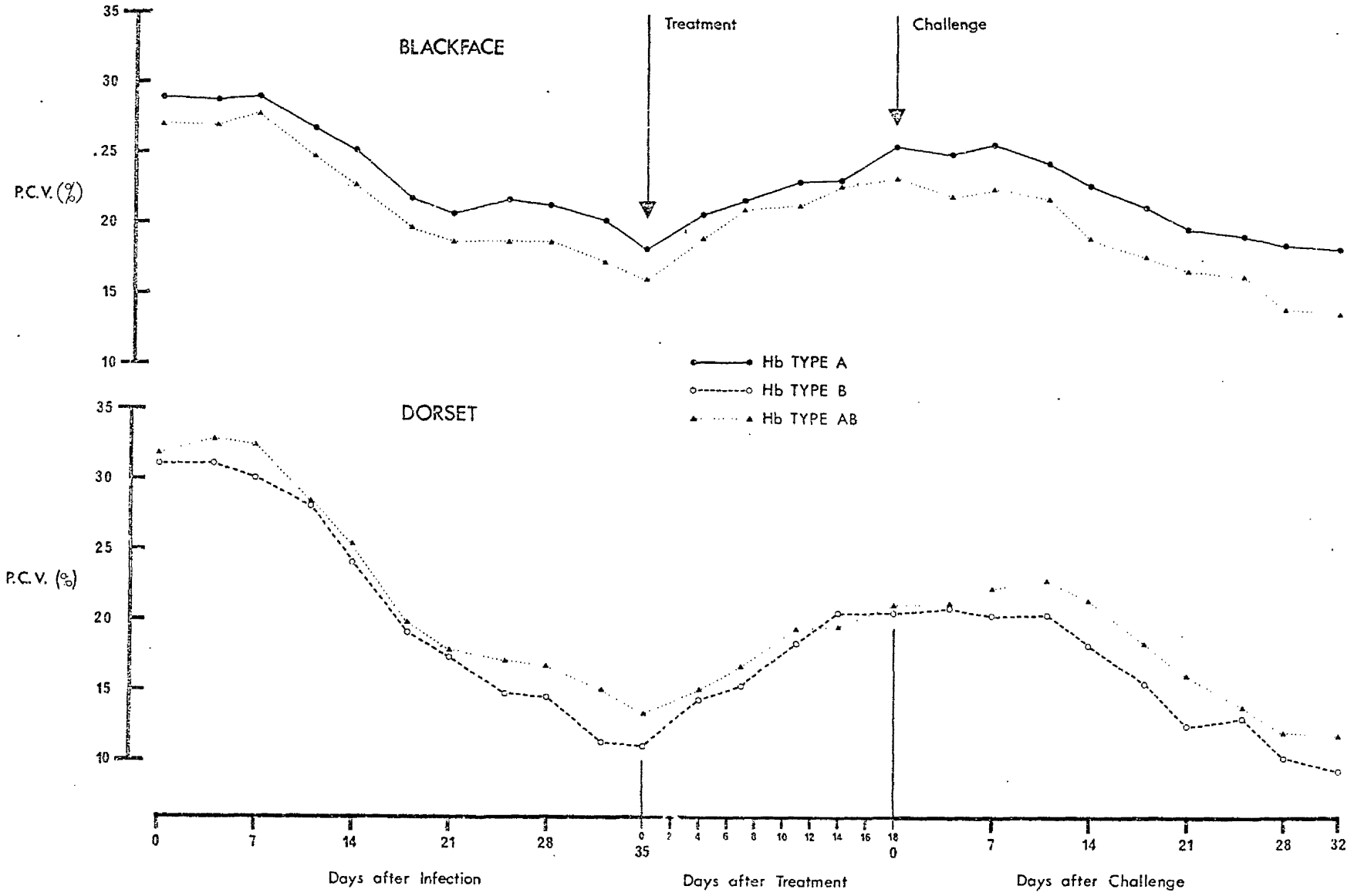
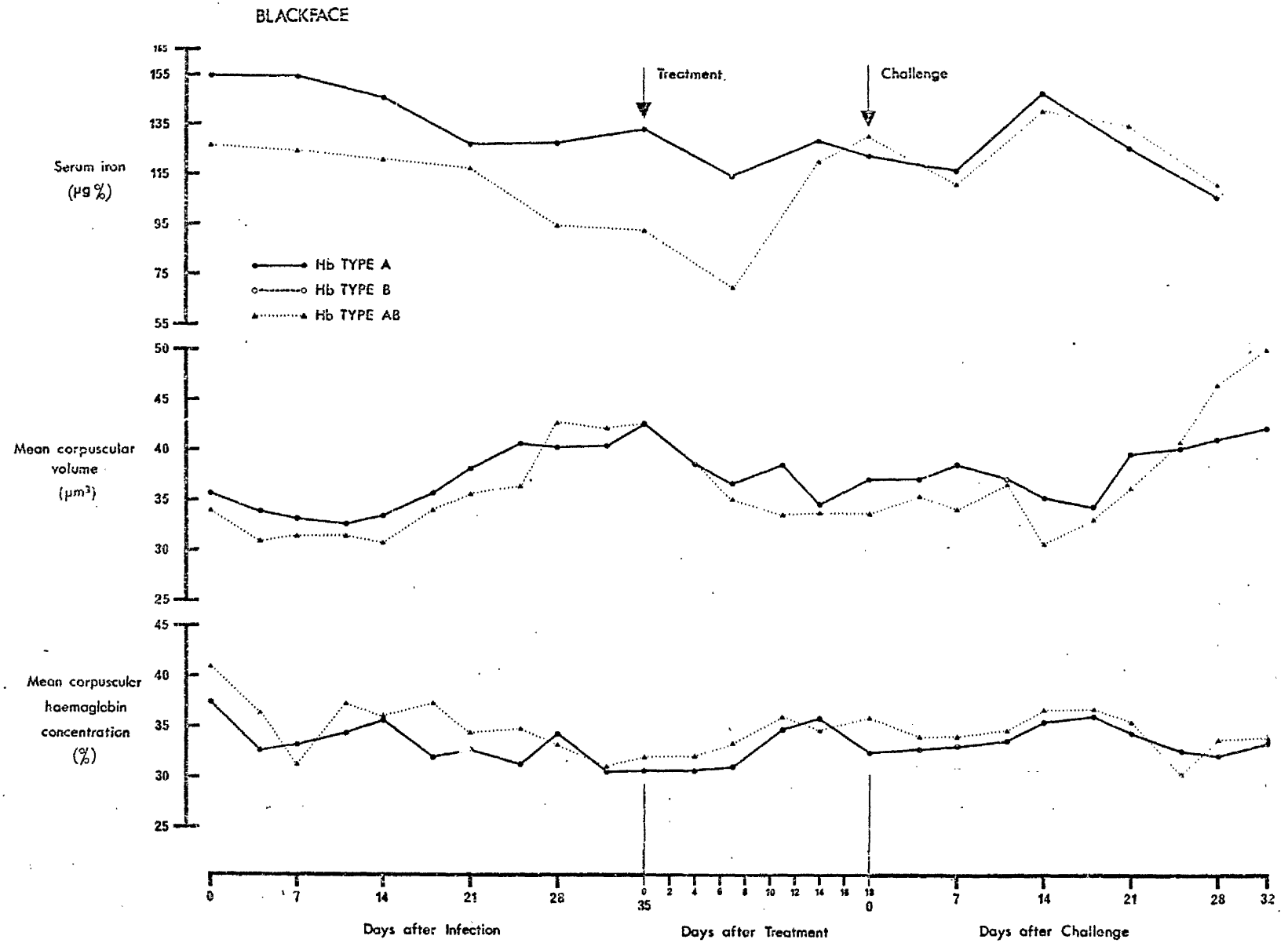
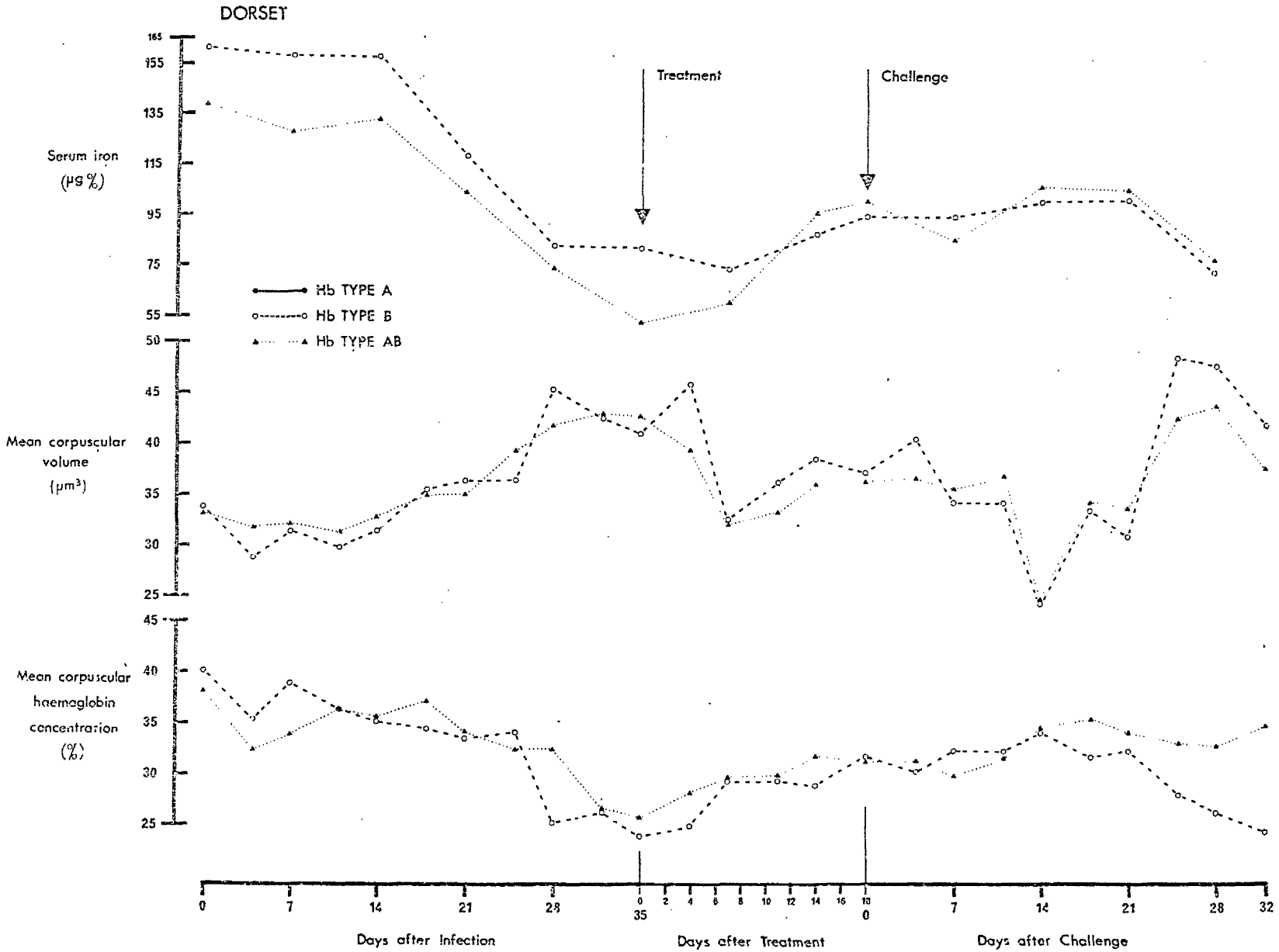


Figure 2. Changes in MCHC, MCV and Serum Iron Levels Following Infection, Anthelmintic Treatment and Reinfection with H. contortus.




DORSET
Treatment
Challenge
Serum iron (µg %)
Hb TYPE A
Hb TYPE B
Hb TYPE AB
Mean corpuscular volume (µm³)
Mean corpuscular haemoglobin concentration (%)
Days after Infection
Days after Treatment
Days after Challenge

Further indications of the more severe disturbances experienced by the Dorsets included their higher MCV and lower MCHC and serum iron levels during the week preceding treatment.

The effect of the anthelmintic was dramatic, resulting in immediate improvements in the PCV of all animals and particularly those worst affected by earlier infection, i.e. the HbB Dorsets. Indeed, a notable feature of the post-treatment data was the inverse relationship between the benefit derived from worm removal and the severity of anaemia existing beforehand - PCV's of HbA and HbAB Blackfaces increased by 30% and 44% respectively, those of HbAB and HbB Dorsets by 69% and 91%; hence, differences apparent between breeds before treatment narrowed, and by reinfection values for all groups containing the B allele were similar, although HbA Blackfaces retained their overall advantage. Also worth mentioning is the fact that reductions in MCV and elevations in MCHC and serum iron which accompanied improvements in PCV were likewise best demonstrated by those sheep formerly most anaemic.

Reinfection was followed by progressive reductions in PCV which despite the heavier challenge were delayed in onset until about the 14th-18th days; as a general rule the severity of anaemia recorded 32 days after challenge was only marginally more severe than that recorded immediately before treatment, but as before

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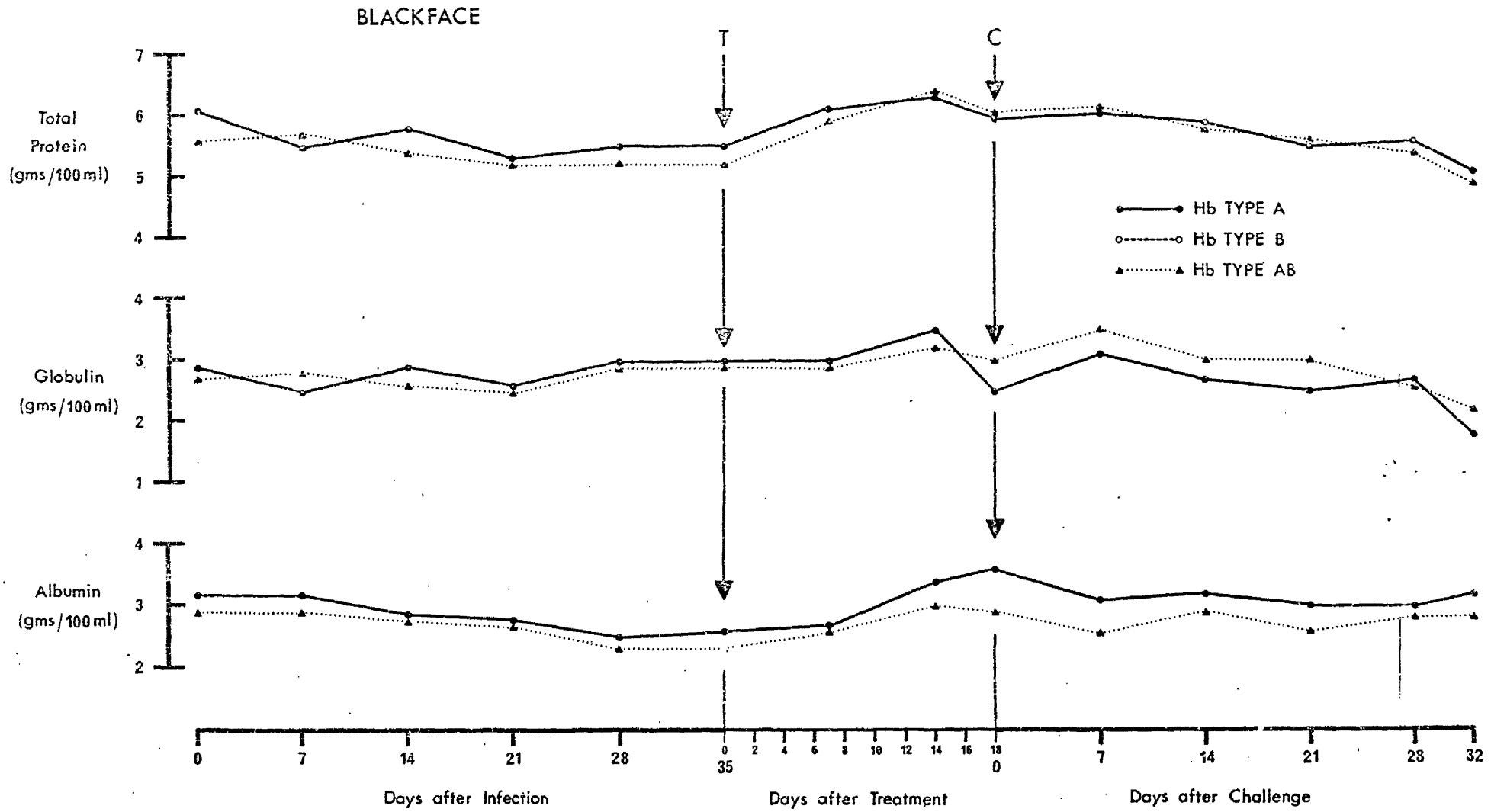
Dorsets were more seriously affected than Blackfaces. With the renewed development of anaemia MCV values increased and MCHC and serum iron levels generally fell; these features were again most apparent in sheep of the Dorset breed.

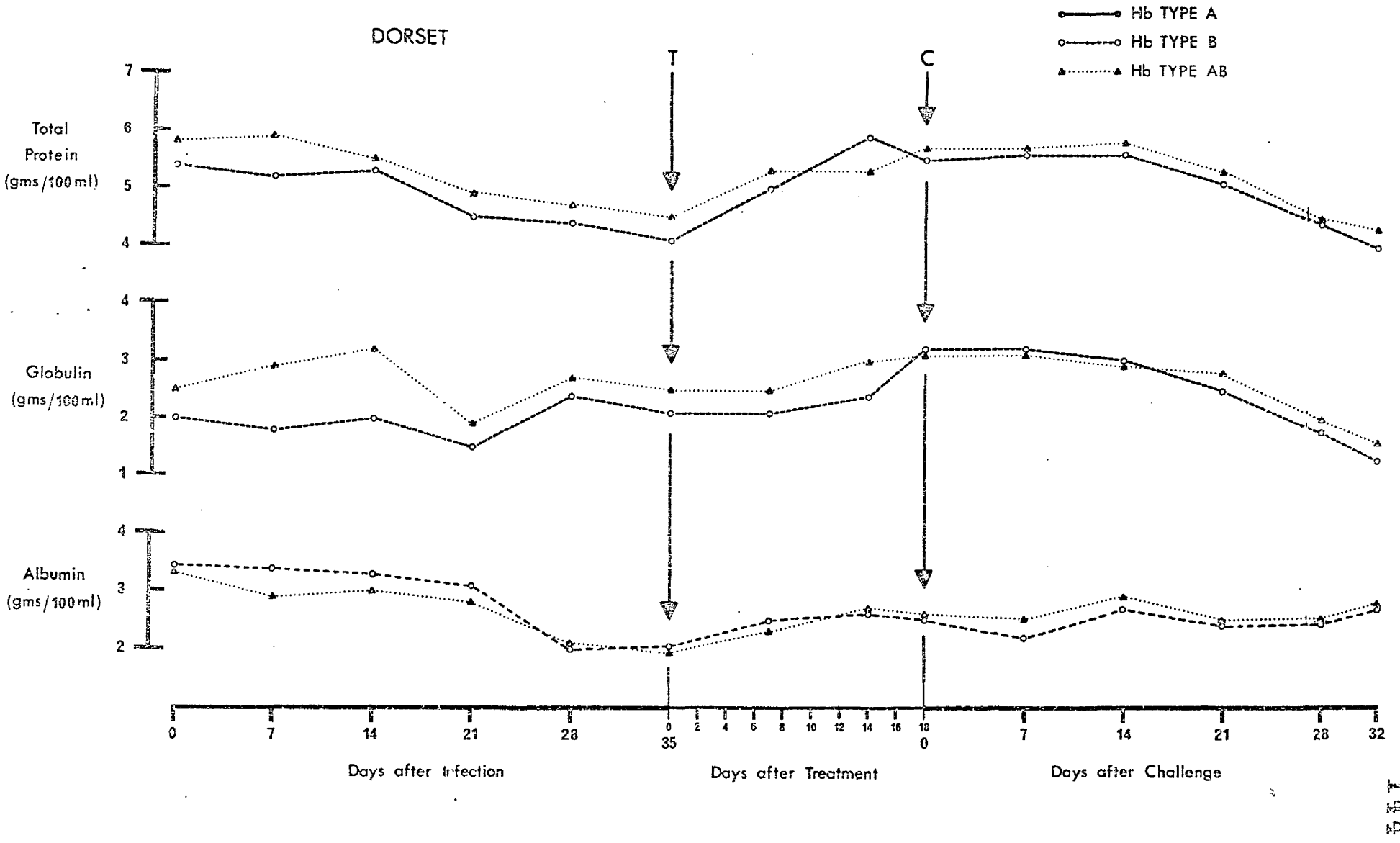
The changes observed in the challenge controls were much more severe, all animals experiencing sharp reductions in PCV from about the 8th day after infection; by necropsy the average values for the HbA Blackfaces had fallen from 31 ± 0.6% to 17 ±0.3% and those of the HbAB Blackfaces from 30 ± 1.0% to 16 ± 0% (Table 12, Appendix 2). Measurements of red cell counts, haemoglobin concentrations and serum constituents were omitted.

Total serum proteins and albumin, which at the outset were similar in all animals, fluctuated thereafter in the same cyclic fashion as the haematolgoical indices (Figure 3). Both fell progressively from about the second week after initial infection, improved rapidly following anthelmintic treatment, but deteriorated yet again after challenge. In common with the PCV changes, HbB Dorsets were worst affected by each infection, but benefited most from treatment.

Elevated serum globulin levels were recorded in all groups, were never particularly marked in any, and especially in the Dorsets often fell to values considerably lower than those existing before infection - particularly just before treatment and necropsy.

Figure 3. Serum Protein Values Following Infection, Anthelmintic Treatment and Reinfection with H. contortus.




DORSET
Hb TYPE A
Hb TYPE B
Hb TYPE AB
T
C
Total Protein (gms/100ml)
Globulin (gms/100ml)
Albumin (gms/100ml)
Days after Infection
Days after Treatment
Days after Challenge

Parasitological findings

Faecal egg counts were performed daily, and the results plotted in Figure 4. Small numbers (50-100 epg) first appeared in the faeces of individuals of both breeds 16 days after the primary infection and in progressively increasing numbers in all animals until treatment. It is apparent however that the ultimate level achieved varied with breed and haemoglobin type, highest counts being associated with the Dorsets and within this breed the HbB group ($p < 0.05$); counts were also higher in HbAB Dorsets than HbAB Blackfaces ($p < 0.05$) which in turn had somewhat greater values than their HbA counterparts.

Within 3 days of treatment egg counts were negative in all animals, and remained so until 19 days after reinfection, when small numbers again appeared in one HbB Dorset. Three days later all Dorsets were positive, but Blackfaces remained negative until day 24 when a few eggs were detected in three animals. Eggs were present in the faeces of all sheep by day 26 and passed in increasing numbers up to the termination of the experiment. Highest counts were recorded in the HbB Dorsets and lowest in HbA Blackfaces, but while little difference existed between the two HbAB groups, egg outputs were significantly lower in all groups ($p < 0.05$-0.02) than at the corresponding stage of the primary infection. They were also significantly lower than the values recorded for the corresponding challenge controls ($p < 0.001$), which first became positive 16 days after infection and increased progressively throughout the remainder of the experiment (Table 12, Appendix 2).

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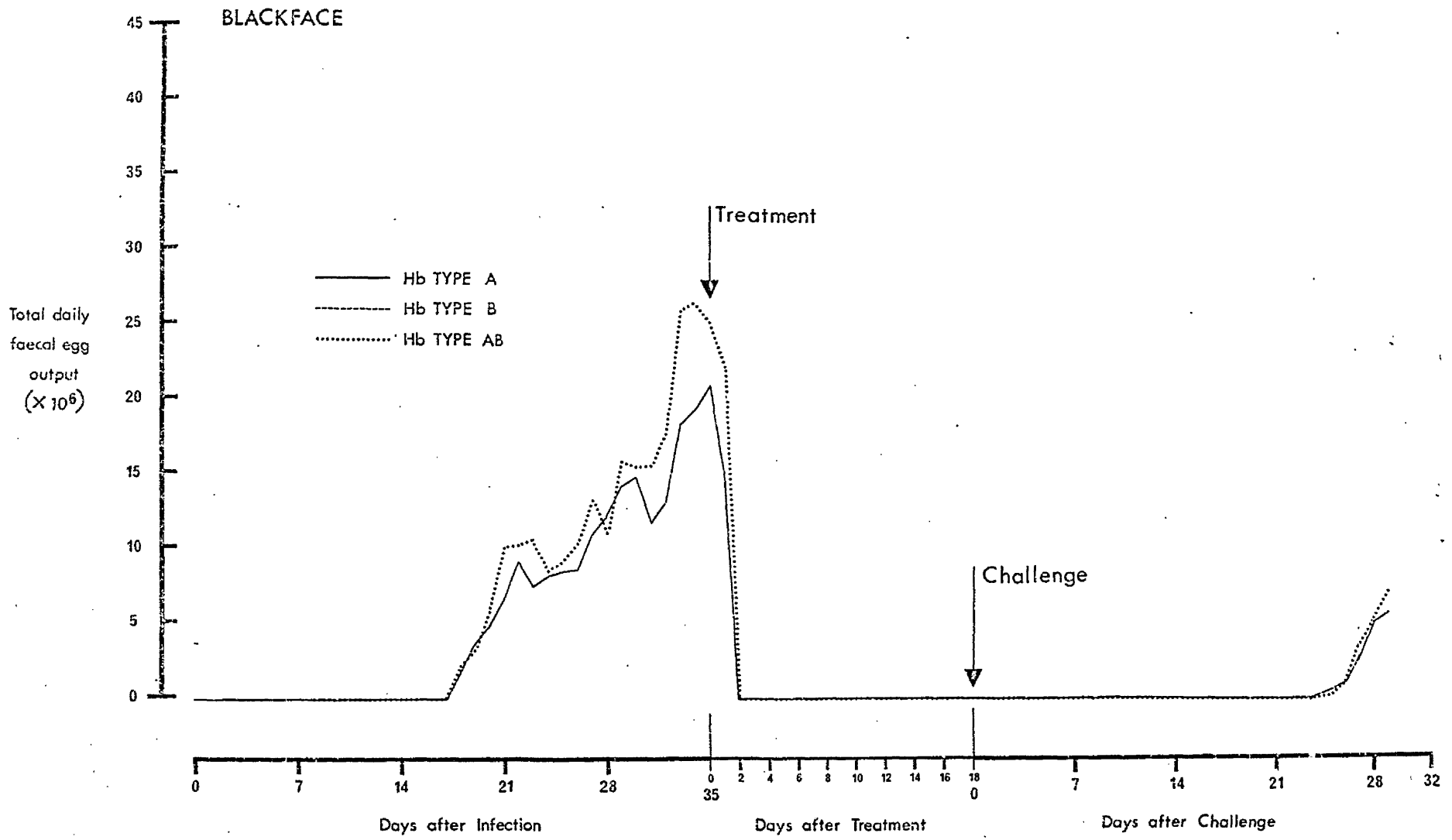
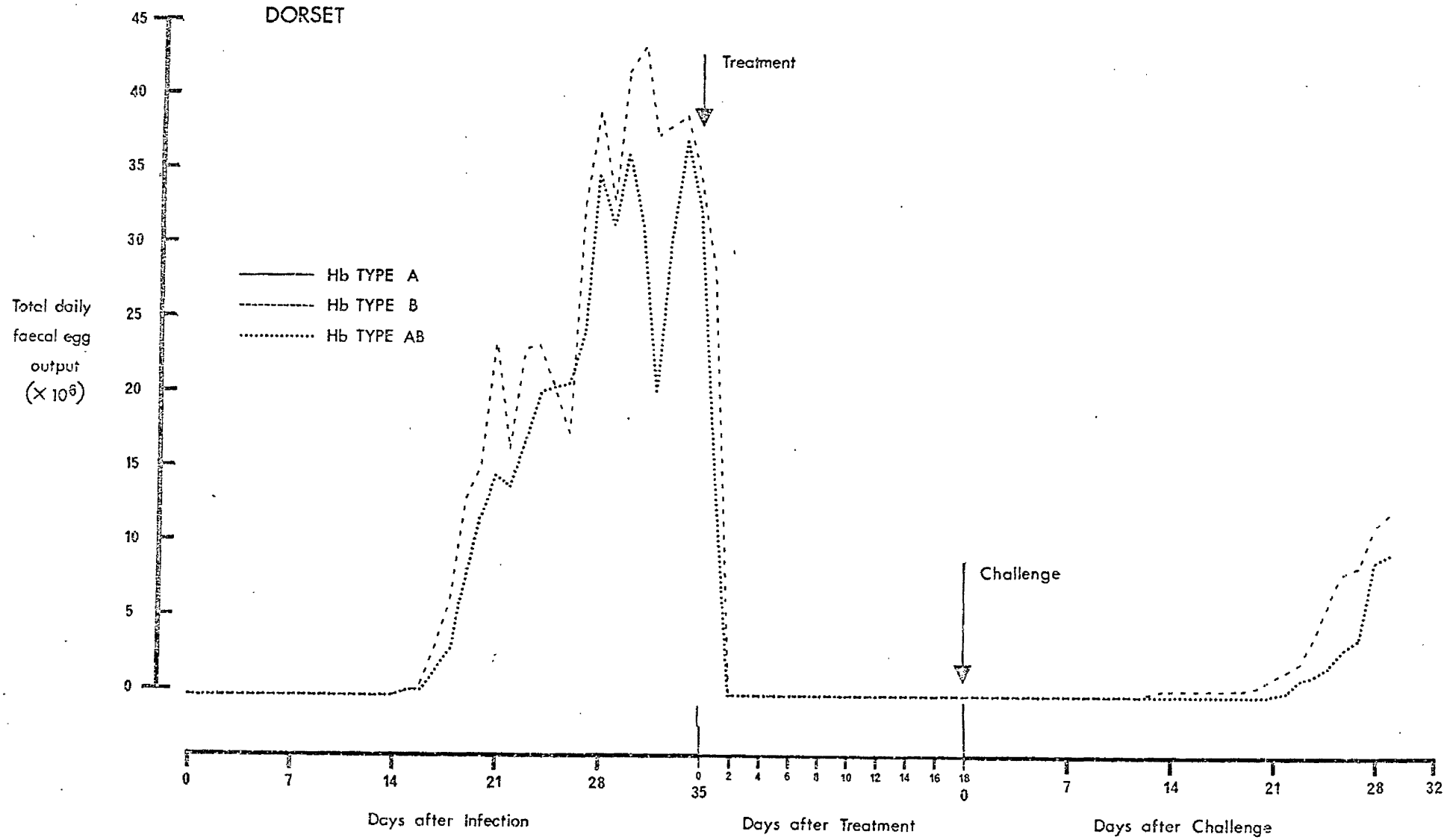
Figure 4. Faecal Egg Output Following Infection, Anthelmintic Treatment and Reinfection with H. contortus.




DORSET
Treatment
Challenge
Hb TYPE A
Hb TYPE B
Hb TYPE AB
Total daily faecal egg output (× 10⁶)
45
40
35
30
25
20
15
10
5
0
0 7 14 21 28
Days after Infection
0 2 4 6 8 10 12 14 16 18
35
0
Days after Treatment
7 14 21 28 32
Days after Challenge

Details of the number and size of the worms established from the second infection and of the worm burdens of the challenge controls are given in Table 1. These reveal a number of important points. Firstly, both groups of challenge controls had lower worm burdens (representing 17% and 24% respectively of the administered larvae in the HbA and HbAB animals) than their respective reinfected counterparts (29% and 35%); in the case of the sheep with HbA this difference was statistically significant ($p < 0.05$). The absence of controls for the Dorsets precludes a direct comparison of the response of this breed to primary and secondary infections, but on the basis of the blood loss measurements, it would appear that in these animals also, the percentage "take" from the second infection (about 40% and 45% respectively for the HbAB and HbB groups) was at least as high as the estimated burdens from the primary infection (35% and 40% respectively). Secondly, although none of the re-infected sheep appeared to have acquired any immunity, more worms were nevertheless recovered from the Dorsets than from the Blackface sheep ($p < 0.05$) and the general pattern of recovery paralleled closely that recorded in the earlier experiment (Table 1, Section 1) using the same number and batch of larvae, i.e. HbB > HbAB Dorsets > HbAB > HbA Blackfaces. Finally, the mean worm lengths of the challenge controls were significantly greater than those of the corresponding reinfected sheep ($p < 0.001$) but similar to the mean length of the worms recovered after the primary infection from the experimental animals employed for the previous studies (Table 1, Section 1).

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TABLE 1.

Worms Recovered at Necropsy.

		Sheep No.	Worms Recovered	% Recovery	Mean Group Length (mm) Male	Female
Blackface HbA		56	2700	27.0		
		57	2750	27.5		
		91	3100	31.0		
Mean			2850	28.5	11.6	16.3
S.E.			129	1.3	1.1	2.6
Blackface HbAB		80	3800	38.0		
		82	2400	24.0		
		92	4250	42.5		
Mean			3483	34.8	12.1	16.0
S.E.			557	5.6	1.7	2.6
Dorset HbAB		482	4900	49.0		
		521	3500	35.0		
		538	3850	38.5		
Mean			4083	40.8	12.5	16.0
S.E.			421	4.2	0.9	1.2
Dorset HbB		477	4750	47.5		
		547	4350	43.5		
		549	4700	47.0		
Mean			4600	46.0	11.8	16.6
S.E.			129	1.3	1.1	1.4
Blackface HbA	Challenge Controls	68	1400	14.0		
		73	1600	16.0		
		95	2000	20.0		
Mean			1667	16.7	13.1	19.2
S.E.			176	1.8	0.9	1.3
Blackface HbAB		69	2200	22.0		
		72	2500	25.0		
Mean			2350	23.5	12.6	19.0
S.E.			150	1.5	1.2	1.5

Pathophysiological data

Blood volumes and red cell kinetics

Plasma, red cell and blood volumes were determined on four occasions during this investigation, i.e. immediately before and 28 days after the primary and challenge infections. The average values obtained on each occasion are illustrated in Figure 5. The only feature of any significance before infection was the sizeable advantage held by Dorsets by virtue of their superior bodyweight. However, this was short-lived and by the 28th day total red cell masses were similar in all groups, though smaller relative to bodyweight in Dorsets, particularly those of the HbB genotype. Hence, like the first experiment, red cell depletion was more extensive in Dorsets than in Blackfaces (54% and 34% respectively, $p < 0.05$) which in turn were more affected in the presence of a B allele (HbA and HbAB group reductions were 26% and 42% respectively, $p < 0.05$). Plasma volumes remained steady in all groups with the result that blood volumes contracted by about 15% in relation to weight. Anthelmintic treatment produced substantial improvements in the red cell mass of all sheep, especially the Dorsets, but since plasma volumes fell at the same time both processes presumably contributed to the

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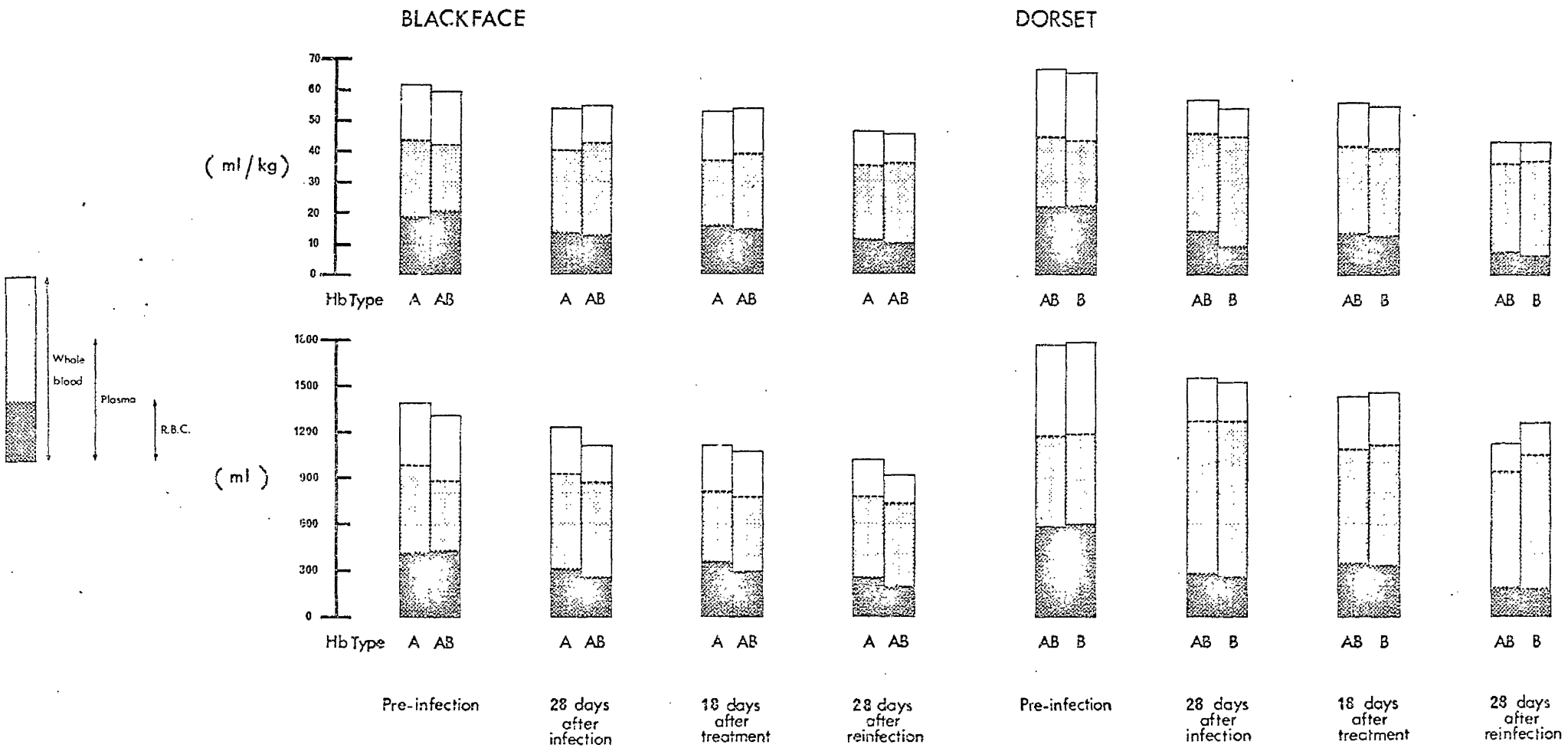
Figure 5. Plasma, Red Cell and Blood Volumes Following Infection, Anthelmintic Treatment and Reinfection with *H. contortus*.



elevations in haematocrit recorded over this period. Red cell volumes deteriorated yet again after reinfection and at the termination of the experiment were lower in all groups than at the corresponding time after initial infection. On the other hand, plasma volumes were maintained at values similar to those recorded after treatment but lower than those found at the same stage of the primary infection, while blood volumes, as a result of reductions in red cell mass, were lowered relative to both pre- and post-treatment figures.

The information given in Figure 6 and Tables 2 and 3 details some of the changes in red cell turnover recorded during this experiment. Haemorrhage into the gut was not measured until 28 days following administration of the first dose of larvae, but from the data obtained thereafter it is evident that the severity of bleeding and iron loss resulting from the primary infection increased with each substitution of a B for an A allele and that HbAB Dorsets were more severely affected than Blackfaces of the same haemoglobin type. It is also relevant that the blood losses recorded here for each group were of a similar order to those in the earlier experiment utilising the same dose of larvae, a finding which when considered against the background of the other features described suggests considerable group variation in worm establishment. In the absence of actual counts

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Figure 6. Abomasal Haemorrhage after Infection, Anthelmintic Treatment and Reinfection with H. contortus.

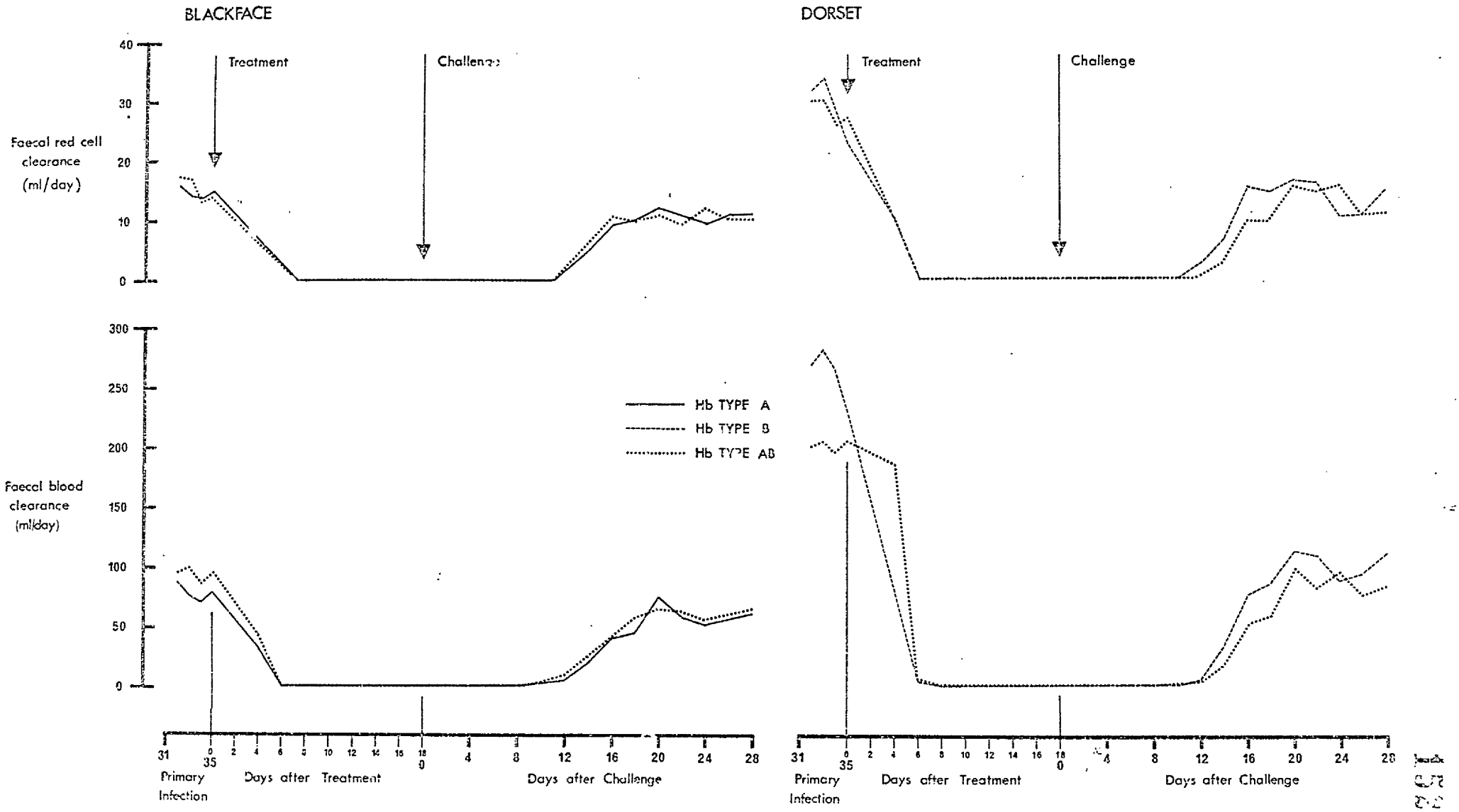

TABLE 2.

Faecal "Clearances" of Blood and Red Cells and Iron Absorption Data 21-28 Days After Reinfection.

	Sheep No.	Average daily ^{51}Cr clearance (ml) Blood	Red Cells	Average daily ^{59}Fe clearance (ml) Blood	Red Cells	Haemoglobin iron (mg/day) Lost in Gut	Excreted in Faeces	Reabsorbed
	56	62.3	11.0	67.5	12.6	11.9	11.1	0.8
Blackface HbA	57	46.5	8.5	51.3	10.3	10.3	11.2	
	91	61.1	10.9	68.5	15.3	14.3	15.3	
Mean		56.6	10.1	62.4	12.7	12.2	12.5	0.3
S.E.		5.1	0.8	5.6	1.5	1.2	1.4	0.3
	80	57.8	10.9	61.8	11.4	12.3	12.8	
Blackface HbAB	82	63.0	9.4	103.6	17.4	13.1	16.4	
	92	59.6	12.5	71.8	10.4	11.1	12.8	
Mean		60.1	10.9	79.1	13.1	12.2	14.0	
S.E.		1.5	0.9	12.6	2.2	0.6	1.2	
	482	73.3	12.2	75.8	11.9	13.8	13.9	
Dorset HbAB	521							
	538	111.2	18.1	112.3	18.0	21.2	21.4	
Mean		92.3	15.2	94.1	15.0	17.5	17.7	
S.E.		19.0	3.0	18.3	3.1	3.7	3.8	
	477	108.0	16.3	100.0	15.5	19.0	18.0	1.0
Dorset HbB	547	128.6	13.7	143.5	17.5	14.5	16.5	
	549	92.7	13.9	92.0	13.2	17.3	17.1	0.2
Mean		109.8	14.6	111.8	15.4	16.9	17.2	0.4
S.E.		10.4	0.8	16.0	1.2	1.3	0.4	0.3

TABLE 3.

Faecal "clearances" of Blood and Red Cells and Iron Absorption Data 32-35 Days After Infection.

	Sheep No.	Average daily ^{51}Cr clearance (ml)		Average daily ^{59}Fe clearance (ml)		Haemoglobin iron/(mg/day)		
		Blood	Red Cells	Blood	Red Cells	Lost in Gut	Excreted in Faeces	Reabsorbed
	56	46.3	9.1	40.5	8.0	8.2	7.2	1.0
Blackface HbA	57	88.1	16.1	79.6	14.5	18.3	16.6	1.7
	91	104.7	20.2	76.3	14.7	19.8	14.4	5.4
Mean		79.7	15.1	65.5	12.4	15.4	12.7	2.7
S.E.		17.4	3.2	12.5	2.2	3.6	2.8	1.4
	80	107.4	17.1	98.4	15.6	19.3	17.7	1.6
Blackface HbAB	82	67.8	12.7	62.6	11.7	12.8	11.8	1.0
	92	109.8	16.8	100.8	15.4	17.6	16.6	1.0
Mean		95.0	15.5	87.3	14.2	16.6	15.4	1.2
S.E.		13.6	1.4	12.4	1.3	2.0	1.8	0.2
	482	130.2	20.9	128.4	20.5	28.6	28.2	0.4
Dorset HbAB	521	221.8	31.5	194.6	27.6	28.8	25.3	3.5
	538	252.4	33.9	204.3	27.4	37.9	30.6	7.3
Mean		201.5	28.8	175.8	25.2	31.8	28.0	3.7
S.E.		36.7	4.0	23.8	2.3	3.1	1.5	2.0
	477	206.4	29.0	197.9	27.8	31.8	30.4	1.4
Dorset HbB	547	314.3	30.8	274.6	27.5	25.4	22.2	3.2
	549	249.2	26.4	238.4	25.3	25.8	24.8	1.0
Mean		356.6	28.7	237.0	26.9	27.7	25.8	1.9
S.E.		31.4	1.3	22.2	0.8	2.1	2.4	0.7

any suggestion regarding differences in worm burden must be purely speculative, but taking the figure of 0.06 ml as the approximate blood loss per worm, it may be tentatively concluded that of the 8-10,000 larvae given to these sheep, approximately 1300 and 1600 became established respectively in the HbA and HbAB Blackfaces compared with 3500 and 4300 in the HbAB and HbB Dorsets, representing 17%, 22%, 35% and 43% of the administered larvae. When related to total egg output during the 4 days preceding treatment these figures give an estimated egg output of 10-15,000/female worm in all groups, which compares favourably with that recorded in the previous section.

Removal of worms by anthelmintic treatment led predictably to a rapid and complete cessation of abomasal bleeding in all animals (Figure 6). This situation continued until about 12 days after challenge when haemorrhage recommenced, reaching a peak in all groups around day 21 which was maintained until necropsy. However, three points merit mention with regard to the blood loss resulting from reinfection. Firstly, despite the heavier challenge, haemorrhage was less severe and group differences less pronounced than at the corresponding stage of the primary infections. Secondly, the average blood loss per worm (0.014-0.024) and incidentally also the egg output/female (2000-5000/day) were considerably lower than expected and undoubtedly lower than in the first infections which were performed using the same number and batch of larvae as

infection of the animals used in the first experiment described in this thesis. Finally, and as observed previously, cumulative red cell losses were substantially greater than deficits in red cell mass; Hb B Dorsets, for example, lost in excess of 220 ml through haemorrhage, while reductions in red cell volume averaged only 155 ml, and HbA Blackfaces lost 145 ml in association with average circulating deficits of 110 ml. These figures again suggest that red cell synthesis was enchanced in all animals but especially in those experiencing greatest haemorrhage.

Direct information on red cell synthesis was obtained from measurements of plasma iron turnover made at four strategic stages of the investigation (Figure 7). Before infection all animals had healthy serum iron concentrations, extended ^{59}Fe plasma clearances and rates of plasma iron turnover compatible with normal sheep. Four weeks later each of these indices was dramatically altered - serum iron levels were lower, plasma ^{59}Fe clearances shorter and plasma iron turnover rates elevated. The extent of these changes was closely related to the severity of haemorrhagic stress, HbA Blackfaces and HbB Dorsets, for example, exhibiting three- and five-fold increases respectively in plasma iron turnover with other groups responding in an intermediate manner.

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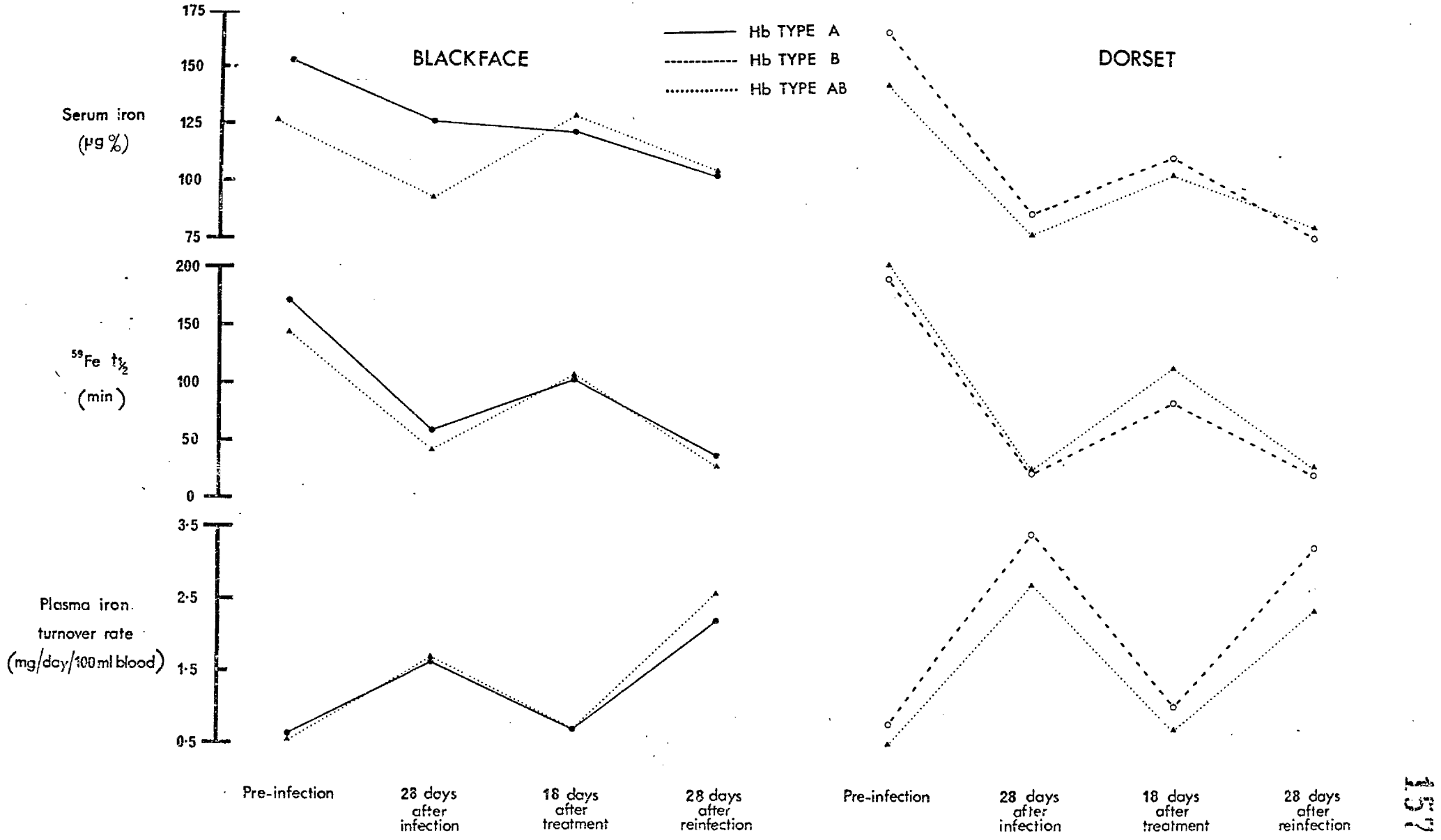
Figure 7. Ferrokinetic Indices Following Infection, Anthelmintic Treatment and Reinfection with H. contortus.



Anthelmintic treatment produced some minor improvement in serum iron levels, plasma ^{59}Fe half-lives lengthened and turnover rates fell in consequence to values approaching but still somewhat higher than those recorded before infection. Reinfection was followed by yet another increase in the plasma iron turnover rate of all animals, highest values again being noted in HbB Dorsets and lowest in HbA Blackfaces. Nevertheless when these figures are compared both with those obtained at the corresponding stage of the primary infection and following treatment it would appear, perhaps paradoxically, that it was the Blackface sheep which responded more positively to reinfection. The two factors contributing to the greater acceleration of erythropoiesis in this breed were firstly their more healthy serum iron levels and secondly the ability to further increase their rates of plasma iron clearance. Dorsets, on the other hand, suffered substantial reductions in serum iron during the second infection and hence although iron clearance from the plasma was probably maximal, turnover rates tended to fall.

Albumin pools and catabolic rate

The results of the albumin pool determinations, made concurrently with those of blood volumes are illustrated in Figure 8 while the measurements of albumin degradation and faecal "clearance" of radioiodide

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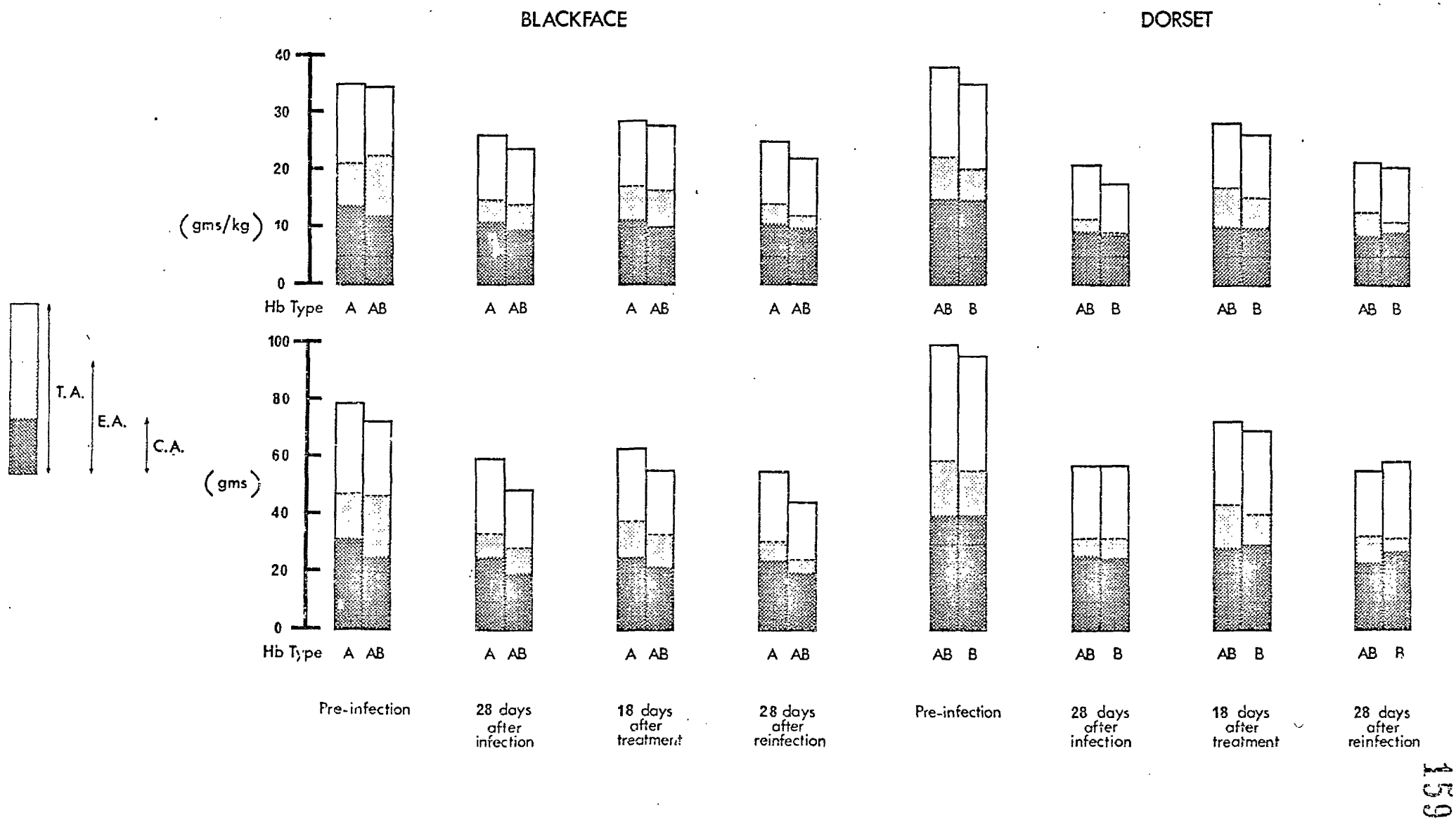
Figure 8. Albumin Pools Following Infection, Anthelmintic Treatment and Reinfection with H. contortus.



during the intervening periods are shown in Figure 9 and Tables 4 and 5. Initially the relative amounts and distribution of protein were similar in individuals of each breed although the heavier HbAB Dorsets had larger total pools than the corresponding Blackfaces ($p < 0.05$). In the course of the primary infection all animals experienced varying degrees of hypercatabolism accompanied by excessive gastrointestinal protein leak with resultant hypoalbuminaemia and depletion of albumin pools; as in previous experiments intravascular pools were preferentially maintained and hence EA:CA ratios fell. All these changes were more pronounced in the Dorset sheep, and in both breeds, in animals carrying an allele for HbB.

Anthelmintic treatment was rapidly followed by sharp reductions in fractional catabolic rates and faecal "clearances". This quiescent period persisted until reinfection by which time the serum albumin levels and pool sizes of all groups, but especially those formerly most adversely affected, showed considerable improvement; significantly the major benefit derived from this re-charging process accrued extravascularly with the result that EA:CA ratios (but not the individual pools) returned to near normal values. The changes in albumin catabolism and pools which accompanied the second infection were of a similar nature but much less dramatic than those described for the primary infection, animals of all groups suffering further hypercatabolism and gastrointestinal protein loss from

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Figure 9. Albumin Catabolism and Faecal Clearance of Radioiodide Following Infection, Anthelmintic Treatment and Reinfection with H. contortus.

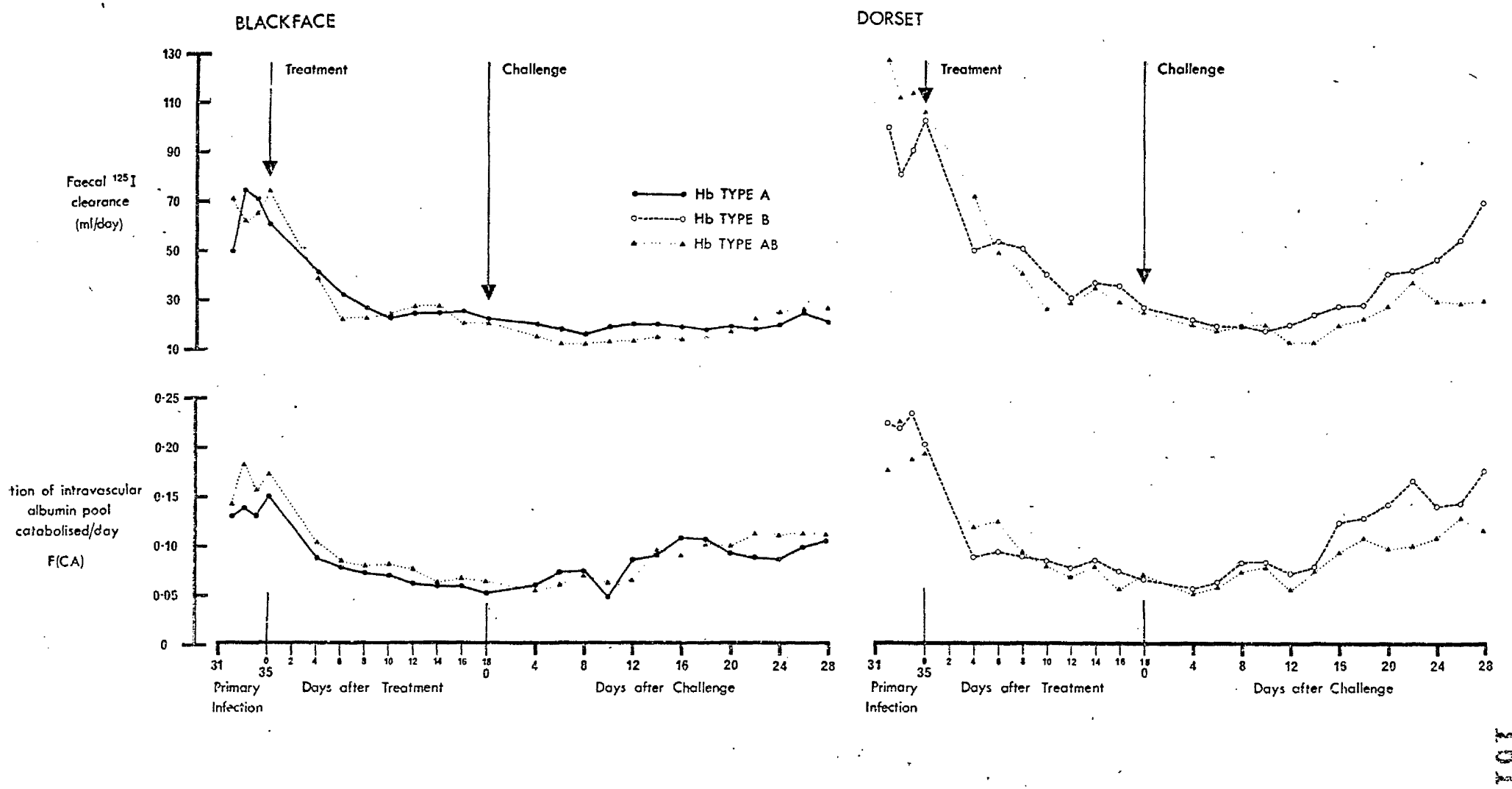

TABLE 4.

Albumin Catabolism Before and After Infection.

	Sheep No.	Before infection: Serum albumin (g%)	Before infection: Apparent $t_{\frac{1}{2}}$ (hr)	32-35 days after infection: Serum albumin (g%)	32-35 days after infection: Apparent $t_{\frac{1}{2}}$ (hr)	F(CA)	Faecal activity (ml plasma)
	56	3.3	448	2.4	240	0.100	48.6
Blackface HbA	57	3.1	480	2.6	288	0.141	75.5
	91	3.2	385	2.4	180	0.165	63.8
Mean		3.2	438	2.5	236	0.135	62.6
S.E.		0.1	28	0.1	31	0.020	7.8
	80	2.6	414	2.2	188	0.257	76.2
Blackface HbAB	82	3.2	460	2.3	256	0.116	58.6
	92	2.9	420	2.3	220	0.120	62.9
Mean		2.9	431	2.3	221	0.164	65.9
S.E.		0.2	14	0.0	20	0.046	5.3
	482	3.1	370	2.4	189	0.126	94.5
Dorset HbAB	521	3.5	460	1.7	246	0.224	111.2
	538	3.5	430	2.1	220	0.224	133.2
Mean		3.4	420	2.1	218	0.191	113.0
S.E.		0.1	27	0.2	17	0.033	11.2
	477	2.9	560	2.0	174	0.201	92.1
Dorset HbB	547	3.4	480	1.6	176	0.219	82.8
	549	3.8	380	2.3	153	0.228	108.7
Mean		3.4	473	2.0	168	0.216	94.5
S.E.		0.3	52	0.2	7	0.008	7.6

TABLE 5.

Albumin Catabolism After Treatment and Reinfection.

	Sheep No.	11-18 days after treatment: Serum albumin (g%)	Apparent $t_{\frac{1}{2}}$ (hr)	F(CA)	Faecal activity (ml plasma)	21-28 days after reinfection: Serum albumin (g%)	Apparent $t_{\frac{1}{2}}$ (hr)	F(CA)	Faecal activity (ml plasma)
	56	3.2	660	0.070	21.7	3.1	382	0.100	15.5
Blackface HbA	57	3.0	502	0.043	22.5	2.7	376	0.078	22.0
	91	3.1	500	0.062	22.7	3.1	336	0.094	22.3
Mean		3.1	554	0.058	22.3	3.0	365	0.091	19.9
S.E.		0.1	53	0.008	0.3	0.1	14	0.007	2.2
	80	2.5	460	0.074	26.1	2.6	390	0.082	8.7
Blackface HbAB	82	2.9	448	0.057	20.5	2.5	354	0.106	13.1
	92	3.2	432	0.068	23.6	3.2	345	0.147	37.0
Mean		2.9	447	0.066	23.4	2.8	363	0.112	19.6
S.E.		0.2	8	0.005	1.6	0.2	14	0.019	8.8
	482	2.6	480	0.053	19.7	2.4	350	0.114	21.6
Dorset HbAB	521	2.6	644	0.069	37.2				
	538	2.7	504	0.080	24.5	2.6	364	0.115	25.1
Mean		2.6	543	0.067	27.1	2.5	357	0.115	23.4
S.E.		0.0	51	0.008	5.2	0.1	7	0.001	1.8
	477	2.9	552	0.052	28.3	2.4	324	0.147	42.3
Dorset HbB	547	2.1	480	0.088	35.1	2.4	279	0.200	73.1
	549	2.9	384	0.085	29.4	3.0	231	0.123	31.8
Mean		2.6	472	0.075	30.9	2.6	278	0.157	49.1
S.E.		0.3	49	0.011	2.1	0.2	27	0.023	12.4

about the 17th day onwards. These disturbances which again were most apparent in HbB Dorsets and least in HbA Blackfaces were reflected in lower albumin pools at necropsy.

DISCUSSION

The results from the the first part of this experiment confirm that the response of sheep to primary infections with H. contortus is determined by the breed of the animals concerned. This is shown by the development of more severe clinical and pathophysiological disturbances in Finn Dorset sheep than in animals with the same haemoglobin type belonging to the Scottish Blackface breed. Also evident, but less marked, was that within both breeds substitution of an HbA for an HbB allele resulted in some reduction in the severity of the disease.

Although the number of worms which became established from these infections was not measured, the nature and severity of the disease experienced by individuals within each of the experimental groups were such that they could only be explained by substantial differences in worm burdens. On the basis of abomasal blood loss, which in the author's view provides a reliable index of worm burden during a primary infection it would appear that worm recovery ranged from about 1300 in the HbA Blackfaces to 4300 in the HbB Dorsets with other groups intermediate, i.e. a trend very similar to that recorded in an earlier experiment where animals were infected with the same number and batch of larvae.

Since there was nothing exceptional about any of the details examined during this period of the investigation

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that has not been covered already in the previous discussion, there is little to be gained by reiteration. There are however two points which merit emphasis. Firstly, and in common with the earlier study, the true severity of disease experienced by all animals, but especially those most seriously affected, was masked by compensatory adjustments to red cell and protein synthesis and also to some extent by changes in protein distribution. Secondly, given a field situation, it is unlikely that any of the physiological advantages displayed by the Dorset sheep before infection would have proved anything but a short-term buffer against the greater functional disturbances which they would have undoubtedly encountered as a result of their greater susceptibility to infection.

The effects of anthelmintic treatment were predictable in view of the well-proven efficiency of the drug involved, resulting in a rapid and essentially completed cessation of abomasal bleeding and excessive albumin catabolism, and an equally dramatic disappearance of worm eggs from the faeces of all animals. These changes were accompanied by substantial improvements in haematological and biochemical indices, especially in those sheep formerly most adversely affected; this can be considered a natural consequence of the greater rates of erythropoiesis and albumin synthesis recorded in these animals at the time of treatment.

In retrospect the response to reinfection was perhaps not entirely unexpected but nevertheless was disappointing

both from an immunological and practical standpoint. However, before discussing the implications of these findings and attempting an explanation, a brief synopsis of the features which emerged from the secondary infection and the respects in which they differed from the primary infection is in order.

The first and most obvious point is that as judged by worm numbers, none of the sheep derived any immunity from the earlier infection - indeed if anything they were more susceptible to the second than to the first dose of larvae. Even so they were not equally susceptible and the fact that the spectrum of establishment from the primary infection was mirrored in the response to reinfection was in itself noteworthy. The second point is that although not reflected in a reduced worm establishment, there was no doubt that when compared with the challenge controls, and for that matter the sheep used in the first experiment, these animals were able to exert considerable immunological pressure on the second dose of larvae which was effective inasmuch as it (a) delayed their development and stunted their growth, (b) reduced their reproductive and haematophagic activities and (c) thereby lessened their pathogenic effects. The third point is that on the basis that the renewal of haemorrhage was delayed until about the 12th day after reinfection, it would appear that the immune response was first directed against the 3rd and 4th stage larvae. It should be stressed however, that at necropsy

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only adult (albeit stunted) worms were recovered, a finding which tends to suggest that unlike the strain of larvae used by Dineen and co-workers[19] which becomes arrested at the 4th stage in previously infected Merino sheep, the strain used in these experiments does not possess this propensity.

It is difficult to provide a rational explanation for the failure of these sheep to resist reinfection but three possibilities may be considered. The first is that because of their age (7 months old) the sheep were immunologically immature from the outset and hence subsequently unresponsive to reinfection; the second is that they were immunologically "exhausted", either as a result of prolonged antigenic stimulation, or because of the metabolic strain imposed by the necessity to replace blood constituents lost through haemorrhage; and finally, it is possible that as a result of the anthelmintic treatment and consequential cessation of antigenic stimulation, any immunity engendered by the first infection rapidly waned. In the author's opinion the first two possibilities may be largely eliminated from consideration, since as the results in the following section demonstrate, individual animals of a similar age to those employed in the present study, even when anaemic, are quite capable of resisting reinfection and moreover of removing an existing infection of adult worms when the second infection is superimposed on the first. The implication of this is quite clear, namely in the absence of continuous antigenic

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stimulation, immunity to this parasite as judged by worm numbers, rapidly disappears. This is supported by the recent work of Benitez-Usher[23] showing that the successful immunising schedule based on irradiated larvae as employed by Jarrett and Urquhart and their colleagues[3-5,11] breaks down even in mature animals when the larvae are removed with an anthelmintic. It also confirms a general impression left by the work of several authors that anthelmintics may impair the development and maintenance of immunity to a number of important gastrointestinal nematodes. For example, Roberts and Keith[24] and Ross[25] have demonstrated the failure of calves to develop an immunity to natural or experimental challenge with H. placei when earlier infections are terminated by drug therapy, and essentially similar results have been recorded in respect of O. circumcincta infections of sheep by Thomas and Boag[26] and by Reid and Armour[27].

The relevance of these findings to the field cannot as yet be assessed, but with the present state of knowledge it would seem unwise to follow the extreme recommendation proposed by Dineen and his colleagues[14], i.e. that animals should be treated only when showing clinical signs of haemonchosis. Such a statement assumes (a) that sub-clinical haemonchosis does not impair productivity, and (b) that under natural conditions the animal is not continuously exposed to reinfection. Neither assumption is valid - subclinical infections with H. contortus have

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a profound adverse effect upon growth[28] and clearly, apart from periods of drought grazing animals will be under continuous, and in many areas, heavy challenge. This being so it is unlikely that treated animals will suffer serious interruption of antigenic stimulation and hence impairment of immunity; furthermore as the results of this study demonstrate, even when reduced to the extent of allowing worm re-establishment, immunological control may remain sufficiently functional for several weeks after treatment to seriously impair the biotic and pathogenic potential of the worms.

SUMMARY

The experiment reported in this section was designed to examine and compare the influence of haemoglobin type and breed on the response of sheep to primary and secondary infections with H. contortus.

The results of the primary infections again showed that animals with HbA experienced less severe clinical and pathophysiological disturbances and passed fewer worm eggs in their faeces than similarly infected animals with HbAB and HbB, and that Scottish Blackface sheep were less affected than Finn Dorsets belonging to the same haemoglobin type; as before variations in the severity of disease were such that they could only be explained by substantial differences in resistance to worm establishment.

A similar pattern emerged during the course of the second infections, but when compared with the challenge controls none of the reinfected animals had acquired an immunity as judged by worm numbers. Nevertheless resistance to reinfection was still highest in HbA Blackfaces and lowest in HbB Dorsets, and all reinfected sheep were better able to retard the development and growth and reduce the reproductive and haematophagic activities of their parasite populations than animals experiencing primary infections.

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SECTION 3

STUDIES ON THE SELF-CURE OF *Haemonchus contortus* INFECTIONS AND ITS POSSIBLE RELATIONSHIP TO HAEMOGLOBIN TYPE AND BREED.

INTRODUCTION

The term "self-cure" was first used by Stoll[1] to describe an abrupt fall in the faecal egg counts of two sheep infected with Haemonchus contortus and grazing contaminated pasture, but it was some time later, and largely through the work of Gordon and Stewart in Australia, before the implications of the phenomenon in the epidemiology of haemonchosis were recognised and its aetiology investigated. The observations of Gordon[2,3] on the nature and timing of the reaction under field conditions in New South Wales drew attention to the following points. First, reductions in faecal egg counts, while reflecting partial or complete expulsion of worm burdens did not necessarily confer resistance to reinfection. Second, the reaction occurred simultaneously in almost every sheep in the flock irrespective of age and previous exposure, and in different flocks grazing pasture several miles apart. Third, its occurrence was associated with periods of rainfall sufficient to favour both the development and survival of infective larvae and the fresh growth of pasture.

Gordon's observations raised speculation as to the cause of self-cure , and over the years two basic theories have evolved. Perhaps the best substantiated of these is the "immunological" mechanism proposed by Stewart, i.e. that worm expulsion is a manifestation of an anaphylactic reaction in the abomasal mucosa triggered by allergens released during the third ecdysis of larvae freshly acquired

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by already infected animals. This explanation is favoured by a number of observations, the most compelling being: (a) that the reaction may be produced experimentally in animals maintained indoors when infective H. contortus larvae (or larvae of other abomasal trichostrongyles, e.g. Ostertagia circumcincta and Trichostrongylus axei) are superimposed on an existing adult population[4,5]; and (b) that expulsion is initiated within 7-10 days of reinfection, is preceded by a transient elevation in blood histamine, and accompanied by raised antibody levels and increased peristalsis and oedema of the abomasum[4,6-8]. These findings, especially when considered against the background of Gordon's epidemiological observations have led to widespread acceptance of the "immunological" theory of self-cure and by analogy, the viewpoint that it is the intake of infective larvae made available on pasture by the favourable climatic conditions prevailing after rain which initiates the phenomenon in grazing animals.

While there is no doubt that self-cure may be reproduced by larval challenge, this in itself does not preclude the involvement of other factors under field conditions, and in this context a number of authors have suggested that it is not the intake of infective larvae which initiates the reaction, but rather the consumption of freshly growing grass or an "anthelmintic factor" therein. Until recently, support for this "non-immunological" hypothesis was indirect, being based on observations which although not entirely inconsistent

with the immunological theory, tended to detract from its merits, e.g. the simultaneous occurrence of self-cure in adult sheep and lambs, and the failure of the reaction to confer resistance to subsequent infection[2,9]. Direct evidence for the occurrence of self-cure under conditions precluding re-exposure to infective larvae has been obtained only within the last few years with the demonstration by Allonby and Urquhart[10] that infected sheep folded over paddocks free of Haemonchus larvae expelled their worm burdens at the same time as similar animals grazing adjacent but contaminated pasture. Since worm expulsion followed periods of rainfall and coincided with a marked growth of grass these authors concluded that climatic and pastoral changes per se were sufficient stimuli for the reaction; at present, however, it is not known whether the aetiological factor acts directly on the worms or indirectly by altering their environment in the abomasum.

In an earlier part of this thesis, evidence was presented which indicated that the resistance of sheep to natural and experimental infection with H. contortus was related to haemoglobin type, sheep of Hb type A being more resistant than similar animals belonging to the HbB genotype[11-14]. Early attempts to explain this phenomenon referred to the greater "natural resistance" of the A type animals both to worm establishment and to the effects of such establishment, but the first suggestion of the possible involvement of self-cure was made by Jilek and Bradley[12] following

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investigations of the relationship between haemoglobin type and resistance to H. contortus under field conditions in Bermuda. These authors noted rapid and periodic reductions in faecal egg counts during an 18-month study involving Florida Native and Rambouillet ewes and postulated that the lower counts recorded in the former was a reflection of a greater frequency of HbA types among this breed. The idea that the advantages exhibited by HbA type animals was related to self-cure was later followed up by Allonby and Urquhart[15], who in the course of studies in Kenya, demonstrated that Merino sheep of this genotype self-cured more frequently and more effectively than their HbB counterparts.

From the above account it is clear that while a great deal is now known about the conditions necessary for the induction of self-cure the relative contribution of infective larvae and growing grass in its aetiology remains in sufficient doubt to warrant further investigation under carefully controlled conditions. Equally apparent is that the association between self-cure and the phenomenon of breed and haemoglobin type resistance to Haemonchus has yet to be verified experimentally and its significance compared under the conditions of re-exposure to infective larvae and parasite-free grazing.

The investigations described in this section represent an attempt to unravel some of the complexities of self-cure and confirm its genetic basis. Two experiments were

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performed, each involving animals reared parasite-free and of known age, breed and haemoglobin type. The first was an attempt to induce self-cure indoors by experimental reinfection, and by radioisotopic methods to monitor its effect upon worm activity; in the second, exposure of infected animals to freshly growing grass uncontaminated by Haemonchus larvae was employed to initiate worm expulsion; the design of both experiments was such as to allow comparison of the response observed on the basis of breed and haemoglobin type.

MATERIALS AND METHODS

Experimental animals and design

Two experiments were conducted involving a total of 45 worm-free wethers reared and haemoglobin typed by the methods described earlier. The first, which represented an attempt to reproduce self-cure indoors by experimental reinfection utilised 20 Scottish Blackface and Finn Dorset sheep which were approximately 8 months old. Eight animals of each breed (4 HbA and 4 HbB) were individually infected with 500 H. contortus larvae/kg bodyweight and challenged 28 days later with an inoculum containing 20,000 larvae; the remaining 4 sheep, comprising 2 HbAB Blackface and 2 Finn Dorsets (1 HbA and 1 HbB) received only the second inoculum and all animals were necropsied and their worm burdens determined 23 days following administration of this preparation. Blood analyses were performed twice weekly, faecal egg counts determined daily, and red cell and albumin turnover monitored continuously following injections of ^{51}Cr-labelled erythrocytes and radioiodinated albumin on the 15th day of the experiment; the sheep were confined throughout in metabolism cages, fed hay and water ad lib, and dosed orally each day with 10 ml 0.75% KI.

The object of the second experiment was to ascertain whether freshly growing grass, uncontaminated with Haemonchus larvae could elicit self-cure. For this purpose, 25 parasite-free wethers were used, consisting of 9 Scottish Blackface (2 HbA and 7 HbAB), 9 Finn Dorsets (4 HbA and 5 HbB) and

7 Suffolks (all HbB); apart from the Suffolks which were 2 years old, all the sheep were aged 8-10 months. With the exception of two Suffolks which were to act as "tracers" each sheep was infected with 7,000 H. contortus larvae; all animals were maintained indoors on a diet of hay and water during the following 5 weeks, at which point the "tracers" together with 19 of the infected sheep were introduced into the first of 6 fenced paddocks containing freshly growing and parasite-free pasture; the remaining 4 infected sheep (i.e. 2 HbAB Blackface, 1 HbB Dorset and 1 HbB Suffolk) were kept indoors. The sheep at grass were moved to a fresh paddock every 5 days, thus obviating the risk of reinfection. Blood samples for analyses and faeces for egg counting were collected every 3-4 days throughout and worm burdens determined at necropsy 32 days after introduction to grass (i.e. 67 days after infection).

Experimental paddocks

One hectare of permanent grass ley (situated in the Warren Park of Garscube Estate, Bearsden, Glasgow) which had not been grazed during the previous year, was dressed with nitrogenous fertiliser around mid-April 1974 and divided by wire fencing into 6 equal plots; sheep were introduced on to the first of these paddocks on the 4th June by which time fresh grass was readily available.

Blood analyses

The following blood and serum analyses were performed by the methods outlined earlier:- PCV, Hb, RBC, MCV, MCHC, total serum protein , albumin, globulin and iron.

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Parasitological techniques

The methods used for culturing of larvae and determination of faecal egg counts and worm burdens were described earlier.

Radioisotopic procedures

Labelled red cells and albumin (containing respectively about 700 μCi ^{51}Cr and 300 μCi ^{125}I) were prepared and injected by the methods outlined earlier. Blood, plasma, urine and faeces were collected at regular 24-hour intervals thereafter, and samples of each prepared for radioassay as previously described. Radioactivity measurements were made using an automatic gamma-scintillation spectrometer, corrections for decay and separation of isotopic mixtures being based on the activities of standard solutions prepared from the labelled materials.

Red cell and albumin turnover calculations

Red cell survival was assessed and gastrointestinal red cell and iron losses monitored by analysis of blood and faeces for ^{51}Cr. The catabolic rate of albumin was assessed from the shape of the exponential part of the plasma ^{125}I activity curve and also by calculating the fractional catabolic rate (F(CA). A full account of the procedures adopted for measuring these parameters was given earlier.

RESULTS

EXPERIMENT 1

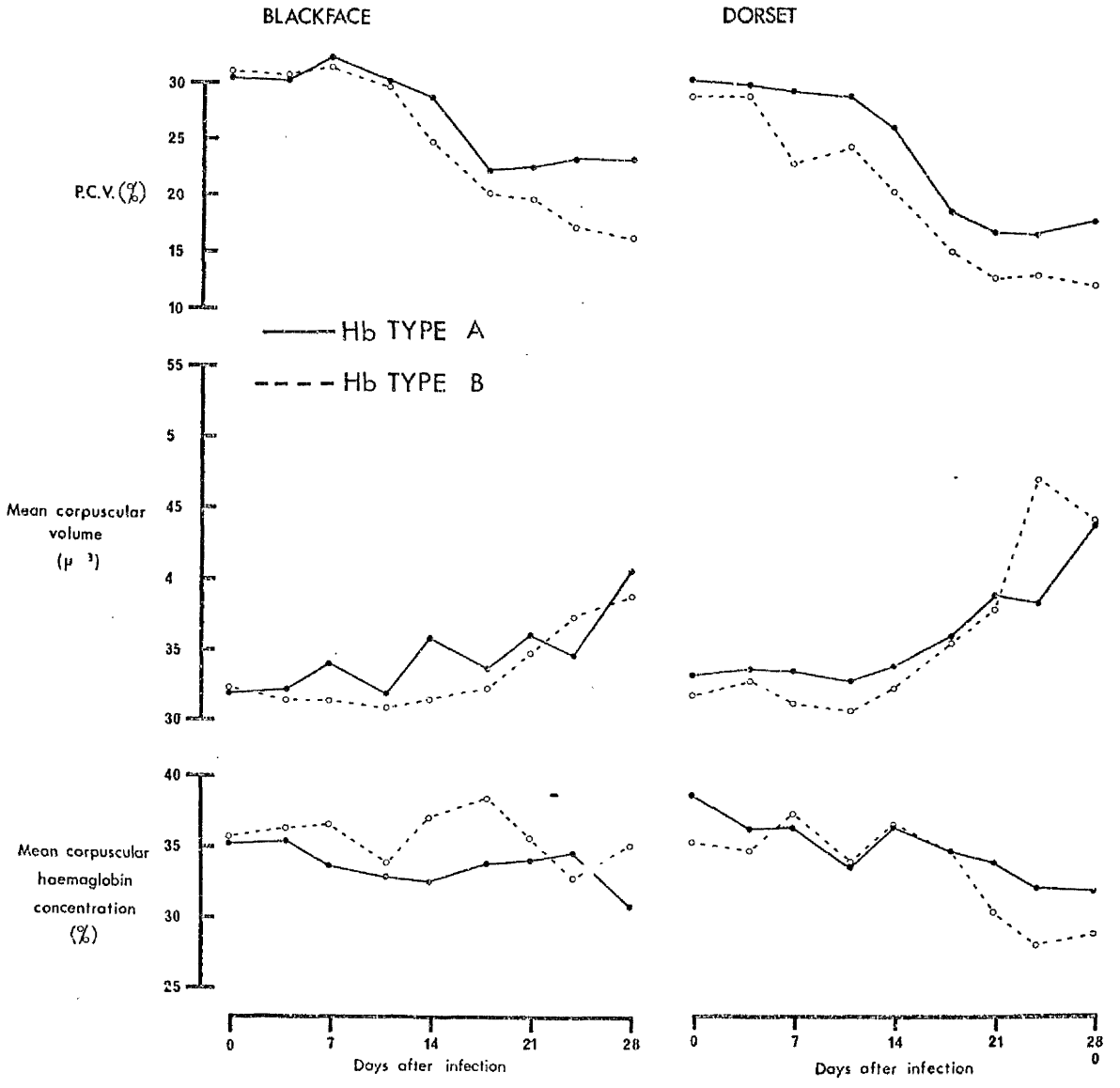
Scottish Blackface and Finn Dorset wethers of known haemoglobin type were each infected with 500 H. contortus larvae/kg bodyweight and along with worm-free controls challenged 4 weeks later with 20,000 larvae. The course of each infection was monitored by haematological, biochemical and radioisotopic methods; faecal worm egg counts were measured continuously and worm burdens determined at necropsy 23 days after administration of the second dose of larvae. To facilitate the presentation of results, responses to the first and second infections are described separately on the basis of the average changes recorded, but some individual data are included to illustrate features of particular interest; the remainder are given in Appendix 3.

Primary infections

Haematological and biochemical observations

The information given in Figure 1 illustrates the haematological changes which accompanied the first infection. Values for each parameter were initially very similar in all animals but the anaemia which developed progressively from about the 7th day thereafter was clearly more severe in Dorset than in Blackface sheep of comparable haemoglobin type and within each breed in animals with HbB. This is particularly well demonstrated by the PCV values which fell by 60% and 40% respectively in the HbB and HbA Dorsets ($p < 0.05$) and by 48% and 23% in the comparable Blackfaces ($p < 0.001$) over the 28 days following infection; differences between groups of the

Figure 1: Haematological Values Following Infection with 500 H. contortus Larvae/kg Bodyweight.



same haemoglobin type were also statistically significant in favour of the Blackface sheep at the time of challenge ($p < 0.05$).

During the early stages of infection the trends observed with regard to red cell counts and haemoglobin concentrations closely paralleled those described for PCV, but from about the 18th day onward macrocytosis and hypochromia became progressively more apparent, particularly in the Dorset sheep and in animals with HbB.

Serum iron concentrations were well maintained at around 130-170 µg% in all animals during the first week of infection, but thereafter deteriorated progressively to values ranging from 90-125 µg%. Although no significant breed or haemoglobin type differences were recorded, the fact that HbB and HbA Dorsets suffered reductions in the order of 43% and 36% and their respective Blackface counterparts 19% and 14%, suggests a clear breed and possible haemoglobin type interaction.

The serum protein changes recorded during the study (Figure 2) show that all animals experienced progressive reductions in total protein and albumin concentrations from about the first week after infection closely in line with those described for PCV, i.e. HbB Dorsets became the most, and HbA Blackfaces the least hypoproteinaemic and hypo-albuminaemic. However, the only statistically significant differences recorded before challenge were the higher albumin levels of Blackface over Dorset sheep belonging to the same haemoglobin type ($p < 0.05$-0.001). Serum globulins changed

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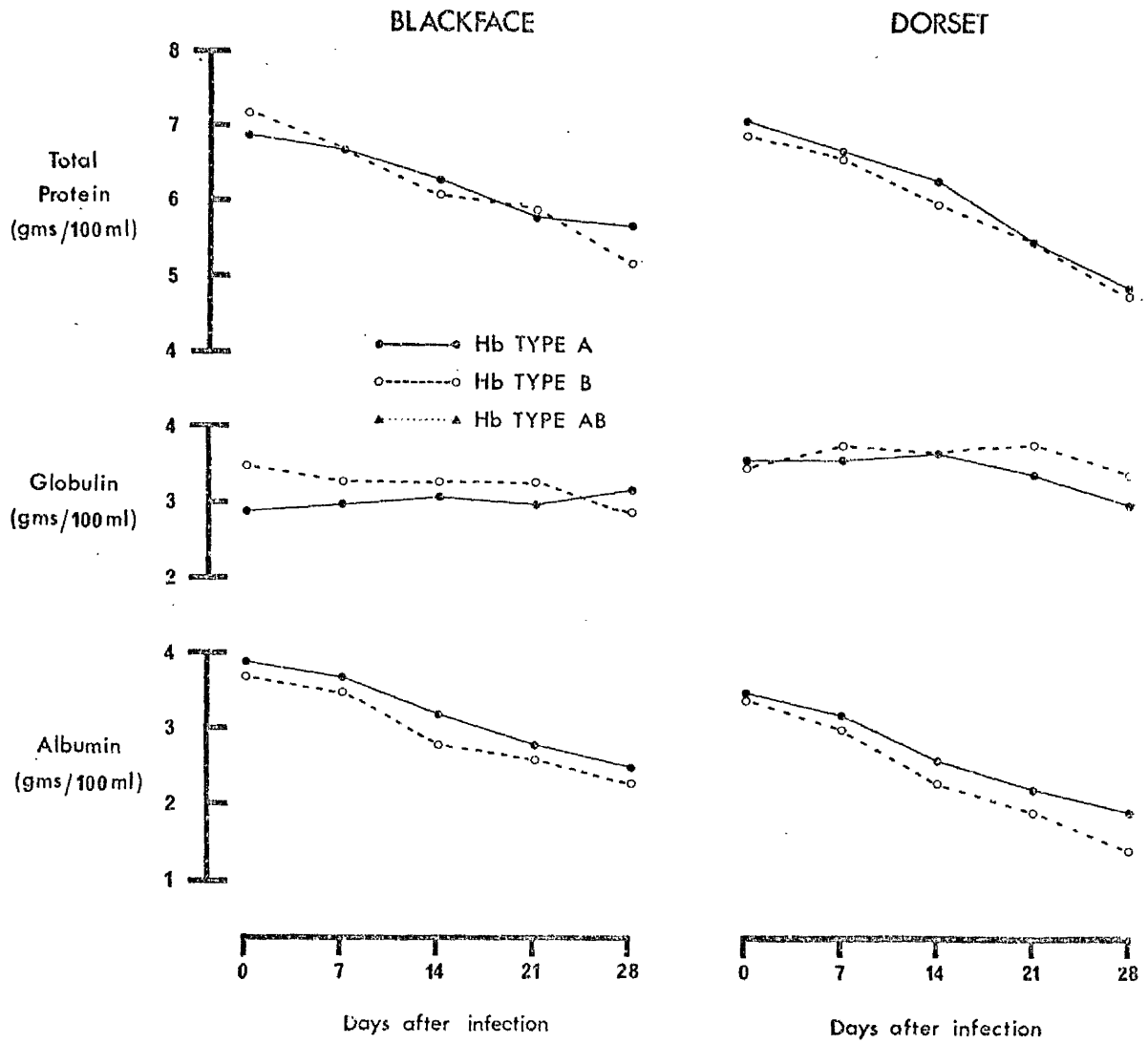
Figure 2: Serum Protein Concentrations Following Infection with 500 H. contortus Larvae/kg Bodyweight.



little with the result that albumin:globulin ratios declined in parallel with the development of hypo-albuminaemia.

Changes in the haematological and biochemical values of the worm-free controls were minimal; apart from slight reductions in PCV, red cell counts and haemoglobin which in all likelihood were caused by blood sampling, these indices remained within the normal range for sheep.

Production of HbC

Before infection haemoglobin C was present in small amounts (1-3%) in the blood of both HbA groups, but formed a progressively increasing proportion of the total haemoglobin during the subsequent 3-4 weeks. At the time of reinfection the average HbC percentage in Blackface sheep was 11% compared with 26% in the Dorsets, representing total concentrations of 0.8 g and 1.5 g% respectively; both differences were highly significant in favour of the Dorset sheep ($p < 0.01$). These changes were accompanied by corresponding reductions in both the proportion and concentration of HbA and in common with previous experiments the extent of A-C switching was closely related to the severity of the anaemia.

Parasitological data

The daily faecal worm egg outputs of the sheep are given in Tables 16 and 17 of the Appendix. Eggs first appeared in the faeces of two Dorsets (1 HbB and 1 HbA) 17 days after infection and in all animals of this breed by day 19. By this time all the HbB but only one of the HbA Blackfaces were positive, the remainder of the

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latter first passing eggs on day 20. Following the onset of oviposition the egg output of each sheep increased steadily reaching a maximum just before reinfection. At this stage the average egg outputs of the HbB and HbA sheep of each breed were similar, but significantly higher in Dorsets (mean 40 million/day) than in Blackfaces (mean 18 million/day) of comparable haemoglobin type ($p < 0.02$-0.001); throughout the study the faeces of the worm-free controls remained negative.

Red cell and albumin turnover

The results of the red cell and albumin turnover measurements made between the 3rd and 4th weeks after larval administration are summarised in Table 1. Four points merit emphasis with regard to these figures. Firstly, taking the values for the worm-free sheep as the baseline for each index, it is clear that all infected sheep experienced considerable losses of blood and its constituent red cells and albumin into the gut and that this haemorrhage was accompanied by dramatic reductions in the circulating half-lives of both constituents and also by an increased catabolic rate for albumin. Secondly, despite considerable individual variation the extent of these changes was both breed and haemoglobin type related, with Dorsets generally suffering more severe disturbances than Blackfaces of the same haemoglobin type, and within both breeds, sheep with HbB being more adversely affected than those with HbA. Although small group sizes and

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TABLE 1.

Red Cell and Albumin Turnover 3-4 Weeks After Infection.

	Sheep No.	PCV Day 21	Apparent red cell half-life (hr)	Faecal clearance (ml/day) Blood	Red cells	PCV Day 28	Serum albumin Day 21 (g%)	Apparent half-life (hr)	F(CA)	Faecal ^{125}I plasma clearance (ml/day)	Serum albumin Day 28 (g%)
Blackface HbA	51	20	208	105	26	23	2.8	318	0.145	46	2.5
	86	21	167	134	29	22	2.8	342	0.130	37	2.5
	95	22	186	126	28	21	2.6	213	0.216	39	2.2
	97	25	378	27	6	26	3.0	408	0.086	20	2.8
Mean		22.0	235	98	22	23.0	2.8	320	0.144	36	2.5
S.E.		1.1	49	24	6	1.0	0.1	41	0.027	6	0.1
Blackface HbB	43	21	308	92	18	17	2.2	367	0.131	23	2.6
	45	16	138	165	25	13	2.5	318	0.161	38	2.3
	85	20	144	178	33	16	3.4	327	0.168	33	2.9
	90	20	310	95	19	18	3.2	354	0.146	56	2.3
Mean		19.3	225	133	24	16.0	2.8	342	0.152	38	2.5
S.E.		1.1	49	23	4	1.0	0.3	11	0.008	7	0.1
Dorset HbA	57	15	67	384	52	16	2.1	186	0.258	53	1.6
	58	22	126	153	38	23	2.5	342	0.095	39	2.5
	59	14	80	275	43	14	2.0	231	0.206	71	1.8
	62	17	58	376	62	17	2.0	300	0.188	67	1.7
Mean		17.0	83	297	49	17.5	2.2	265	0.187	58	1.9
S.E.		1.8	15	54	5	1.9	0.1	35	0.034	7	0.2
Dorset HbB	3	12	54	428	56	12	1.9	153	0.298	165	1.3
	40	14	93	213	31	14	1.8	181	0.213	67	1.4
	54	10	48	483	53	9	1.8	168	0.278	105	1.4
	61	14	86	258	37	12	2.0	203	0.210	87	1.6
Mean		12.5	70	346	44	11.8	1.9	176	0.250	106	1.4
S.E.		1.0	11	65	6	1.0	0.1	11	0.023	21	0.1
Controls											
Mean		31.0	415	2.5	0.8	28.0	3.4	524	0.066	11	3.3
S.E.		1.0	14	0.3	0.1	0.4	0.1	29	0.010	2	0.2

variation precluded significance with respect to intra-breed haemoglobin type differences, inter-breed differences in most parameters were highly significant in favour of the Blackfaces. Animals of this breed, HbA and HbB types alike, lost significantly less blood and red cells than the corresponding Dorsets ($p < 0.02$ and $p < 0.05$ respectively) and as a result their red cell and albumin half-lives were higher ($p < 0.02$ and $p < 0.05$; and $p < 0.001$ and $p < 0.05$ respectively) and rates of albumin catabolism lower ($p < 0.01$ and $p < 0.05$ respectively). Finally, although the degree of anaemia and hypoalbuminaemia exhibited by any individual were to some extent determined by the severity of bleeding and rate of albumin catabolism the correlation was at best extremely tenuous, presumably because the animals were synthesising red cells and albumin at rates far in excess of normal. Even in the absence of direct measurements, the importance of increased synthesis of these constituents can be readily appreciated when the minor changes in PCV and serum albumin levels are viewed against the greatly accelerated rates of loss or degradation in the gut. Considering the magnitude of these disturbances it is remarkable that so many sheep, and particularly the Dorsets were able to prevent the development of much more severe anaemia and hypoalbuminaemia than actually recorded.

Secondary infections

It is well established from field and experimental work in Australia[16] that the response of infected sheep to a

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second dose of H. contortus larvae is far from uniform, but usually falls into four basic categories, i.e. (a) loss of the existing worm population with little or no establishment of the new infection - "self-cure and protection"; (b) loss of the existing infection with subsequent establishment of the new population - "classical self-cure"; (c) maintenance of the existing infection but no establishment of subsequent infections and (d) temporary suppression of egg production followed by hyperinfection. For convenience the responses exhibited by the sheep used in this experiment are classified according to these patterns.

Self-cure and protection

This reaction, which is illustrated in Figure 3 and Table 2 was observed in two of the HbB and one of the HbA Blackfaces (Nos. 43, 45 and 51) and also in one HbA Dorset (No. 58). In these animals administration of the second dose of larvae was followed by a sharp decline in egg output closely paralleled by an equally striking reduction in abomasal haemorrhage. In all cases the most dramatic changes in both indices occurred between about the 4th and 10th days after challenge during which time average daily egg outputs fell from 21 to 3 million and erythrocyte losses from 20 to 5.6 ml/day. Further features of the reaction included a gradual decline in HbC and elevation in HbA, a reduction in the fractional catabolic rate of albumin, progressive improvements in PCV and serum albumin and iron levels, and perhaps significantly, the persistence of slight macrocytosis.

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Figure 3: Effect of Reinfection on PCV, Faecal Egg Output and Gastrointestinal Haemorrhage of a Sheep Previously Infected with 500 H. contortus Larvae/ kg Bodyweight.

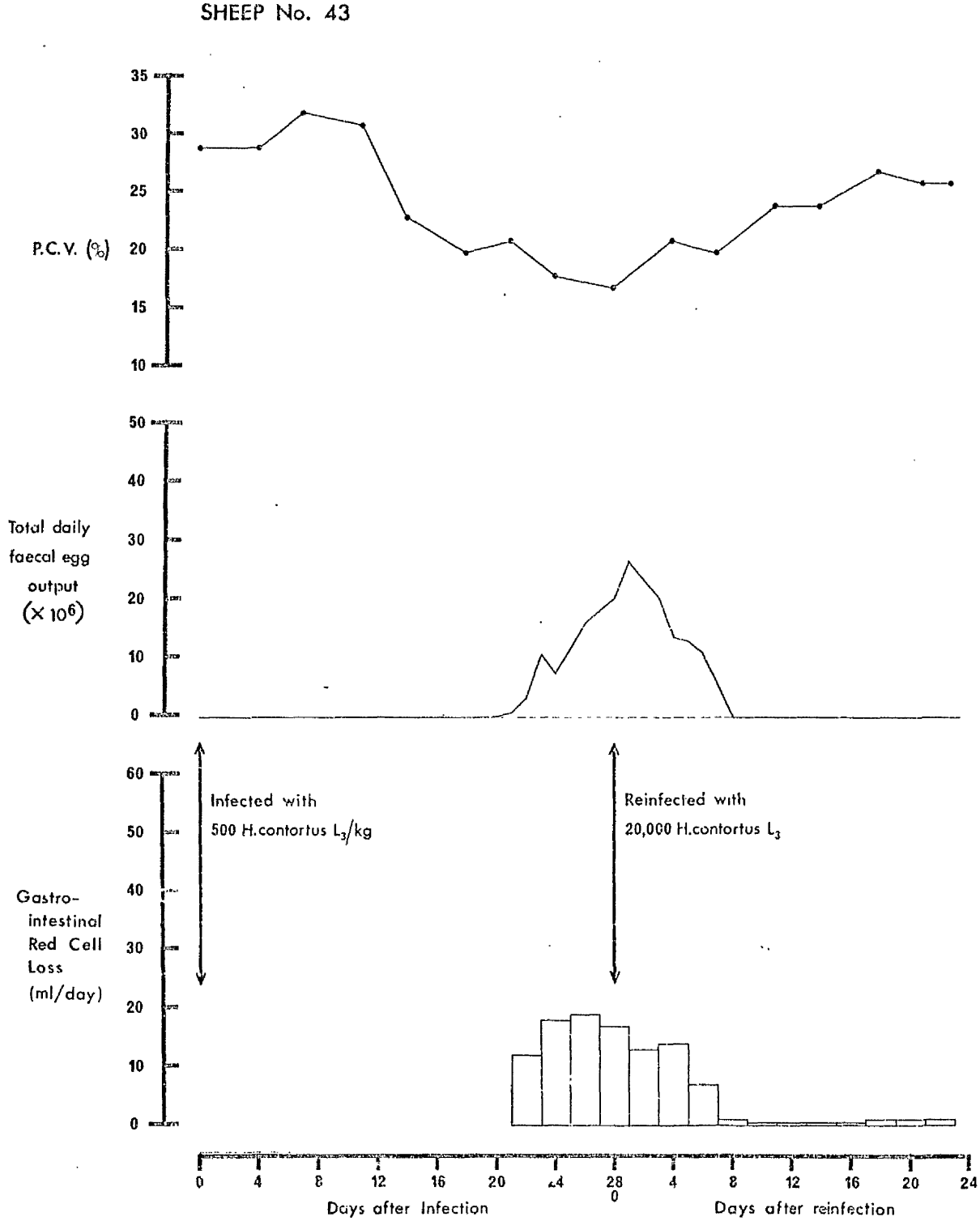

TABLE 2.

Self-Cure and Protection

Indices of Infection at Challenge(1) and Necropsy(2)

Sheep No.	43		45		51		58	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
PCV (%)	17	26	13	28	23	32	23	33
MCV (μ^3)	39	39	37	38	41	41	39	40
MCHC (%)	36	34	35	34	31	32	33	37
Serum iron (µg%)	91	132	100	134	105	120	102	124
Red cell clearance (ml)	16	2	17	1	30	2	32	1
F(CA) (%)	19	6	15	5	14	5	10	4
Serum albumin (g%)	2.6	2.8	2.3	2.7	2.5	2.9	2.5	3.4
Faecal egg output ($x10^6$)	21	0	16	0	26	0	29	0.1

These findings are all consistent with elimination of the majority of the existing worms and challenge larvae and this was confirmed at necropsy by the presence of only 163 ± 94 (range 0-350) H. contortus.

Classical self-cure

In three HbA (Nos. 86, 95 and 97) and one HbB (No. 85) Blackface sheep, the challenge larvae induced temporary reductions in egg output (from 21 to 4 million/day), abomasal haemorrhage (from 21 to 10 ml/day) and rates of albumin degradation (from 14.8 to 8.5%/day), indicative of expulsion of the majority of the established worm burdens (Figure 4 and Table 3). As before these events occurred more or less simultaneously and generally between the 4th and 10th days after the new larval intake, but unlike the group described above, the red cell losses and acceleration of albumin catabolism suffered by these sheep increased progressively from about day 13, reaching levels by necropsy which on average were similar to those recorded at the time of challenge; egg output however remained depressed throughout the entire reinfection period. Haematological and biochemical changes generally reflected the severity of abomasal bleeding. The transient decline in blood loss following challenge was accompanied by equally short-lived improvements in PCV and serum albumin levels (from 22 to 25% and from 2.4 to 2.7 g% respectively) and with the subsequent resumption of haemorrhage both indices either remained static or deteriorated progressively. These changes were accompanied by further elevations in HbC levels

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Figure 4: Effect of Reinfection on PCV, Faecal Egg Output and Gastrointestinal Haemorrhage of a Sheep Previously Infected with 500 H. contortus Larvae/kg Bodyweight.

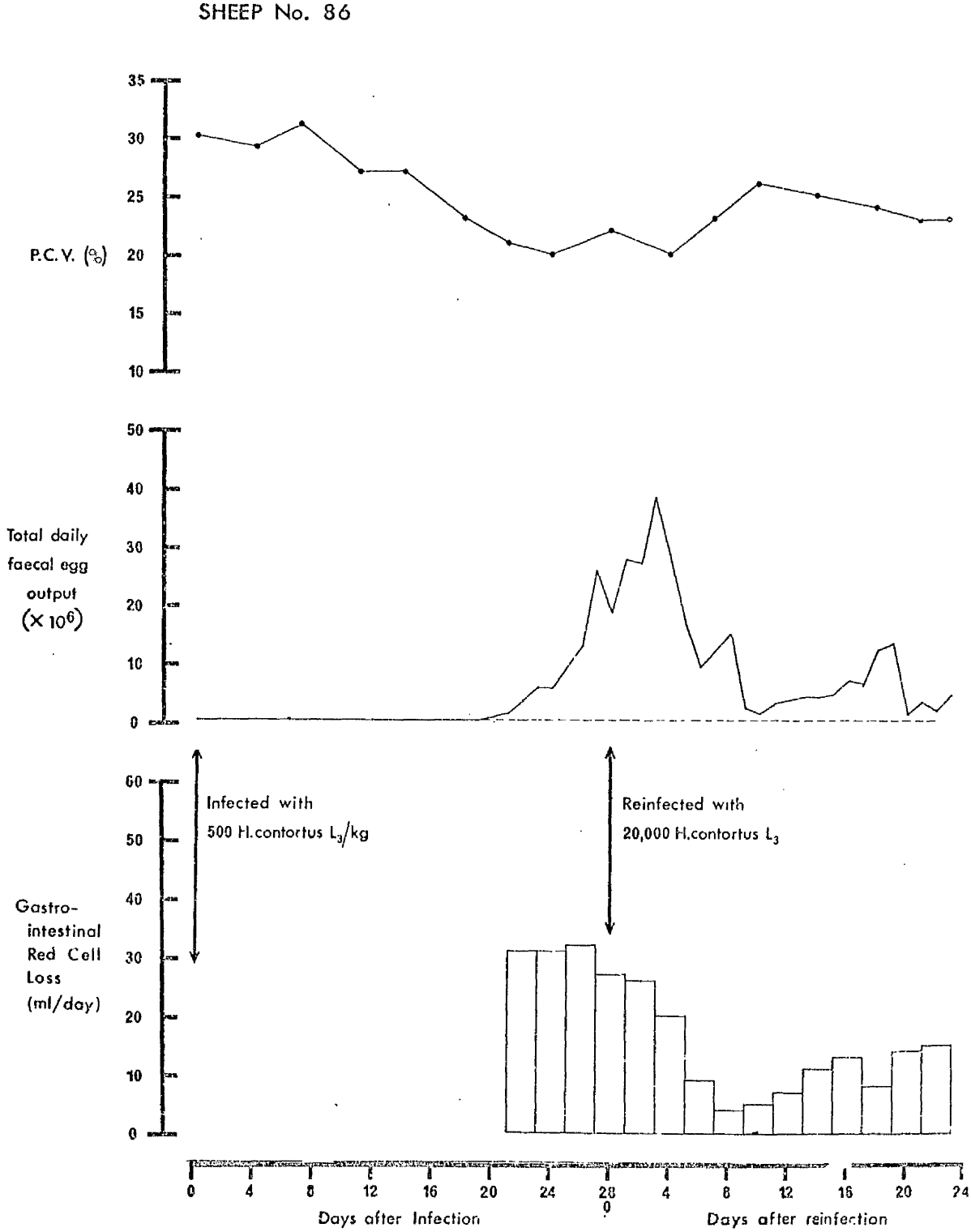

TABLE 3.

Classical Self-Cure

Indices of Infection at Challenge[1] and Necropsy[2]

Sheep No.	85		86		95		97	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
PCV (%)	16	16	22	23	21	18	26	28
MCV (μ^3)	44	44	45	41	40	41	37	41
MCHC (%)	33	33	29	33	29	30	34	31
Serum iron (µg%)	319	73	165	114	118	89	129	138
Red cell clearance (ml)	26	22	27	20	26	31	6	9
F(CA) (%)	19	14	13	17	24	26	8	7
Serum albumin (g%)	2.1	2.3	2.5	2.3	2.2	2.0	2.8	2.6
Faecal egg output ($x10^6$)	18	4	13	5	24	2	8	2

and corresponding reductions in HbA. Macrocytosis persisted throughout, was more pronounced than in the self-cure and protection group, and was associated with reduced serum iron levels. At necropsy each of these animals was harbouring between 2,300 and 5,500 worms (mean 4200).

No loss of the existing or establishment of the new infection

Two HbB type sheep fell into this category, i.e. one Dorset (No. 61) and one Blackface (No. 90). Apart from some slight reductions in red cell loss, egg output and albumin degradation between the 4th and 10th days after reinfection which resulted in temporary and minor improvements in PCV, serum albumin and iron concentrations, parasite activity remained virtually unaltered by the second dose of larvae (Figure 5), suggesting little or no alteration in worm burden. However, as a result of the continuous blood loss PCV, serum albumin and iron levels declined during the latter stages of the investigation and macrocytosis became progressively more dominant (Table 4). At necropsy 6,150 and 2,750 adult worms were recovered.

Temporary suppression of egg production followed by hyperinfection

This was the response exhibited by the vast majority of the Dorset sheep, (see Figure 6 and Table 5) being observed in three HbA (Nos. 57, 59 and 62) and three HbB types (Nos. 3, 40 and 54). During the week preceding challenge the faecal egg output, red cell "clearance" and fractional catabolic rate of albumin averaged 33 million, 49 ml and 23%/day respectively. For a period of 5-15 days after challenge these figures either remained static or declined marginally, but subsequently again rose to

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Figure 5: Effect of Reinfection on PCV, Faecal Egg Output and Gastrointestinal Haemorrhage of a Sheep Previously Infected with 500 H. contortus Larvae/kg Bodyweight.

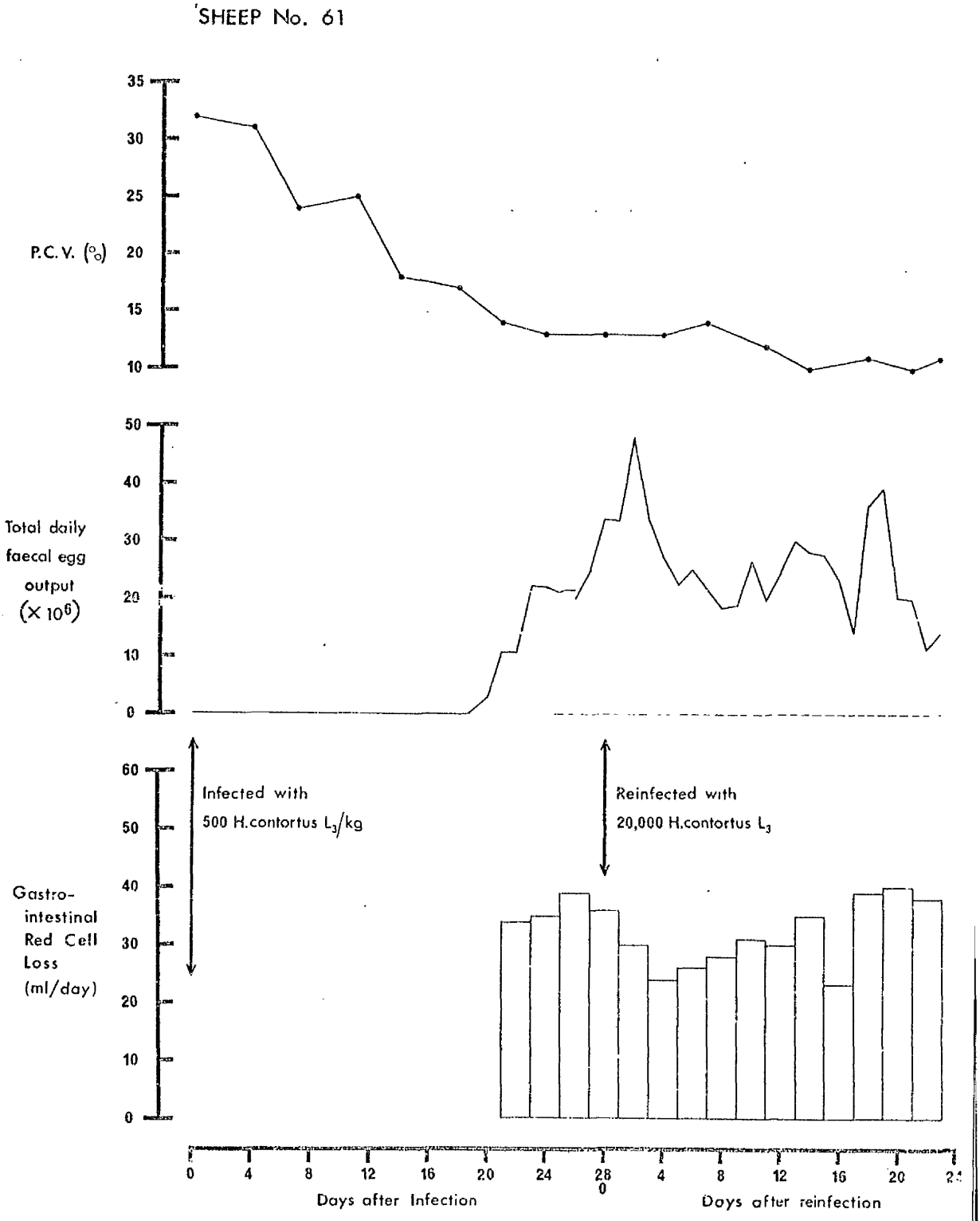


TABLE 4.

No Loss of Existing or Establishment of the New Infection

Indices of Infection at Challenge[1] and Necropsy[2]

Sheep No.	61		90	
	(1)	(2)	(1)	(2)
PCV(%)	13	10	18	14
MCV (μ^3)	45	47	36	43
MCHC (%)	27	30	36	32
Serum iron (µg%)	105	58	110	97
Red cell clearance (ml)	36	35	21	19
F(CA) (%)	28	24	16	19
Serum albumin (g%)	1.6	1.2	2.3	2.0
Faecal egg output ($x10^6$)	34	14	14	2

Figure 6: Effect of Reinfection on PCV, Faecal Egg Output and Gastrointestinal Haemorrhage of a Sheep Previously Infected with 500 H. contortus Larvae/ kg Bodyweight.

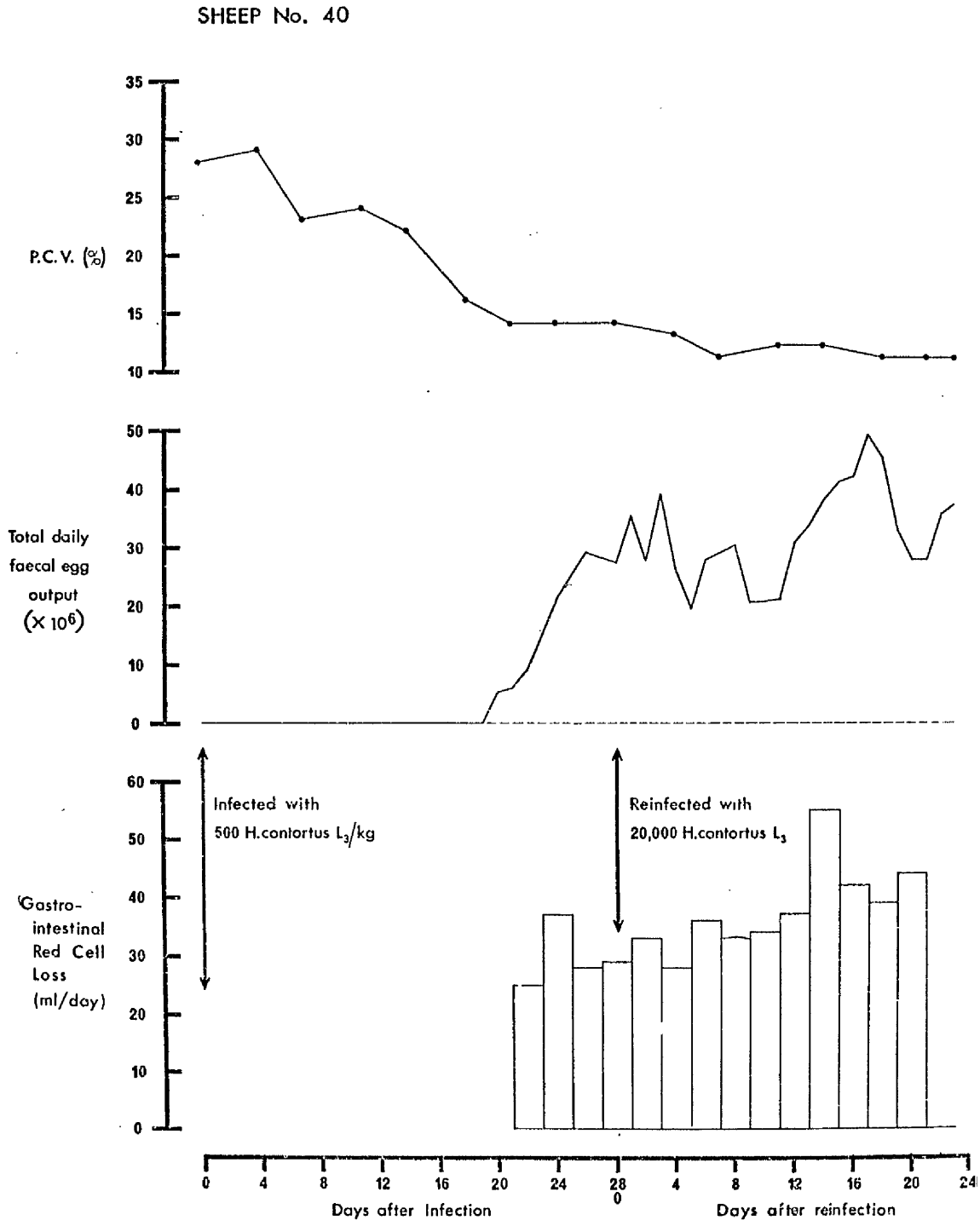


TABLE 5.

Hyperinfection

Indices of Infection at Challenge[1] and Necropsy[2]

Sheep No.	3		40		54		57		59		62	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
PCV (%)	12	8	14	11	9	7	16	13	14	11	17	9
MCV (μ^3)	48	58	39	44	44	55	45	57	46	63	44	47
MCHC (%)	25	20	31	21	33	28	31	29	35	29	29	23
Serum iron (µg%)	70	23	91	59	88	49	132	118	110	79	88	26
Red cell clearance (ml)	49	42	29	39	50	43	54	48	46	63	59	53
F(CA) (%)	44	46	36	27	48	51	25	34	32	35	26	33
Serum albumin (g%)	1.3	0.4	1.4	1.0	1.4	0.5	1.6	1.2	1.8	1.4	1.7	1.2
Faecal egg output ($x10^6$)	60	35	27	37	42	29	45	47	61	47	56	39

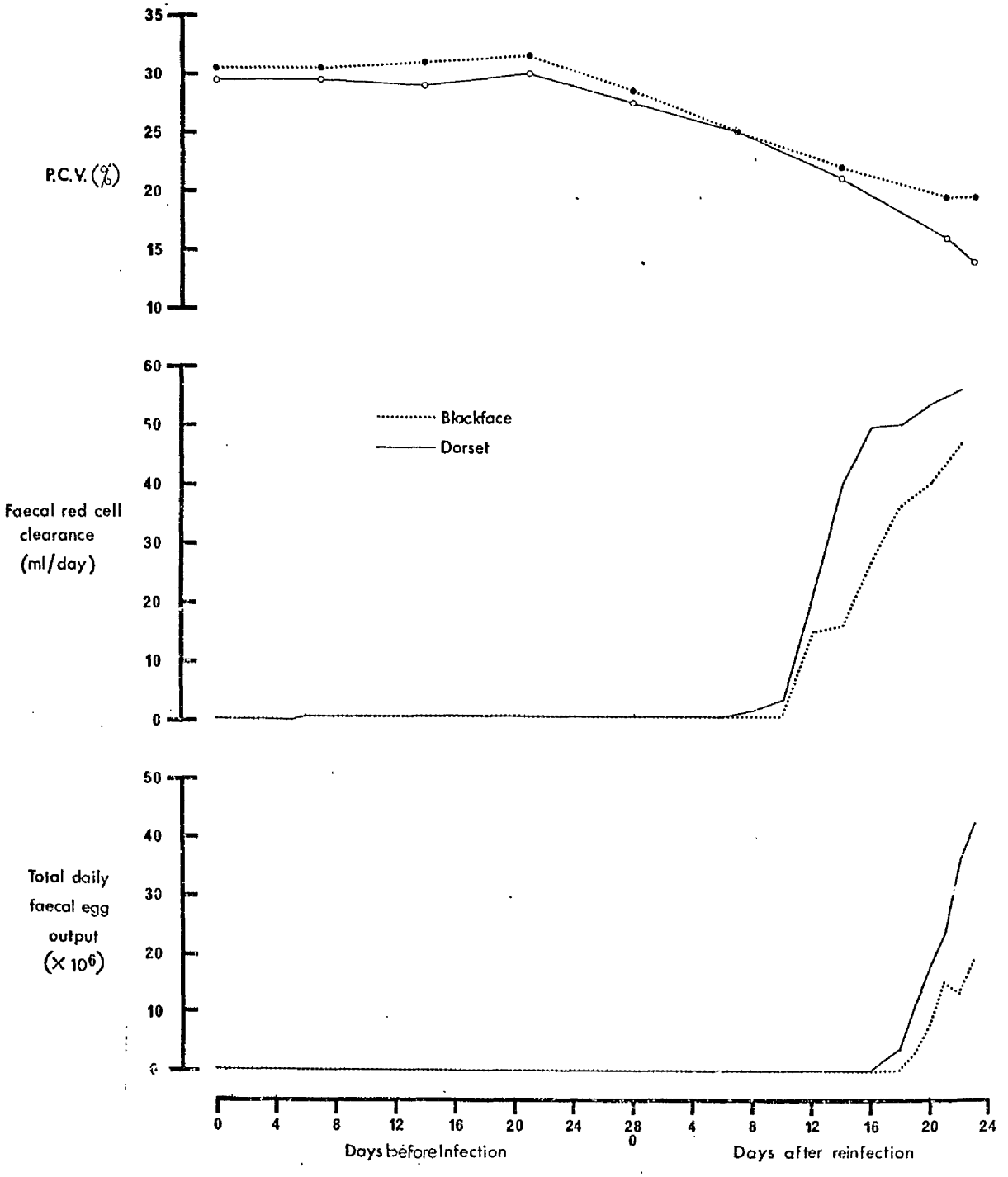
levels which with the notable exception of egg output were higher than those recorded prior to challenge, i.e. 30 million, 52 ml and 38%/day respectively. It should perhaps be stressed at this point, that because of the development of much more severe anaemia during the latter stages of the experiment, the loss of 52 ml of red cells represents a degree of haemorrhage considerably in excess of that recorded before reinfection. Further features of this reaction included the development of severe hypoalbuminaemia, hypoferraemia, macrocytosis, and in some instances, hypochromia. Haemoglobin C appeared in dramatically increased amounts, and by the termination of the experiment formed 55%-95% of the total haemoglobin.

Three animals (two HbB, Nos. 40 and 54); and one HbA, No. 62) had to be necropsied before the termination of the experiment; as the condition of these sheep deteriorated there was a marked fall in faecal egg output and some reduction in the severity of haemorrhage, consistent with elimination of a proportion of the existing worm populations. In these sheep worm recovery (8,750-18,900) may therefore be an underestimate of establishment although the remaining animals in the group had comparable burdens, i.e. 12,650-13,850.

Response of the worm-free sheep to infection with 20,000 H.contortus larvae

The salient features of the effects of 20,000 larvae on the worm-free controls are illustrated in Figure 7; further details are given in the Appendix.

Figure 7: PCV, Gastrointestinal Haemorrhage and Egg Output of Challenge Controls Infected with 20,000 H. contortus La rvae.



Infection of these animals resulted in a progressive anaemia from about day 11, characterised terminally by macrocytosis and hypochromia. Hypoproteinaemia, hypoalbuminaemia and depressed serum iron levels were additional features and as in previous experiments the majority of these disturbances were more pronounced in the Dorsets and particularly in the one HbB animal available. Excessive haemorrhage into the gut commenced in all sheep between the 8th and 10th days increasing rapidly in severity over the following 12 days to reach a peak around necropsy. At this stage Dorsets were losing more red cells than Blackfaces and HbAB types of each breed more than their HbA counterparts.

Eggs first appeared in small numbers on the 17th or 18th day after infection and in progressively increasing numbers during the remainder of the investigation. At necropsy egg output was higher in Dorset than in Blackface sheep of the same haemoglobin type and within each breed in animals with HbAB. The number of worms recovered (mean 6313 ± 1420) reflected the pathophysiological and egg output data, being higher in Dorsets and in sheep with HbAB.

Inter-relationships between blood loss, egg output and worm load

Measurement of worm establishment from each dose of larvae was precluded by the necessity to superimpose the challenge on the primary infections and hence any conclusions regarding the above relationships during the course of the latter can only be based on a speculative assessment of worm burden. Since egg production per unit of blood consumed

compared favourably with that recorded in previous experiments, i.e. about 100,000/ml between the 3rd and 4th weeks (Table 6), it can reasonably be assumed that before challenge the metabolic activities of the individual worms were also comparable and that each removed about 0.06 ml blood/day. On this basis it may be tentatively concluded that about 10% and 16% respectively of the larvae given to the HbA and HbB Blackfaces became established (i.e. 1,600 and 2,600 worms) compared with about 5,000 and 5,600 worms (23% and 30%) in the comparable Dorsets; these figures yield an average daily egg output of 10,000-15,000 per female which again is similar to previous estimates from primary infections.

The same indices calculated during reinfection (Table 7) and based on worm recovery at necropsy suggest a serious impairment in the haematophagic and reproductive activities of the worms present in sheep which exhibited self-cure, i.e. the majority of the Blackfaces. In these animals, blood loss per worm and egg output per female averaged only 0.02 ml/day and 1,600 eggs/day respectively compared with about 0.05 ml/day and 7,000 eggs/day in the challenge controls, while the number of eggs produced per ml blood (44,000/day) was also considerably lower than that recorded for the controls (70,000/day). Even allowing for the fact that the control figures are somewhat lower than expected, being based on the measurements made around the 3rd week it is clear that self-cure, in addition to causing expulsion of adult worms and developing larvae, causes considerable functional embarrassment to the surviving parasites.

Inter-relationship between Blood Loss, Egg Output and Worm Load during the Secondary Infection.

	Sheep No.	Worms Recovered	% Recovery	Average daily Egg Output ($x10^6$)	Average daily Blood Loss (ml)	Average eggs/ml Blood ($x10^3$)	Average eggs/female worm ($x10^3$)	Average Blood Loss/ worm (ml)
Blackface HbA	51	N	N	N	6	N	N	-
	86	4050	10.5	3.4	62	55	1.7	0.015
	95	5500	15.4	2.6	172	15	0.9	0.031
	97	2300	6.3	1.7	31	55	1.5	0.013
Mean		2963	8.1	1.9	68	31	1.0	0.015
S.E.		1185	3.3	0.7	37	14	0.4	0.006
Blackface HbB	43	350	3.5	N	5	N	N	0.014
	45	N	N	N	21	N	N	-
	85	4950	13.3	5.0	115	43	2.1	0.023
	90	2750	7.2	3.0	110	27	2.2	0.040
Mean		2013	6.0	2.0	63	18	1.1	0.019
S.E.		1154	2.8	1.2	29	11	0.6	0.008
Dorset HbA	57	12650	32.0	32.5	481	68	5.1	0.038
	58	300	0.7	0.3	6	50	2.0	0.020
	59	13050	31.2	48.0	560	86	7.4	0.043
	62	11650	26.6	30.2	574	53	5.2	0.049
Mean		9413	22.6	27.8	405	64	4.9	0.038
S.E.		3052	7.4	10.0	135	8	1.1	0.006
Dorset HbB	3	13850	33.1	32.3	636	51	4.7	0.046
	40	8750	25.8	23.3	320	73	5.3	0.037
	54	18900	46.7	28.4	680	42	3.0	0.036
	61	6150	17.2	15.1	365	41	4.9	0.059
Mean		11912	30.7	24.8	500	51	4.5	0.045
S.E.		2825	6.3	3.7	92	7	0.5	0.005
Blackface HbA	1	4050	20.3	14.0	210	67	6.9	0.052
Blackface HbB	65	4650	23.3	17.5	251	70	7.5	0.054
Dorset HbA	52	6200	31.0	23.2	289	80	7.5	0.047
Dorset HbB	56	10350	51.8	44.2	514	86	8.5	0.050
Mean		6313	31.6	24.7	316	75		0.051
S.E.		1420	7.1	6.8	68	4		0.002

TABLE 6.

Inter-relationship between Blood Loss Egg Output and Worm Load during the Primary Infection.

	Sheep No.	Larval Dose	Estimated Worm Recovery	Estimated % Recovery	Average daily Egg Output ($x10^6$)	Average daily Blood Loss (ml)	Average eggs/ml Blood ($x10^3$)	Average eggs/female worm ($x10^3$)
Blackface HbA	51	16360	1750	10.7	16.0	119	134.5	18.3
	86	18520	2233	12.1	10.3	134	76.9	9.2
	95	15680	2100	13.4	15.2	126	120.6	14.5
	97	16100	450	2.8	4.0	27	148.2	17.7
Mean		16665	1633	9.8	11.4	102	120.1	14.9
S.E.		634	407	2.4	2.8	25	15.5	2.1
Blackface HbB	43	16020	1533	9.6	11.0	92	119.6	14.3
	45	15680	2750	17.5	14.8	165	89.7	10.8
	85	17270	2967	17.2	15.1	178	84.8	10.2
	90	18060	3167	17.5	11.7	95	123.2	7.4
Mean		16758	2604	15.5	13.2	133	104.3	10.7
S.E.		553	367	2.0	1.1	23	9.9	1.4
Dorset HbA	57	19090	6400	33.5	32.1	384	83.6	10.8
	58	20500	2550	12.4	19.6	153	128.1	15.4
	59	21860	4583	21.0	31.4	275	114.2	13.7
	62	23860	6267	26.3	33.8	376	89.9	10.8
Mean		21328	4950	23.3	29.2	297	104.0	12.7
S.E.		1016	900	4.5	3.3	54	10.4	1.1
Dorset HbB	3	21800	7133	32.7	55.6	428	129.9	15.6
	40	13860	3550	25.6	17.9	213	84.0	10.1
	54	20450	7417	36.3	27.9	483	62.7	7.5
	61	15860	4300	27.1	31.2	258	120.9	14.5
Mean		17993	5600	30.4	33.2	346	99.4	11.9
S.E.		1874	981	2.5	8.0	65	15.8	1.9

There is also some evidence to suggest that a similar but less effective control was exerted by the Dorset sheep which became hyperinfected, blood loss per worm being reduced to 0.04 ml and egg output per female to 5,000 eggs/day compared with 0.05 ml and 8,000 eggs/day in the challenge controls; these figures are probably an underestimate since some animals became moribund prior to necropsy and in all likelihood lost a proportion of their established worm populations.

EXPERIMENT 2

Scottish Blackface, Finn Dorset and Suffolk sheep of established haemoglobin type were individually infected with 7,000 H. contortus larvae and with the exception of four animals which were kept indoors, introduced 5 weeks later along with two "tracer" sheep into paddocks containing freshly growing parasite-free grass. The course of infection in each animal was monitored by PCV and faecal egg counts and worm burdens were determined at necropsy about 9 weeks after infection.

PCV values were somewhat variable before infection but this was neither related to the breed nor haemoglobin type of the animals concerned (Table 8). About 10 days after larval administration these indices fell progressively, reaching their lowest values around the 6th week. At this stage Blackface and Dorset sheep with HbA were less anaemic than their counterparts with the HbB gene ($p < 0.05$-0.01), and also incidentally than the HbB Suffolks ($p < 0.05$). During the latter 3 weeks of the study, PCV's of all the grazing sheep showed marked and relatively equal improvements, but this feature was not observed in the group kept indoors with the result that by necropsy these animals were more anaemic than any of the groups at grass.

Egg counts rose steadily from the 19th day after infection, reached a peak around the 5th week, and thereafter declined in all groups, although less noticeably in the HbB Dorsets and Suffolks, and the sheep kept indoors (Tables 9 and 10).

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TABLE 8.
Packed Cell Volumes (%)

	Sheep No.	Weeks after infection 0	1	2	3	4	5	6	7	8	9
Blackface	78	39	35	34	28	24	21	21	26	30	30
HbA	86	33	34	30	24	20	21	24	26	29	30
Mean		36.0	34.5	32.0	26.0	22.0	21.0	22.5	26.0	29.5	30.0
S.E.		3.3	0.5	2.0	2.0	2.0		0.5		0.5	
	61	34	26	31	22	17	18	17	17	22	22
Blackface	67	34	32	32	22	14	14	16	15	20	21
HbAB	81	33	32	31	26	19	18	18	20	26	30
	967	32	30	28	21	18	19	20	21	23	24
Mean		33.3	30.0	30.5	22.8	17.0	17.3	17.8	18.3	22.8	24.3
S.E.		0.5	1.4	0.9	1.1	1.1	1.1	0.9	1.4	1.3	2.0
	433	35	32	33	31	28	25	24	24	25	27
Dorset	440	36	34	34	28	21	20	20	19	22	23
HbA	446	38	37	37	29	25	23	23	22	22	21
	508	39	39	36	31	27	26	24	24	28	29
Mean		37.0	35.5	35.0	29.8	25.3	23.5	22.8	22.3	24.3	25.0
S.E.		0.9	1.6	0.9	0.8	1.6	1.3	1.0	1.2	1.4	1.8
	430	33	30	26	22	20	22	18	18	20	20
Dorset	435	35	33	31	25	19	20	20	17	21	23
HbB	441	34	33	30	24	19	20	16	20	22	24
	444	35	33	33	27	16	18	18	20	22	24
Mean		34.3	32.3	30.0	24.5	18.5	20.0	18.0	18.8	21.3	22.8
S.E.		0.5	0.8	1.5	1.0	0.9	0.8	0.8	0.8	0.5	1.0
	39	34	32	31	27	25	20	21	23	27	28
Suffolk	48	31	31	31	27	22	20	18	21	23	25
HbB	57	29	28	25	22	17	16	13	16	18	19
	74	31	31	30	27	21	20	19	21	24	23
Mean		31.3	30.5	29.3	25.8	21.3	19.0	17.8	20.3	23.0	23.8
S.E.		1.0	0.9	1.4	1.3	1.7	1.0	1.7	1.5	1.9	1.9
Suffolk HbB	55	34	33	33	28	23	21	23	20	20	17
Blackface HbAB	62	36	33	30	25	20	18	20	14	12	11
Blackface HbAB	84	33	31	31	23	16	16	16	14	14	14
Dorset HbB	503	33	33	32	27	24	21	23	22	25	27
Mean		34.0	32.5	31.5	25.8	20.8	19.0	20.5	17.5	17.8	17.3
S.E.		0.7	0.5	0.7	1.1	1.8	1.2	1.7	2.1	3.0	3.5
Suffolk HbB		35	36	33	35	37	37	35	36	34	38
Suffolk HbB		38	37	37	38	36	35	35	36	36	35
Mean		36.5	36.5	35.0	36.5	36.5	36.0	35.0	36.0	35.0	36.5
S.E.		1.5	0.5	2.0	1.5	0.5	1.0	-	-	1.0	1.5

TABLE 9.

Faecal Egg Counts ($x10^3$) of Sheep at Grass.

Days after infection	Blackface HbA 78	86	Mean ±S.E.	Blackface HbAB 61	67	81	967	Mean ±S.E.
19	-	0.2	0.1±0.1	0.9	0.8	1.9	0.1	0.9±0.4
22	0.4	0.4	0.4±0.0	10.7	2.7	6.5	2.8	5.7±1.9
26	1.5	1.5	1.5±0.0	12.3	5.3	8.5	7.6	8.4±1.5
29	2.7	2.2	2.5±0.3	6.4	5.6	7.5	7.3	6.7±0.4
33	5.5	6.5	6.0±0.5	9.0	10.2	9.2	8.6	9.3±0.3
36	4.2	6.2	5.2±1.0	25.6	10.9	11.4	6.4	13.6±4.2
40	3.5	5.2	4.4±0.9	18.1	11.2	8.9	4.9	10.8±2.8
43	2.6	2.4	2.5±0.1	14.3	9.3	3.2	1.8	7.2±2.9
47	2.5	3.2	2.9±0.4	19.5	6.2	1.2	3.2	7.5±4.1
50	4.5	1.9	3.2±1.3	14.5	8.8	1.3	3.7	7.1±2.9
54	1.9	1.4	1.7±0.3	15.0	8.1	1.3	2.0	6.6±3.1
61	0.2	0.5	0.4±0.2	15.6	11.5	1.2	2.9	7.8±3.4
63	0.1	0.8	0.5±0.4	11.3	12.5	1.2	1.1	6.5±3.1

Days after infection	Dorset HbA 433	440	446	508	Mean ±S.E.	Dorset HbB 430	435	441	444	Mean ±S.E.
19	0.5	4.3	2.0	1.1	2.0±0.8	2.9	1.2	1.3	3.2	2.2±0.5
22	1.5	6.2	6.2	2.1	4.0±1.3	11.3	6.1	6.5	6.5	7.6±1.2
26	0.9	5.0	12.5	2.1	5.1±2.6	11.2	9.5	10.8	12.4	11.0±0.6
29	1.1	14.2	16.8	3.3	8.9±3.9	14.1	9.5	14.4	20.6	14.7±2.3
33	1.2	10.8	17.9	4.8	8.7±3.7	33.9	14.0	19.9	18.1	21.5±4.3
36	2.5	16.1	17.4	3.4	9.9±4.0	11.3	14.5	24.3	21.2	17.8±3.0
40	1.2	20.9	10.6	5.7	9.6±4.2	18.4	10.8	7.2	16.4	13.2±2.6
43	0.7	18.6	6.6	4.0	7.5±3.9	17.2	7.4	3.2	8.7	9.1±2.9
47	1.4	10.3	5.5	4.6	5.5±1.8	22.6	7.6	6.3	6.7	10.8±3.9
50	1.3	8.2	11.0	2.9	5.9±2.3	14.1	6.0	4.6	8.1	8.2±2.1
54	1.0	5.4	10.0	3.3	4.9±1.9	15.3	6.8	4.7	6.7	8.4±2.4
61	0.7	3.3	9.8	2.0	4.0±2.0	18.8	5.2	4.2	6.3	8.6±3.4
63	0.7	3.2	7.3	4.1	3.8±1.3	16.8	5.8	5.6	6.0	8.6±2.8

Days after infection	Suffolk HbB 39	48	57	74	Mean ±S.E.
19	2.2	2.6	3.1	2.9	2.7±0.2
22	9.1	4.9	7.4	4.4	6.5±1.1
26	9.3	5.4	8.0	5.5	7.1±1.0
29	8.8	7.1	10.9	6.9	8.4±0.9
33	15.1	9.1	8.7	5.9	9.7±1.9
36	16.6	15.8	12.1	4.9	12.4±2.7
40	7.7	6.4	11.8	7.3	8.3±1.2
43	9.0	4.8	12.9	8.2	8.7±1.7
47	6.2	9.6	14.5	NS	10.1±2.4
50	14.8	9.3	10.5	5.8	10.1±1.9
54	21.0	12.3	8.3	8.9	12.6±2.9
61	16.6	10.5	9.7	3.4	10.1±2.7
63	11.5	8.2	8.8	7.7	9.1±0.8

TABLE 10.

Faecal Egg Counts ($x10^3$) of Sheep Maintained Indoors

Days after infection	Suffolk HbB 55	Blackface HbAB 62	HbAB 84	Dorset HbB 530	Mean ±S.E.
19	0.8	0.1	1.5	1.9	1.1±0.4
22	4.0	1.2	9.7	2.4	4.3±1.9
26	4.0	3.9	10.9	5.0	6.0±1.7
29	4.6	2.8	14.8	5.0	6.8±2.7
33	6.0	4.2	16.4	8.5	8.8±2.7
36	7.5	5.8	11.2	15.3	10.0±2.1
40	5.0	4.5	14.4	5.8	7.4±2.3
43	5.5	10.1	10.6	4.7	7.7±1.5
47	6.7	4.4	11.9	4.9	7.0±1.7
50	4.8	8.3	12.9	1.4	6.9±2.5
54	2.4	9.0	15.2	11.1	9.4±2.7
61	3.4	8.0	11.8	2.9	6.5±2.1
63	3.9	8.1	12.2	3.3	6.9±2.1

HbB Dorsets had the highest and HbA Blackfaces the lowest counts throughout with other groups intermediate. It is also worth mentioning that by necropsy the average egg count of HbA Blackfaces had fallen from a peak of 6,000 e.p.g. to 500 e.p.g., HbA Dorsets from 10,000 e.p.g. to 3,800 e.p.g., while all other groups, including the HbB Dorsets were still passing 7,000-9,000 e.p.g.

Worm burdens at necropsy reflected the terminal egg counts (Table 11). Less than 1,000 worms were recovered from the HbA and HbAB Blackface and HbB Dorset groups compared with in the region of 2,500 parasites from the HbB Dorsets and Suffolks and the group kept indoors. Differences between HbA and HbAB Blackfaces were not significant but on average the latter had four times as many worms; HbA Dorsets however had significantly lower burdens than their counterparts with HbB ($p < 0.05$) and the HbAB Blackfaces at grass fewer worms than those kept indoors. Both "tracer" sheep maintained their worm-free status during the grazing period.

TABLE 11.

Worm Recovery 9 Weeks after Infection with 7,000 H. contortus Larvae.

Group	Sheep No.	Worms Recovered	% Recovery
Blackface HbA	78	150	2.1
(at grass)	86	300	4.3
Mean		225	3.2
S.E.		75	1.1
	61	1550	22.1
Blackface HbAB	67	700	10.0
(at grass)	81	450	6.4
	967	800	11.4
Mean		875	12.5
S.E.		237	3.4
	433	350	5.0
Dorset HbA	440	900	12.9
(at grass)	446	1250	17.9
	508	850	12.1
Mean		838	12.0
S.E.		185	2.7
	430	1800	25.7
Dorset HbB	435	800	11.4
(at grass)	441	3600	51.4
	441	2400	34.3
Mean		2150	30.7
S.E.		585	8.3
	39	2700	38.6
Suffolk HbB	48	3150	45.0
(at grass)	57	2000	28.6
	74	1750	25.0
Mean		2400	34.3
S.E.		321	4.6
Blackface HbAB	62	3050	43.6
Blackface HbAB	84	1100	15.7
Suffolk HbB	55	2400	34.3
Dorset HbB (all indoors)	530	2750	39.3
Mean		2325	33.3
S.E.		439	6.1
Suffolk HbB	30	N	-
("tracers")	47	N	-
Mean		N	-
S.E.		N	-

DISCUSSION

The experiments reported in this section were designed with two principal objects in mind, namely to examine the possible association between the host's haemoglobin type and breed and the occurrence of self-cure, and to attempt an assessment of the relative contributions made to the induction of self-cure by larvae and freshly-growing grass. The results obtained can only be described as conflicting, but taking each of the experiments in turn the following conclusions seem in order.

The first point to be made is that self-cure can be induced by reinfecting sheep carrying an existing population of adult worms, thus confirming Stewart's[4] original observations. Secondly, the nature and timing of the underlying changes in worm activity (reinfection was followed after 4-6 days by a sudden and dramatic fall in faecal egg output and abomasal haemorrhage), are consistent with the hypothesis that the phenomenon, at least when produced in this way, is caused by an immediate-type hypersensitivity reaction in the abomasum triggered by antigenic materials released by the newly acquired larvae during the 3rd ecdysis[4,17]. Thirdly, and as noted by previous authors, the reaction is not necessarily accompanied by complete worm expulsion neither is it synonymous with resistance to reinfection[4,5,14,16,17]; indeed in only 25% of the sheep which exhibited self-cure

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was worm expulsion complete and in only 50% was the reaction followed by protection against renewed larval intake. Furthermore, judging by their terminal worm burdens, and assuming that these originated from the second dose of larvae, it is debatable whether sheep susceptible to reinfection were any less susceptible than the challenge controls. By the same token it would be unwise to assume on the basis of worm recovery that the immunological status of the two groups was the same; as the measurements of egg output and blood loss clearly demonstrate, the biotic and haematophagic activities of the parasites in the reinfected sheep were severely depressed and hence although both groups were similarly infected (the average worm burden of the reinfected sheep was 4,200 compared with 4,350 in the challenge controls), the former experienced by far the less severe functional disturbances - a point well exemplified by the PCV values during this period. Hence in some respects the response to reinfection, at least in the case of individual Scottish Blackface sheep, was the same irrespective of whether the primary infection was terminated with anthelmintic.

The final point to emerge from the first experiment was that the occurrence of self-cure was in no way related to the host's haemoglobin type, but was highly correlated with breed, being observed in 7 of the 8 Blackface sheep (4 HbA and 3 HbB) and in only one of the Dorsets (HbA) which as a group were uniformly susceptible to hyperinfection

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(6 out of 8 animals were affected in this manner). This finding is in sharp contrast to the recent report by Allonby and Urquhart[15] that under natural conditions HbA Merinos self-cure more frequently and effectively than their HbB counterparts, but it would be unrealistic to suggest that this disparity is anything other than a reflection of differences in experimental conditions or the breeds of sheep used. However, it is also worth stating that although the Dorsets showed no apparent resistance to reinfection as judged by worm establishment, they were nonetheless able to exert some control over the parasites' blood-sucking and reproductive activities, though somewhat less effectively than the Blackfaces.

In attempting to explain the contrasting abilities of the Blackface and Dorset breeds to exhibit self-cure, it should be recalled that the latter were much more susceptible to the primary infection and at challenge were more anaemic and experiencing considerably greater haemorrhage than the Blackfaces (320 ml/day compared with 120 ml/day). In the author's opinion it would be surprising if losses of this magnitude did not adversely affect the immune response since quite apart from the accompanying metabolic strain, these represent significant depletion of circulating antibody which could be important in aiding worm expulsion. It is also possible that being subjected to a much higher level of antigenic stimulation during this infection, the Dorset sheep had reached a stage of desensitisation or immunological exhaustion[16,18].

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The results of the second experiment are more difficult to interpret but appear to indicate that self-cure can occur in the absence of reinfection in sheep grazing on rapidly growing pasture. However, unlike the phenomenon induced by reinfection which was characterised by a precipitous fall in egg count over a period of 6-8 days, the reaction at grass was notable for the fact that it occurred only gradually over about 4 weeks following the change of diet. Thus, while in general these findings confirm the original and more extensive studies reported by Allonby and Urquhart[10], they do nonetheless differ inasmuch as these authors observed an effect similar to that described here following reinfection. A further feature which emerged from this experiment was that although the occurrence of self-cure was again more closely dependent upon the breed than the haemoglobin type of the animals concerned, its effectiveness as judged by faecal egg counts and worm burdens was greater in HbA than in HbAB and HbB type sheep; this latter finding confirms the recent report by Allonby and Urquhart[15] that HbA Merinos self-cure better than HbB sheep.

The mechanism underlying self-cure at grass is unknown but on the basis of the present experiment appears unlikely to be the same as that initiated by reinfection; what appears more likely is that worm expulsion is caused either as a result of an "anthelmintic factor" as proposed by Gordon[2] or physiological disturbances in the abomasum induced by the sudden change of diet. Alternatively, it is possible,

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as Allonby and Urquhart[10] have suggested, that self-cure at grass and upon reinfection are essentially similar in aetiology, the former being caused by some as yet undefined factor in new pasture capable of producing an anaphylactoid state in the abomasum. The fact that self-cure as observed in the present experiment was less dramatic than that recorded under Kenyan conditions may therefore simply reflect differences in pasture composition, but clearly further work is needed before a proper assessment can be made of the relative contributions of grass, larvae and the host's genetic constitution to the induction and expression of the reaction.

SUMMARY

In this section a detailed examination was made of the relationship between host genetic factors and the occurrence and effectiveness of self-cure of H. contortus infections in sheep exposed to reinfection or to rapidly growing parasite-free grass.

The results obtained demonstrated that self-cure occurred under both conditions, suggesting that the reaction could be both immunological and non-immunological in origin, the former in all likelihood being a manifestation of a hypersensitivity reaction in the abomasum and the latter due to the presence of an anthelmintic or anaphylactoid-type factor in the grass.

Whether induced by larvae or grass the phenomenon was more closely associated with the breed than with the haemoglobin type of the animals concerned, being observed in the majority of the Scottish Blackface sheep of each haemoglobin type but in only a minority of the Finn Dorsets and Suffolks; even if unable to expel their existing worm populations most sheep were able to impair the reproductive and haematophagic activities of parasites derived from subsequent infections.

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SECTION 4

STUDIES ON *Ostertagia circumcincta* INFECTIONS IN SCOTTISH BLACKFACE SHEEP OF DIFFERENT HAEMOGLOBIN TYPE.

INTRODUCTION

Evidence was presented in previous sections of this thesis that the susceptibility of sheep to Haemonchus contortus was at least partially determined by the animal's genetic constitution and in particular by its haemoglobin type, animals of Hb type A being more resistant to infection as judged by a variety of clinical, pathophysiological and parasitological parameters than similar sheep with HbB. No obvious explanation could be given for this interaction, but the possibility was raised that the lower oxygen affinity of HbB provided a more suitable environment for the establishment of this particular parasite which requires substantial amounts of red cells for survival.

To test the validity of this hypothesis the studies described for Haemonchus require to be extended to a parasite which as far as is known neither sucks blood nor causes significant anaemia, and for a number of reasons it seemed most appropriate to use Ostertagia circumcincta for this purpose. In the first place, while there are certainly a number of major differences between the two species with regard to their development and ensuing structural damage within the host[1-4], both reside in the abomasum and hence are at least comparable from the standpoint of predilection site. Secondly, of all the gastrointestinal nematodes, O. circumcincta is the one most commonly associated with outbreaks of ovine parasitic gastroenteritis in temperate regions and is therefore of considerable economic importance[5-17].

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Thirdly, like Haemonchus there is some evidence of genetic resistance to Ostertagia in grazing sheep. Stewart et al.,[18] noted that Romney Marsh sheep were more resistant than Southdowns, Shropshires and Hampshires as judged by faecal egg counts, and this was later confirmed by Ackert[19] who also demonstrated, again on the basis of egg counts, that Cheviots were more resistant than any of these lowland breeds. Subsequent work by Scrivner in the United States[20-22] provided more convincing evidence of an interaction between breed and Ostertagia infections under natural conditions, noting lower faecal egg counts and worm burdens in sheep of the Targhee and Panama breeds than in Suffolks, Hampshires and Rambouillets, and demonstrating transmission of resistance between a Targhee ram and his progeny. Quite apart from the limitations imposed by reliance on field observations and egg counts, (these have already been discussed elsewhere), in none of these experiments was haemoglobin typing performed, and hence it has yet to be established whether the variations attributed to breed were in fact more a reflection of differences in the haemoglobin type of the animals concerned. This certainly remains a possibility since in each case considerable variation was encountered in the response of individuals within the breeds investigated.

Finally, and in common with Haemonchus, the functional disturbances caused by larval and adult stages of Ostertagia may be readily detected and quantified by clinical and radioisotopic methods and therefore accurately related to

parasite development and burden. The life-cycle and pathogenesis of ovine ostertagiasis have been described by a number of workers[1,2,14,23-30], but most information on these aspects has come from the detailed work of Armour and his colleagues[4] and Holmes and his co-workers[31,32], in Scottish Blackface sheep. Using doses of 100,000 larvae, Armour investigated the structural and biochemical consequences of infection in parasite-free sheep and their relationship to the kinetics of larval and worm establishment. These studies revealed (a) that a proportion (up to 25%) of an established infection fails to develop beyond the 4th larval stage, remaining dormant or inhibited within the gastric glands, (b) that large numbers of worms are lost following emergence of immature adults, i.e. from about the 16th day after infection, and (c) that as in the case of bovine ostertagiasis[33,34], plasma pepsinogen levels provide a useful index of the extent of infection and abomasal damage associated with the histotrophic phase of the parasite; these levels were shown to rise from the onset of infection, reaching a peak around day 16 and thereafter declining in association with worm expulsion and regression of gastric lesions. The studies of Holmes and co-workers were concerned more with the disturbances in host function, and in particular with the changes in plasma protein and nitrogen metabolism accompanying Ostertagia infections. Using heavier infections (300,000 and 900,000 larvae) and radioiodinated albumin and ^{51}Cr-labelled plasma protein to monitor albumin turnover and

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gastrointestinal plasma leak, these authors related the development of hypoalbuminaemia to a marked increase in the catabolic rate of albumin and loss of plasma into the gut; such changes were most noticeable between the 1st and 3rd weeks after infection, accompanied by reduced nitrogen balances and significant elevations in plasma pepsinogen and blood urea levels, and in more heavily infected animals, diarrhoea and inappetence. It is also worth stating that contrary to reports from South Africa[28] and Poland[35] in none of these studies was anaemia recorded. While there is no obvious explanation for this discrepancy, it should be stressed that the South African work was conducted on massively infected and obviously very young Merinos (each was infected with 270,000 larvae, and although of unspecified age, weighed only 14 kg) which possibly suffered acute gastric haemorrhage as a result of larval development. The Polish workers used a group of 3-month old sheep belonging to the Polish long-wool breed; these sheep were heavily infected (400,000 larvae) and the haematological changes compared with similar animals infected with only 50,000 larvae; both groups developed relatively mild anaemia which for some unknown reason was of similar degree in all animals (PCV's dropped from about 30% to 24%). Clearly it is difficult to construct a reasonable hypothesis with regard to the anaemia which on occasions attends this parasite, but it seems clear that at least in the case of the Scottish Blackface breed which has been more extensively

studied than any other, Ostertagia circumcincta is not haematophagic and therefore a suitable parasite for investigating further the possible influence of haemoglobin type in resistance to gastrointestinal helminths.

The approach adopted in the experiment reported here was essentially the same as that described in earlier sections, the basic aim being to ascertain whether resistance, either to the establishment or to the effects of this parasite, or both, was related to the haemoglobin type of the animal concerned; because of the considerable background information available on the response of Scottish Blackface sheep, the studies were restricted to this breed.

MATERIALS AND METHODS

Experimental animals and design

Nine Scottish Blackface wethers, 6-7 months old, which had been reared and maintained parasite-free from birth were divided into two groups on the basis of haemoglobin type (5 HbA and 4 HbB). Each sheep was injected with ^{125}I-albumin and ^{51}Cr Cl_3 and seven days later infected with 100,000 O. circumcincta larvae. During the experiment the sheep were fitted with faecal bags and maintained in standard metabolism cages, The animals were fed ½ lb of commercial sheep pellets each day, and hay and water were given ad lib. To ensure rapid excretion of ^{125}I from degraded protein, the thyroid was blocked by daily dosing with 15 ml 0.75% KI orally, starting 5 days prior to the injection of the isotopes and continuing throughout the experimental period.

All animals were necropsied 16 days after infection and their worm burdens determined as described earlier.

Blood analyses

Blood samples were collected for both haematological and biochemical estimations. The values recorded were packed cell volume and total serum protein, albumin and globulin; these estimations were conducted using techniques identical to those described earlier.

Plasma pepsinogen levels were determined by a method essentially similar to that of Edwards, Jepson and Wood[36] in which plasma was incubated at a pH of 2.0 with bovine serum albumin substrate (Armour's Fraction V_2, Armour

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Pharmaceutical Co.Ltd., Eastbourne, England) for 24 hours at 37°C. The liberated tyrosine, non-precipitable with trichloracetic acid was estimated spectrophotometrically with Folin-Ciocalteau reagent (British Drug Houses, Poole, England). The enzyme activity was expressed in milli-Units (mU) i.e. μ mols tyrosine liberated per litre of plasma per minute x 1000.

Labelling procedures

^{125}I-labelled albumin

Labelled albumin was prepared by trace-labelling a buffered solution containing 600 mg ovine albumin with 10 mCi Na^{125}I. 2 g bovine albumin was added as "carrier" and the preparation dialysed for 2 days against 40 litres of saline. A solution containing approximately 350 μCi ^{125}I was injected into each sheep.

^{51}Cr-labelled protein

Protein labelling with ^{51}Cr was first described by Gray and Sterling[37] who tagged bovine albumin *in vitro* with ^{51}chromic chloride. Waldmann[38] later introduced ^{51}Cr-labelled albumin to demonstrate leakage of albumin into the gut on the basis that chromium is not absorbed on oral administration but appears quantitatively in the faeces[39]. The same author also suggested that such preparations might be useful for metabolic studies but this was subsequently invalidated, firstly by its more rapid elimination from the circulation than ^{131}I-albumin[40], and secondly by the lability of the chromium-protein bonding,

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i.e. when injected intravenously much of the tag is eluted from albumin and becomes attached principally to the gamma$_2$ globulins[41,42]; indeed the studies of Van Tongeren and co-workers[42,43] indicated that the plasma disappearance of ^{51}Cr-albumin was probably more a reflection of transferrin than of albumin degradation and differed little from that of ^{51}Cr Cl_3 itself. Hence while it is clear that albumin labelled with ^{51}Cr is in itself of little value for precise kinetic measurements, it remains a useful tool for estimating gastroenteric protein leak, and when used in conjunction with ^{125}I-albumin provides a basis for correlating alterations in albumin metabolism with plasma loss into the gut.

Since there is no advantage in labelling albumin in vitro (in fact the technique apart from causing considerable protein denaturation is tedious and necessitates removal of unbound isotope), the simplest and preferred method consists of injecting ^{51}Cr Cl_3 directly into the circulation[44]. This results in the attachment of ^{51}Cr to a number of the plasma proteins and hence faecal clearances based on this isotope cannot be regarded as absolute but rather as indices of plasma loss into the gut.

In the present experiments ^{51}chromic chloride was obtained as a sterile solution from the Radiochemical Centre, Amersham, England, and the plasma proteins were labelled in vivo by injecting approximately 800 µCi ^{51}Cr into each sheep.

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Injection and sampling procedures

^{125}I-albumin and Cr Cl_3 solutions were injected together from separate syringes via a 2-way tap and jugular catheter. The first blood sample was taken 15 minutes later, a further six samples at regular intervals over the following 3 days, and subsequent samples daily. 1.0 ml plasma was obtained for radioactivity determinations. The total urine and faeces excreted during each 24-hour period were collected and the ^{51}Cr and ^{125}I activities of representative aliquots determined as described previously.

Calculations and expression of results

The catabolic rate of albumin was measured in terms of both the plasma half-life ($t_{\frac{1}{2}}$) of labelled albumin and the fraction of the intravascular pool catabolised each day - F(CA) (see Section 1). Faecal clearances of ^{51}Cr, calculated by dividing the total daily faecal activity by the activity per ml plasma were used as indices of gastrointestinal plasma leak.

RESULTS

Scottish Blackface sheep with HbA and HbB were individually infected with 100,000 third-stage Ostertagia circumcincta larvae and the course of infection monitored and compared by haematological, biochemical and radio-isotopic methods; worm establishment was measured 16 days after infection; results for individual animals are given in Appendix 4.

Clinical observations

Clinical signs of parasitic gastritis were not observed in any of the sheep, and throughout the experiment all animals continued to eat normally and pass pelleted faeces.

Haematological and biochemical observations

Haematological indices remained steady throughout the study as did total serum protein and globulin concentrations. Albumin levels however fell slightly in all animals - from 3.4 to 2.8 g% in the HbA group, and from 3.2 to 2.4 g% in their HbB counterparts, while plasma pepsinogen values increased approximately fourfold, i.e. from about 1,500 to 6,500 mU ($p < 0.01$) and from about 1,250 to 5,500 mU ($p < 0.02$) respectively (Figure 1). The only consistently significant group difference recorded was that of PCV which was higher in the HbA animals ($p < 0.05$-0.01) throughout the study.

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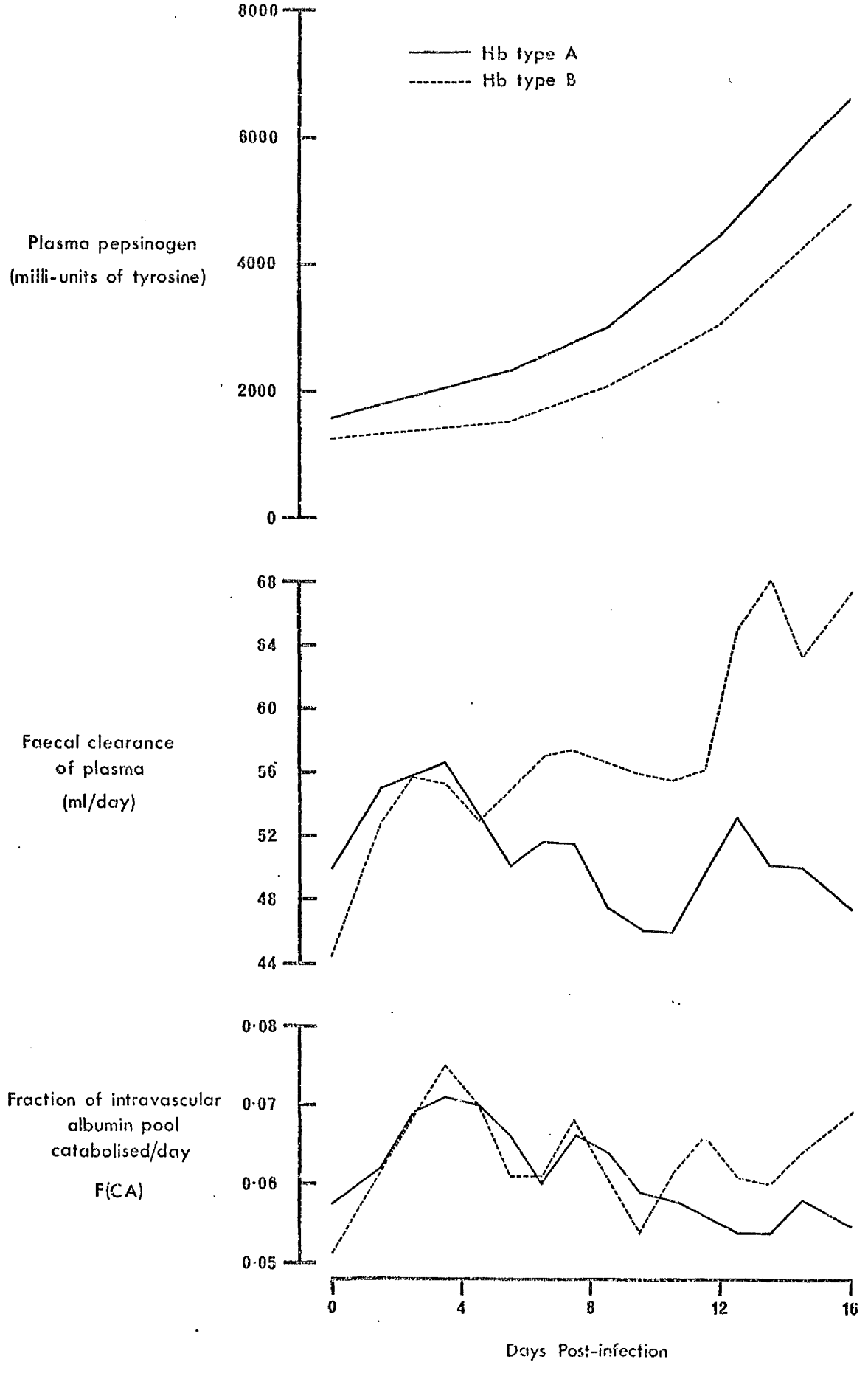
the Plasma Pepsinogen Levels and Rates of Albumin
Degradation in Scottish Blackface Sheep.



Albumin catabolism

Average F(CA) values changed little during the course of infection and no significant differences were apparent between the groups (Figure 1). Nevertheless, a degree of hypercatabolism was noted during the first week, particularly in the HbB group, and whereas this was subsequently maintained in these animals, catabolic rates of the HbA group declined progressively reaching levels similar to and in some cases somewhat lower than those recorded prior to infection; comparison of preinfection and necropsy figures revealed a significant increase only in the HbB group ($p < 0.05$). Alterations in fractional rates of catabolism were reflected in similar changes in $t_{\frac{1}{2}}$, average figures for the HbB group being 409 ± 37 hours compared with 515 ± 34 hours in HbA animals - a difference which just failed to reach the level of significance by the "t"-test.

Plasma loss into the gut

The trends exhibited with regard to faecal plasma clearance were similar to those described for albumin catabolism, with all animals showing an early rise during the first 4-5 days of infection followed in the case of the HbA group by a gradual return to pre-infection values, and in the HbB group by a further and more or less progressive increase up to the time of necropsy. None of these changes or group differences was statistically significant.

Parasitological findings

The numbers of O. circumcincta present in both groups of sheep at post-mortem are shown in Table 1. In this table

TABLE 1.

Worm Counts at Necropsy* of Scottish Blackface Sheep Given a Single Dose of 100,000 Ostertagia circumcincta Larvae.

Sheep No.	Hb Type	Total	Adult Stages		Developing Stages		Inhibited early 4th Stages	
			Lumen	Mucosa	Lumen	Mucosa	Lumen	Mucosa
33	A	16,800	13,800	-	-	-	3,000	-
34	A	18,650	6,800	-	2,350	-	8,750	750
35	A	22,300	13,300	50	500	-	6,400	2,050
37	A	20,750	13,550	-	1,250	-	5,950	-
40	A	13,250	7,500	-	550	-	5,000	200
Mean		18,350	10,990	10	930	-	5,820	600
S.E.		1,579	1,574	10	407	-	937	389
31	B	19,950	14,500	300	150	-	4,700	300
36	B	21,950	18,150	50	450	-	3,050	250
38	B	22,400	19,500	50	-	-	150	2,700
39	B	25,900	25,300	-	600	-	-	-
Mean		22,550	19,363	100	306	-	1,975	813
S.E.		1,237	2,243	68	137	-	1,148	633
P		N.S.	<0.02	N.S.	N.S.	-	<0.05	N.S.

* All sheep necropsied 16 days after infection.

apart from the total worm burdens the parasites present were classified into 3 stages: a) mature adults; b) developing stages, i.e. late 4th, 4th moult and 5th stage; and c) inhibited larval stages.

The mean total number of worms present in the HbB group was greater than in the HbA sheep, but there was no significant difference between these values. However, the mean number of adult Ostertagia in the HbA sheep was significantly less ($p < 0.02$), while the mean number of inhibited 4th stage larvae was significantly greater ($p < 0.05$) than in the HbB sheep.

DISCUSSION

The absence of significant clinical changes in the majority of animals confirms the original and more detailed work of Armour and his colleagues[4] who demonstrated that a dose of 100,000 O. circumcincta larvae was below the threshold necessary to produce overt signs of ostertagiasis in Scottish Blackface sheep. Nevertheless all animals did experience some degree of functional impairment, manifest by reduced serum albumin and elevated globulin concentrations, increased plasma pepsinogen levels, and accelerated rates of albumin degradation and gastroenteric protein leak. These changes, which have been well documented by a number of workers (see Introduction) were, with the exception of the pepsinogen values which increased at a similar rate in both groups, marginally more severe in the sheep with HbA. It would be unrealistic however to suppose on the strength of these findings that the effects of this parasite are influenced by haemoglobin type.

On the other hand, the results of this study suggest that haemoglobin type, or more likely factors related thereto, does have a profound influence on the parasites' development. This is clearly shown in Table 1 by the much higher proportion of inhibited 4th stage larvae and lower proportion of adult worms recovered from the HbA as compared with the HbB type sheep. Since there was no difference between the two groups in total worm establishment, the suggestion from these findings is that the presence of HbA is in some way associated with retardation of development.

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The mechanism of inhibition of parasite larvae is highly controversial, and although long ascribed to the development of host immunity during the grazing season[25,45-49], in the case of O. ostertagi has recently been shown by Armour and his colleagues[50-52] to be primarily a function of the larvae themselves, and more specifically of the environmental conditions under which they are maintained. Nevertheless as Armour and Bruce[52] have pointed out, the phenomenon is probably multifactorial in aetiology depending upon the interaction between host, parasite and environment. This being the case it is not unreasonable that one such factor is the host's genetic constitution and that the genetic link with inhibition may be immunological. Nor is it surprising that the same genetic pool imparts resistance to two closely related parasites; this point has already been clearly established by the work of Scrivner[22] who showed that sheep selected for resistance to Ostertagia sp. were also resistant to H. contortus, and also by earlier studies on the inheritance of resistance to bacterial infections[53].

It could of course be argued that the differences recorded between HbA and HbB sheep were merely a reflection of the particular time at which they were necropsied and that the inhibited larvae would in any case have resumed their development and along with the adults been subsequently expelled. This is a valid criticism since primary infections of O. circumcincta are notoriously labile in Scottish Blackface

sheep[4]. However, as the results stand there would appear to be some justification for suggesting that the parasites' biotic potential might be reduced, and in heavier infections its pathogenic effects lessened in HbA as compared with HbB animals. Clearly, further and more detailed work is required to elucidate these points and establish their relationship, if any, to the well-recognised phenomenon of breed resistance to this parasite, but on the basis of the evidence available it would appear that the association between haemoglobin type and resistance to helminthic parasites is not confined to those known for their blood-sucking activities.

SUMMARY

The experiment reported in this section was designed to compare the responses of HbA and HbB Scottish Blackface sheep to infection with O. circumcincta, a parasite considered to be non-haematophagic. In terms of disease, no clear-cut differences were observed between the groups, but the differential worm counts performed 16 days after infection revealed smaller numbers of adults and more inhibited larvae in HbA than in HbB sheep.

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SECTION 5

INTER-RELATIONSHIP BETWEEN HAEMOGLOBIN TYPE, BREED AND THE IMMUNE RESPONSE OF SHEEP TO NON-PARASITIC ANTIGENS.

INTRODUCTION

The literature on the relationship between haemoglobin type in sheep and resistance to parasitic infections is reviewed in the General Introduction of this thesis.

Considering the amount of work which has been carried out, it is strange that so little attention has been directed towards elucidation of the genetic control of immune responses in the animals involved. Observed differences in the pathophysiology have been related more to variation in undefined mechanism of "resistance" rather than to variation in specific immunological events in the host-parasite relationships concerned.

It has been recognised for some time that immune responses are under direct genetic control[1,2]. This situation provides a basis for explaining some of the variations in susceptibility to parasites and has been implicated by Wassom, Dewitt and Grundmann[3] in their analysis of the incidence of Hymenolepis citelli in wild Peromyscus maniculatus and by Wakelin[4] who presented evidence that differences in the susceptibility of mice to Trichuris muris are related to genetically determined differences in the ability to bring about immune expulsion of the parasite.

In previous chapters of this thesis, evidence was presented that differences in the susceptibility of sheep to the nematode H. contortus are linked with the animal's

haemoglobin type. The pathophysiological and parasitological observations made during the course of primary infections are completely in line with the respective parasite burdens. In other words, these differences are broadly speaking immunological in origin. This raises the question: are HbA type animals generally speaking 'good responders', i.e. do they give a better immune response than HbB types to a wide range of antigenic stimuli?

The present chapter deals with some preliminary studies on Scottish Blackface and Finn Dorset sheep of different haemoglobin type to investigate the antibody response to a number of non-parasitic antigens.

Experimental animals and design

Three experiments were conducted involving a total of 33 worm-free wethers reared and maintained as described earlier. All of the sheep were haemoglobin typed at the beginning of the experiment.

In the first experiment, 8 Scottish Blackface (4 HbA and 4 HbB) and 8 Finn Dorset sheep (4 HbA and 4 HbB) which were approximately 6-7 months old were each injected intravenously with 200 mg of ^{125}I-labelled horse gammaglobulin and similarly reinjected 22 days later with 400 mg of the same labelled protein. At the time of the first injection each sheep also received 200 mg ^{131}I sheep albumin to act as a control non-foreign plasma protein, and throughout the study 10 ml of 0.75% KI was administered orally. Radioactivity analyses were performed on plasma samples collected twice daily for the first 32 days of the experiment and thereafter daily for the remainder of the experimental period. It was hoped that the characteristics of the elimination of this antigen from the circulation might be used as an index of the immune response of the experimental sheep.

The object of the second and third experiments was to study the antibody response of 9 HbA and 8 HbB Blackface sheep to both rabbit red blood cells and human serum albumin (HSA). These responses were measured in the first case by the haemagglutination reaction and in the second by the

Farr test, which gives a measure of antigen-binding capacity. Each sheep was injected intravenously with 5 ml rabbit red cells (a 10% suspension in saline) and subcutaneously with 1 ml of 5% human serum albumin (50 mg/ml) daily for 10 days and serum samples for antibody assay taken 1, 2, 3 and 4 weeks after the final injection.

EXPERIMENT 1

Elimination of ^{125}I horse gammaglobulin from the circulation of Blackface and Finn Dorset sheep

When a heterologous plasma protein is injected into the circulation of an animal, its disappearance from the plasma follows a characteristic pattern (see Figure 3, Section 1).

Over the first few days there is a fairly steep fall. This corresponds to the loss of the protein from intravascular to extravascular compartments, i.e. a period of equilibration. A slower decline takes place over a period of several days. This represents the result of normal catabolism. The third phase, where there is a sharp drop in plasma concentration corresponds to "immune catabolism". The onset of this phase depends on the presence of circulating antibody and, therefore, the time of onset will depend on the antigen, the amount injected and the immune competence of the recipient animal.

The patterns of antigen elimination and the antibody response described above have been established in rabbits and other laboratory animals[5-11], but to the author's knowledge no similar measurements have been made on sheep.

In the present preliminary study the mean "half-life" of the injected antigen was determined from the slope of the disappearance curve from day 4 onwards. It was considered that any significant degree of immune catabolism occurring during this time would show up as a shortening of the mean half-life.

Materials and methods

The antigen used in the present experiment was a commercial preparation of horse gamma-globulin (Cohn Fr. II Pentex Incorp., USA). The methods of labelling, injection, sampling and counting were essentially as described in the earlier sections of this thesis.

Results

The results of this experiment are summarised in Table 1; individual values are given in Appendix 5. It must be said that no very clear picture emerges from these figures. This is perhaps due to the large scatter within groups for which, at present, no explanation can be offered. Technical faults in injection, sampling and counting can be ruled out since the control measurements made with ^{131}I sheep albumin covering the period of the primary injection of antigen gave consistent results in all groups.

Although not significant by the "t" test the A type animals of both breeds appear to show a shortening of the half-life of the injected gamma globulin at the second injection. No such effect is seen in the B type

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TABLE 1.

Mean Half-Lives ($t_{\frac{1}{2}}$) of ^{125}I-Labelled Horse Gamma Globulin in Blackface and Fim Dorset Sheep of Different Hb Type.

	No. of animals	Half-life ($t_{\frac{1}{2}}$) Primary injection (hr)	Secondary injection (hr)
Blackface HbA	4	285±29	171±66
Blackface HbB	4	198±42 N.S.	229±73 N.S.
Dorset HbA	4	291±87	153±59
Dorset HbB	4	180±84 N.S.	168±71 N.S.

animals. This might be considered as evidence of a better immune response by the A types.

In retrospect it might have been better to follow the elimination of a very small amount of antigen in the reinfection study.

EXPERIMENT 2

Antibody response to rabbit red cells of HbA and HbB Scottish Blackface sheep

The animals involved and the immunisation procedures have already been described. The details of the serological examinations are given below:

Materials

1. Phosphate buffer pH 7.2 (PBS)
   36.0 g sodium chloride
   7.4 g Disodium hydrogen orthophosphate (anhydrous)
   2.15 g Dihydrogen orthophosphate
   Diluted to 5 litres with distilled water and stored at $4^{\circ}C$.
2. Rabbit red cells.

Test procedure

The rabbit red cells were washed 3 times in PBS and diluted to 2% with the same solution. 0.2 ml test serum were diluted with 0.8 ml PBS in a test tube and incubated for 30 minutes at $56^{\circ}C$. Normal sheep serum was similarly prepared for use as a standard. 0.5 ml PBS were added to each well of an agglutination plate; 0.5 ml of each test serum was added to the first well, mixed, and 0.5 ml removed and added to the next well.

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Each sample was diluted in this way 10 times to give a range of dilutions of 1:5 to 1:2560. 0.5 ml rabbit red cells were then added to each well with gentle mixing and the plate left for one hour in an incubator at 37°C. The plates were stored at 4°C and read the next morning.

The end point of titration was determined by observing the settling pattern of the red cells rather than their actual agglutination or clumping. In those dilutions where an excess of antibody is present, the cells settle to form a mat covering the whole of the bottom of the well. If no antibody is present the cells slide down to form a neat round button in the centre. Since a doubling dilution of the antibody was used, a series of patterns intermediate between an even carpet and a button were present in the region of the end point. The highest dilution just prior to the "button" pattern was taken as the actual end point.

Results

The mean haemagglutination titres for the two groups of sheep are shown in Table 2, while individual results are shown in the Appendix.

It is evident from these results that in both groups the titre of agglutinating antibodies was highest at day 7, i.e. 17 days after the start of the immunising schedule. This is perhaps not surprising, as such antibodies are likely to appear early in a course of immunisation. Application of the t-test to the results revealed no significant difference between the results of the two

TABLE 2.

Haemagglutinin Titres in Sera of Blackface Sheep of Different Haemoglobin Types Immunised with Rabbit r.b.c's.

	Sheep No.	Days after last Injection 7	14	21	28
Hb type A	9	947±175	437±87	296±143	200±42
Hb type B	8	800±105	380±80	290±130	138±33
p		N.S.	N.S.	N.S.	N.S.

groups over the experimental period. It should be mentioned, however, that the results at day 7 were distorted by the presence of two very poor responders in the A type group (33, 34). In fact 6 of the 9 animals in this group had titres of 1:1280, whereas 6 of the 8 HbB animals had titres of 1:640.

EXPERIMENT 3

Antibody response to human serum albumin of HbA and HbB Scottish Blackface sheep as measured by antigen-binding capacity (Farr test)

The estimation of antigen binding capacity of serum was devised by Farr[12]. This method, which measures the total antibody (of all immunoglobulin classes) raised against an antigen can be used with albuminous antigens e.g. HSA or BSA which do not precipitate with 50% saturated ammonium sulphate (SAS). These antigens can stimulate excellent antibody responses in common laboratory animals, and the former is a very good stimulant for antibody response in sheep.

The principle of this method is as follows:
If an animal is given injections of HSA it produces antibodies consisting of many immunoglobulin classes. If dilutions of serum from the immunised animal are mixed with HSA which is labelled with ^{131}I, the immunoglobulin molecules become attached to the molecules of the antigen. Some of them may form a precipitate, but others will remain in suspension. However, all immunoglobulins whether antibody to HSA or not can be precipitated out of solution by the addition of

ammonium sulphate. When this is done, those immunoglobulins which have combined with HSA are precipitated taking down the attached HSA with them. Any HSA which has not combined remains in solution. The antigen-antibody complex which precipitates is readily measured in a gamma-ray counting chamber, thus giving a direct indication of the total antigen binding capacity of all immunoglobulin produced by the immunisation schedule.

Materials

1. Borate buffer, pH 8.3-8.5
   12.368 g Boric acid
   19.072 g Sodium tetraborate
   8.768 g Sodium chloride
   Made up to 2 litres. Stored in polythene bottles in refrigerator.
2. Saturated ammonium sulphate. Stored in refrigerator.
3. 20% Trichloroacetic acid (TCA).
4. Normal serum (1:10). One part normal serum to nine parts borate buffer.
5. Normal serum (1:100). One part normal serum to ninety-nine parts borate buffer.
6. Human serum albumin (HSA) trace-labelled with ^{131}I.

This was prepared from commercial HSA (Cohn Fraction V, Pentex Incorporation, USA), by the method of Hunter and Greenwood[13]. The principle of this procedure depends on the addition of an oxidising substance (chloramine-T) which releases free iodine from the thiosulphate-free iodide. The iodine then combines with tyrosine groupings on the protein molecule and the labelled protein can then be

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separated from any residual ^{131}I by passing through a Sephadex G25 column (Pharmacia Fine Chemicals, Uppsala, Sweden).

Apart from albumin and thiosulphate-free radioiodide the following reagents are required:

0.1 M phosphate buffer, pH 7.5
1 mg/ml Chloramine-T in phosphate buffer
2.4 mg/ml Sodium metabisulphite in phosphate buffer
10 mg/ml Potassium iodide in phosphate buffer.

The albumin, dissolved in 0.5 ml PBS together with 0.25 ml chloramine-T were added to the isotope vial, and the contents allowed to interact for 1-3 minutes. 0.1 ml of the metabisulphite solution was then added to convert any free iodine to iodide and 0.2 ml potassium iodide to act as a carrier.

The labelled HSA was then separated on a G25 (coarse) Sephadex column previously equilibrated in 10% buffered saline. Fractions were collected in 2 ml amounts in 4 x ½" tubes.

After the radioactivity of each fraction was measured and the values plotted against the tube number, a few of the most active samples of the HSA were bulked, and the total protein and nitrogen content estimated by the method of Warburg and Christian[14]. The labelled antigen diluted with 1:100 normal serum yielded a value of 0.36 mg N/ml.

Test procedure

A series of dilutions of the test sera ranging from 1:5 to 1:80 were made using 1:10 normal serum as diluent.

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0.5 ml of each dilution was then pipetted into tubes and to four other tubes containing 0.5 ml of 1:10 normal serum, 0.5 ml of the diluted antigen were added. Two further tubes to which 0.5 ml of diluted antigen were added acted as controls. All tubes were mixed and incubated overnight at $4^{\circ}C$.

The following day 1 ml of saturated ammonium sulphate was added except to two tubes containing 1:10 normal serum; to each of these 20% TCA was added. After the addition of the SAS, the tubes were immediately mixed and incubated at $4^{\circ}C$ for 30 minutes. The samples were then centrifuged at $4^{\circ}C$ for 30 minutes, the supernatant discarded and the precipitate washed with 50% SAS and centrifuged only once. After the final washing, all of the tubes were assayed for radioactivity in a gamma detector.

The background activity was subtracted from all counts and the available activity (A) estimated from antigen added control (Ag-add) - the normal serum control precipitate tubes (Ns Ppt), while the bound activity (B) was measured from the experimental precipitate tubes (Ex Ppt) - Ns Ppt. (Figure 1).

The % of bound activity (P) = $\frac{B}{A} \times 100$.

P was plotted against the reciprocal of the antisera dilution on semi-log paper, and from the plot the reciprocal dilution of antiserum which could have precipitated exactly 33% of the antigen was determined. This dilution was designated as the Antigen-Binding Capacity (ABC-33) end point.

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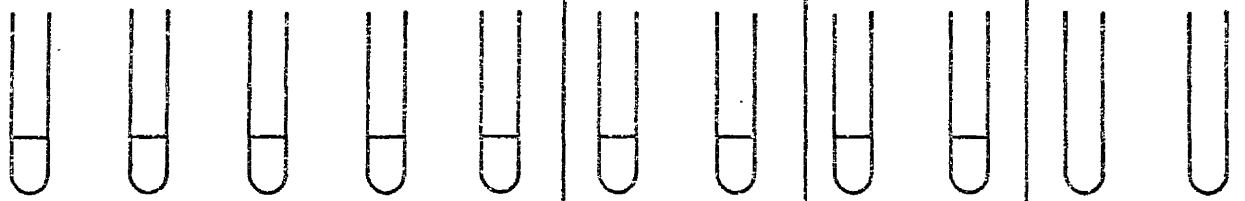
Figure 1: Experimental Procedure for the Determination of Antigen Binding Capacity.

	DILUTIONS (0.5ml)					0.5ml 1:10 Normal serum		0.5ml 1:10 Normal serum			
	1:5	1:10	1:10	1:40	1:80						
											
ANTIGEN (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	MIX AND INCUBATE					OVERNIGHT		AT		40° C	
S.A.S. (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	–	–	–	–
T.C.A. (ml)	–	–	–	–	–	–	–	1.0	1.0	–	–
	Experimental Precipitates					Normal serum Precipitate		T.C.A. Precipitate		Antigen added Control	

Since 0.5 ml of the antiserum dilution designated as the ABC-33 end point would have specifically precipitated 33% of the ^{131}I-HSA N used for this test, the ABC-33 value was calculated as follows:

(ABC-33 end point) x 2 x 0.33 x (AgN) - ^{131}I HSA N bound/ml undiluted serum at the antigen concentration employed.

Results

The mean results of this test are shown in Table 3, while the individual data are given in the Appendix. Although both of the haemoglobin types developed antibody responses to HSA the results show an apparently better immune response in the HbA animals at all the sampling times. The difference was significant by the t-test at 14 and 21 days after completion of the course of injections ($p < 0.05$).

TABLE 3.

Antigen Binding Capacity (33% end-point) of Sera of Blackface Sheep of Different Haemoglobin Types after a Course of Injections of Human Serum Albumin.

	Sheep No.	Days after last Injection 7	14	21	28
Hb type A	9	1.8±0.4	3.9±1.2	1.4±0.3	2.1±0.6
Hb type B	8	1.0±0.5	1.0±0.4	0.4±0.2	0.8±0.3
p		N.S.	<0.05	<0.05	N.S.

DISCUSSION

The parasitological and pathophysiological results presented in earlier sections of this thesis indicated that the superior resistance of HbA type animals to H. contortus was largely attributable to immunological mechanisms.

The object of the work described in the present chapter was to investigate the relationship between Hb type and response to non-parasitic antigens in Blackface and Finn Dorset sheep. No significant differences between the breeds or between the different Hb types within each breed could be demonstrated by a study of the elimination of labelled horse gamma-globulin. However, no previous studies on the application of this method to the measurement of immune response of sheep appear to have been carried out, and some basic work on this problem is necessary.

Among the nine HbA Blackface sheep tested for haemagglutinin response to rabbit red cells when titres were maximal, two were poor responders, six good responders and one intermediate in its response. Of the eight HbB animals, two were good responders and six were intermediate. The experiment on the same animals with human serum albumin provided clearer evidence of a better antibody response by the A type animals.

On the basis of these findings, a significantly better response to HSA and suggestive evidence of better response

to rabbit red cells on the part of HbA type sheep, it is tempting to postulate that some of the genes responsible for haemoglobin type may also influence certain aspects of the immune response. It must be recognised that the genetic control of the immune response is extremely complex and will operate at several different levels. Furthermore, the tests used in the present study are for circulating antibodies and therefore relate to macrophage and B-lymphocyte activity.

The ability of the host to produce antibody to any antigen is some indication of the immune competence of the host and its ability to mount an immunological reaction. This might suggest that HbA sheep which have been shown to be better responders to various antigens may also be better responders to parasitic challenge. Such a state of affairs could explain the differences in pathophysiological and parasitological findings observed in animals of different Hb types. At the moment, however, this must be regarded as speculation. Apart from the present experiments, very little appears to have been done on the possible relationship between relative resistance to parasites and immune competence in the face of other forms of antigenic stimulation.

Very recently Perrudet-Badoux, Binaghi and Biozzi[15] compared the resistance to Trichinella infection of two strains of mice recognised to be 'good' and 'poor' in terms of antibody response. They could find no difference

in parasite burdens between the groups. The authors themselves point out that their system is not ideal for such a study in that antibodies appear ten days after infection when larvae are already established in the muscle. They also emphasise a general point which is of great relevance to all such studies including the preliminary one reported in this thesis, i.e. that antibody responses give an indication of macrophage and B-lymphocyte activity only, and do not reflect cell mediated responses.

Clearly a good deal of work remains to be done before any generalisation can be made about the possibility that genetically determined resistance to any parasite is linked to general immune competence.

SUMMARY

In this section the results of some preliminary studies on the genetic control of antibody responses to non-parasitic antigens are described.

No difference was observed between HbA and HbB type sheep, or between Scottish Blackfaces and Finn Dorsets in respect of immune elimination of horse gamma globulin, but on the basis of a significantly better response to human serum albumin, and suggestive evidence of a better response to rabbit red cells in HbA than in HbB Scottish Blackface sheep, it seemed reasonable to conclude that the advantages exhibited by the latter in relation to parasitic infections were associated with a superior immunological competence.

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GENERAL SUMMARY

That some breeds of sheep and individuals within these breeds thrive better than others in parasite-infested localities has probably been recognised by stockmen for centuries, but it is only within the past 50 years, and largely as a result of field surveys that the existence of genetically-determined differences in host resistance to parasitic infections has become widely recognised. As yet remarkably little is known about the genotypes responsible for resistance or susceptibility and even less of the mechanisms involved. Within the past decade several reports have suggested that resistance to some parasites is associated with the animal's haemoglobin type, sheep with HbA being more resistant as judged by faecal egg counts and venous haematocrits than those with HbB. Since the frequency of these haemoglobin types varies in different breeds it has been tacitly assumed that inter-breed variations in resistance are related to differences in the relative haemoglobin type gene frequencies, but none of these studies has indicated whether such resistance is expressed primarily as a resistance to parasite establishment or resistance to the parasites' specific pathogenic effects.

In view of its potential practical significance it was considered worthwhile to examine the concept of breed and haemoglobin type resistance to parasitic infections and their possible relationship to the immunological status of the host under carefully controlled experimental conditions.

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The work represented in this thesis is essentially a study of the role of genetic factors in resistance to two important gastrointestinal helminth parasites of sheep, i.e. Haemonchus contortus and Ostertagia circumcincta.

The first section deals with the inter-relationship between haemoglobin type and breed and the response of sheep to primary infections with H. contortus. For this purpose worm-free Scottish Blackface and Finn Dorset sheep with different haemoglobin types were each infected with 350 H. contortus larvae/kg bodyweight and their responses monitored and compared by a combination of clinical, radioisotopic and parasitological techniques. The results obtained showed that sheep with HbA developed less severe clinical and pathophysiological disturbances, passed fewer eggs and harboured fewer worms at necropsy than animals with HbB and that Scottish Blackface sheep exhibited similar advantages over Finn Dorsets with the same haemoglobin type. Since variations in the severity of the disease as judged by pathophysiological effects correlated closely with worm numbers it was concluded (a) that genetic resistance operates at the level of parasite establishment which in turn is controlled by the immune response elicited, (b) that although HbA is a useful genetic marker for resistance, the degree of protection with which it is associated is very much influenced by other, and as yet undefined "breed" characteristics, and (c) on the basis of a second experiment demonstrating that sheep of each haemoglobin type were equally susceptible to

the establishment and pathogenic effects of H. contortus when heavily infected (1400 larvae/kg), it would appear that the magnitude of the larval intake is an additional factor involved.

The second and third sections are devoted to an examination of the influence of breed and haemoglobin type on acquired resistance to H. contortus, the former dealing with the acquisition of resistance from primary infections terminated by anthelmintic treatment, the latter with the well-known "self-cure" phenomenon. The results of the third section demonstrated that individuals and breeds with high resistance to primary infections, i.e. animals with HbA and belonging to the Scottish Blackface breed are also more resistant to reinfection than for example HbB and Finn Dorset sheep. In terms of worm establishment this resistance was no greater than that acquired during primary infections possibly due to the interruption of antigenic stimulation caused by anthelmintic treatment, but all reinfected animals were nonetheless able to seriously impair the parasites' biotic and pathogenic potential.

Attempts to induce "self-cure" of H. contortus by exposing infected sheep to reinfection or rapidly growing parasite-free grass showed that the reaction could occur under both conditions. This suggests that the phenomenon may be both immunological and non-immunological, the former in all likelihood being a manifestation of a hypersensitivity reaction in the abomasal mucosa, the latter due to the presence of an anthelmintic or anaphylactoid-type factor in the grass. However, whether produced by larvae or grass the reaction was more closely

associated with the breed than with the haemoglobin type of the animals concerned, being observed in the majority of the Scottish Blackface sheep of each haemoglobin type but in only a minority of the Finn Dorsets and Suffolks; even if unable to expel their existing worm populations most sheep were able to impair the reproductive and haematophagic activities of parasites derived from subsequent infections.

The experiment reported in the fourth section was designed to ascertain whether the response of sheep to non-haematophagic parasites is also related to their haemoglobin type. For this purpose HbA and HbB Scottish Blackface sheep were individually infected with 100,000 Ostertagia circumcincta larvae; resistance to the subsequent disease was compared by biochemical and radioisotopic methods and resistance to worm establishment by measurement of worm counts 16 days after infection. In terms of disease no clear difference was observed between the groups, but the presence of smaller numbers of adult worms and more inhibited larvae in HbA than in HbB sheep was suggestive of a better immune response on the part of the former.

The final section examines the genetic control of antibody production to non-parasitic antigens. No difference was observed between HbA and HbB type sheep, or between Scottish Blackfaces and Finn Dorsets in respect of immune elimination of horse gamma globulin, but on the basis of a significantly better response to human serum albumin, and

suggestive evidence of a better response to rabbit red cells in HbA than in HbB Scottish Blackface sheep, it seemed reasonable to conclude that the advantages exhibited by the former in relation to parasitic infections were associated with a superior immunological competence.

APPENDICES

APPENDIX 1

(A) The Influence of Haemoglobin Type and Breed on the Response of Sheep to Infection with 350 *H. contortus* Larvae/kg Bodyweight.

TABLE 1.

Packed cell volumes (%) following infection with 350 *H. contortus* larvae/kg.

	Sheep No.	0	4	7	11	14	18	21	25	28	32
Blackface HbA	34	33.0	29.0	29.0	30.0	27.0	22.0	21.0	22.0	22.0	20.0
	51	32.0	31.0	31.0	31.0	30.0	24.0	21.0	23.0	21.0	22.0
	60	33.0	30.0	31.0	30.0	30.0	25.0	24.0	23.0	21.0	23.0
	71	33.0	30.0	30.0	26.0	26.0	20.0	19.0	19.0	19.0	19.0
Mean		32.7	30.0	30.3	29.3	28.3	22.8	21.3	21.8	20.8	21.0
S.E.		0.3	0.4	0.5	1.1	1.0	1.1	1.0	0.9	0.6	0.9
Blackface HbAB	48	36.0	32.0	29.0	27.0	24.0	18.0	17.0	17.0	19.0	15.0
	54	30.0	30.0	27.0	26.0	25.0	18.0	17.0	20.0	19.0	19.0
	79	28.0	26.0	25.0	24.0	20.0	20.0	14.0	*	*	*
	93	28.0	28.0	26.0	26.0	23.0	18.0	17.0	17.0	16.0	16.0
Mean		30.5	29.0	26.8	25.8	23.0	18.5	16.3	18.0	18.0	16.7
S.E.		1.9	1.3	0.9	0.6	1.1	0.5	0.8	1.0	1.0	1.2
Blackface HbB	53	30.0	28.0	27.0	25.0	23.0	18.0	16.0	16.0	16.0	16.0
	85	29.0	31.0	28.0	26.0	24.0	18.0	17.0	15.0	16.0	15.0
Mean		29.5	29.5	27.5	25.5	23.5	18.0	16.5	15.5	16.0	15.5
S.E.		0.5	1.5	0.5	0.5	0.5	0.0	0.5	0.5	0.0	0.5

* Died on day 23

TABLE 2.

Packed cell volumes (%) following infection with 350 *H. contortus* larvae/kg.

	Sheep No.	0	4	7	11	14	18	21	25	28	32
Dorset HbAB	473	28.0	29.0	27.0	25.0	24.0	22.0	19.0	19.0	20.0	18.0
	485	36.0	35.0	34.0	31.0	27.0	23.0	18.0	16.0	14.0	13.0
	514	31.0	32.0	31.0	34.0	27.0	18.0	19.0	17.0	17.0	15.0
	520	37.0	33.0	31.0	30.0	27.0	27.0	19.0	16.0	17.0	15.0
Mean		33.0	32.3	30.8	30.0	26.3	22.5	18.8	17.0	17.0	15.3
S.E.		2.1	1.3	1.4	1.9	0.8	1.8	0.3	0.7	1.2	1.0
Dorset HbB	480	32.0	34.0	29.0	27.0	22.0	21.0	15.0	15.0	16.0	12.0
	489	32.0	30.0	27.0	23.0	25.0	18.0	16.0	14.0	15.0	13.0
	501	36.0	34.0	30.0	31.0	24.0	17.0	15.0	14.0	15.0	13.0
	541	32.0	27.0	30.0	28.0	26.0	20.0	19.0	16.0	16.0	12.0
Mean		33.0	31.3	29.0	27.3	24.3	19.0	16.3	14.8	15.5	12.5
S.E.	1.0	1.0	1.7	0.7	1.7	0.9	0.9	0.9	0.5	0.3	0.3

TABLE 3.

Red cell counts ($X10^6$) following infection with 350 *H. contortus* larvae/kg.

	Sheep No.	Days after infection 0	4	7	11	14	18	21	25	28	32
Blackface HbA	34	9.4	8.2	8.2	8.3	6.7	5.9	5.9	4.6	4.6	5.0
	51	10.0	8.9	8.5	8.0	7.4	5.7	5.6	5.5	5.4	5.4
	60	10.3	9.6	8.9	8.0	7.0	6.7	6.1	5.4	5.3	4.9
	71	10.3	9.6	8.9	7.9	7.1	5.3	4.5	4.4	4.4	4.6
Mean		10.0	9.1	8.6	8.1	7.1	5.9	5.5	4.9	4.9	5.0
S.E.		0.2	0.3	0.2	0.1	0.1	0.3	0.4	0.3	0.2	0.2
Blackface HbAB	48	10.1	9.9	9.3	9.4	6.3	5.6	4.7	3.9	4.6	3.2
	54	9.1	9.0	7.6	7.4	7.8	5.3	4.5	4.3	4.5	3.3
	79	7.9	7.8	6.9	7.4	5.7	4.5	3.7	*	*	*
	93	8.5	8.4	6.8	7.2	6.2	5.2	4.9	4.4	3.9	3.3
Mean		8.9	8.8	7.6	7.9	6.5	5.2	4.4	4.2	4.3	3.3
S.E.		0.5	0.5	0.6	0.5	0.4	0.2	0.2	0.2	0.2	0.0
Blackface HbB	53	8.1	8.6	8.2	7.8	6.2	5.8	4.9	4.1	3.6	3.1
	85	9.9	9.1	8.9	8.5	7.0	5.0	5.4	4.0	3.5	3.2
Mean		9.0	8.9	8.6	8.2	6.6	5.4	5.1	4.1	3.6	3.2
S.E.		0.9	0.2	0.4	0.4	0.4	0.4	0.3	0.1	0.1	0.0

* Died day 23

TABLE 4.

Red cell counts ($X10^6$) following infection with 350 *H. contortus* larvae/kg.

	Sheep No.	Days after infection 0	4	7	11	14	18	21	25	28	32
Dorset HbAB	473	8.8	8.3	8.0	8.1	8.1	6.3	5.7	5.2	4.5	4.0
	485	10.9	10.3	8.5	8.0	7.4	7.4	5.4	3.7	3.5	3.3
	514	8.9	9.3	8.8	8.3	7.7	6.9	5.6	4.1	3.4	3.3
	520	10.8	10.5	8.4	9.4	9.1	6.5	5.4	4.1	3.5	3.5
Mean		9.9	9.6	8.4	8.4	8.1	6.8	5.5	4.3	4.2	4.0
S.E.		0.6	0.5	0.2	0.3	0.4	0.2	0.1	0.4	0.2	0.3
Dorset HbB	480	10.7	9.8	8.3	8.7	6.4	5.0	4.4	3.3	3.4	2.4
	489	9.2	8.3	8.1	8.0	7.5	5.2	4.5	3.6	3.2	2.7
	501	9.8	9.7	8.9	8.0	6.5	5.0	3.3	3.5	3.8	3.1
	541	9.7	10.5	9.9	9.5	8.9	6.8	4.6	3.1	3.1	2.1
Mean		9.9	9.6	8.8	8.5	7.3	5.7	4.9	4.2	3.4	2.5
S.E.		0.3	0.5	0.4	0.4	0.6	0.4	0.3	0.1	0.1	0.2

TABLE 5.

Haemoglobin concentrations (g%) following infection with 350 *H. contortus* larvae/kg.

	Sheep No.	Days after infection 0	4	7	11	14	18	21	25	28	32
Blackface HbA	34	10.7	9.5	9.6	9.8	7.7	6.7	6.8	7.0	6.5	6.1
	51	10.7	10.0	10.2	10.3	8.8	7.4	6.4	7.1	6.8	6.3
	60	11.4	9.3	9.8	10.2	9.1	8.1	7.4	7.4	6.8	6.8
	71	10.8	8.8	8.8	8.8	7.5	7.0	6.7	7.0	5.7	5.7
Mean		10.9	9.4	9.6	9.8	8.3	7.3	6.8	7.1	6.5	6.2
S.E.		0.2	0.2	0.3	0.3	0.4	0.3	0.2	0.1	0.3	0.2
Blackface HbAB	48	12.1	10.9	11.3	9.5	7.8	6.1	5.5	5.3	5.9	4.3
	54	10.7	10.3	10.0	9.2	8.2	6.6	5.8	7.0	6.6	5.5
	79	9.5	9.0	8.5	8.3	6.3	5.1	4.5	*	*	*
	93	9.5	9.5	8.9	8.3	7.5	6.4	5.7	6.3	5.7	4.9
Mean		10.5	9.9	9.7	8.8	7.5	6.1	5.4	6.2	6.1	4.9
S.E.		0.6	0.4	0.6	0.3	0.4	0.3	0.3	0.5	0.3	0.3
Blackface HbB	53	10.7	10.1	9.7	9.2	8.5	6.6	6.0	5.9	5.6	4.0
	85	10.3	11.6	10.3	9.0	8.5	6.7	6.3	5.1	4.8	4.1
Mean		10.5	10.9	10.0	9.1	8.5	6.6	6.1	5.5	5.2	4.0
S.E.		0.3	0.8	0.3	0.1	0.0	0.1	0.2	0.4	0.4	0.1

* Died day 23

TABLE 6.

Haemoglobin concentration (g%) following infection with 350 *H. contortus* larvae/kg.

	Sheep No.	Days after infection 0	4	7	11	14	18	21	25	28	32
Dorset HbAB	473	9.7	9.5	9.1	8.7	7.9	7.6	6.7	5.0	4.8	4.4
	485	12.5	11.7	11.6	11.2	9.3	8.1	6.2	5.0	4.2	4.2
	514	10.7	11.2	11.0	11.7	9.4	8.2	6.8	5.3	5.7	4.4
	520	12.7	11.7	11.3	12.0	9.5	7.5	6.8	5.6	4.9	4.5
Mean		11.4	11.0	10.8	10.9	9.0	7.9	6.6	5.2	4.9	4.4
S.E.		0.7	0.5	0.6	0.8	0.4	0.2	0.1	0.1	0.3	0.1
Dorset HbB	480	11.5	11.7	10.8	9.9	8.3	6.4	5.3	5.0	4.4	3.2
	489	11.2	9.2	9.5	9.3	8.7	7.4	6.2	6.1	4.7	4.1
	501	12.8	11.8	11.3	11.4	8.1	6.5	5.1	5.7	4.9	4.2
	541	10.9	11.9	11.1	11.1	10.3	8.1	6.3	5.8	4.7	4.2
Mean		11.6	11.2	10.7	10.4	8.9	7.1	5.7	5.7	4.7	3.9
S.E.		0.4	0.7	0.4	0.5	0.5	0.4	0.3	0.2	0.1	0.2

TABLE 7.

Mean corpuscular volumes (μ^3) following infection with 350 *H. contortus* larvae /kg.

	Sheep No.	Days after infection 0	4	7	11	14	18	21	25	28	32
	473	32.0	34.9	33.7	30.8	29.6	34.7	34.1	36.4	44.4	45.0
Dorset	485	33.2	34.0	40.1	37.5	36.3	30.9	33.5	43.0	40.0	39.4
HbAB	514	36.0	34.3	35.3	36.1	35.0	26.2	34.1	41.5	50.0	45.5
	520	34.4	31.4	36.8	31.3	29.6	40.6	35.1	43.5	48.5	42.9
Mean		33.9	33.7	36.5	33.9	32.6	33.1	34.2	41.1	45.8	43.2
S.E.		0.9	0.8	1.4	1.7	1.8	3.0	0.3	2.2	2.2	1.4
	480	29.8	34.6	34.8	31.2	34.4	42.0	34.4	46.0	46.7	49.1
Dorset	489	34.6	36.1	33.3	28.9	34.4	34.5	35.8	38.5	46.9	47.8
HbB	501	36.9	35.0	33.7	38.9	36.7	34.3	45.1	42.9	39.4	42.2
	541	33.1	25.8	30.2	29.5	29.2	29.6	41.4	51.0	51.6	56.6
Mean		33.6	32.9	33.0	32.1	33.7	35.1	39.2	44.6	46.2	48.9
S.E.		1.5	2.4	1.0	2.3	1.6	3.8	1.3	1.8	0.6	1.7
	34	35.0	35.5	35.5	36.1	40.2	38.3	35.8		48.2	40.2
Blackface	51	32.1	34.9	35.2	38.7	39.0	42.5	37.5	34.5	39.2	39.5
HbA	60	32.0	32.0	34.2	37.5	42.6	37.1	39.7	42.9	39.6	40.7
	71	32.1	32.0	33.9	33.5	36.8	37.6	42.4	42.0	40.3	41.1
Mean		32.8	33.6	34.7	36.5	39.7	38.9	38.9	39.8	41.8	40.4
S.E.		0.7	0.9	0.4	1.7	1.2	1.2	1.4	2.7	2.1	0.3
	48	35.6	32.4	31.3	28.7	28.0	32.3	36.5	43.4	44.4	45.0
Blackface	54	33.0	33.3	35.8	34.9	32.1	33.7	36.8	46.1	36.6	36.9
HbAB	79	35.5	33.5	36.2	32.4	34.8	44.8	37.4			
	93	32.9	33.4	38.5	36.3	36.9	34.6	35.0	38.6	40.6	49.1
Mean		34.3	33.2	35.5	33.1	33.0	36.4	36.4	42.7	40.5	43.7
S.E.		0.8	0.3	1.5	1.7	1.9	2.9	0.5	2.2	2.3	3.6
Blackface	53	37.0	32.4	33.0	31.9	29.1	30.8	32.8	38.6	44.4	51.0
HbB	85	29.1	34.1	31.4	30.4	35.5	35.9	31.6	37.8	45.5	47.5
Mean		33.0	33.3	32.2	31.1	32.3	33.4	32.2	38.2	45.0	49.3
S.E.		4.0	0.9	0.8	0.8	3.2	2.6	0.6	0.4	0.6	1.8

TABLE 8.

Mean corpuscular haemoglobin concentrations (%) following infection with 350 H. contortus larvae/kg.

	Sheep No.	Days after infection 0	4	7	11	14	18	21	25	28	32
	473	34.6	32.8	33.7	34.8	32.9	34.5	34.4	26.3	24.0	24.4
Dorset	485	40.3	33.4	34.1	36.1	34.4	35.2	34.4	41.3	30.0	32.3
HbAB	514	33.4	35.0	35.5	34.8	34.8	45.6	35.8	29.4	33.5	29.3
	520	34.3	35.5	36.5	40.0	35.2	27.8	35.8	31.1	28.8	30.0
Mean		35.7	34.2	35.0	36.3	34.3	35.8	35.1	32.0	29.1	29.0
S.E.		1.6	0.6	0.6	1.3	0.5	3.7	0.4	3.2	2.0	1.7
	480	35.9	34.4	38.5	36.7	37.7	30.5	35.3	33.3	27.5	26.7
Dorset	489	35.0	30.7	33.9	40.4	34.8	41.1	38.8	34.0	31.3	31.5
HbB	501	37.6	34.7	37.7	36.8	33.8	38.2	34.0	40.7	32.7	32.3
	541	40.4	44.1	37.0	39.6	39.6	40.5	33.2	30.5	29.4	25.0
Mean		37.2	36.0	36.8	38.4	36.5	37.6	35.3	34.6	30.2	28.8
S.E.		1.2	2.9	1.0	1.0	1.3	2.4	1.2	2.2	1.1	1.8
	34	32.4	32.8	33.1	32.7	28.5	29.8	32.4	31.8	29.5	30.5
Blackface	51	33.4	32.3	32.9	33.2	30.2	30.8	30.5	29.6	32.4	30.0
HbA	60	34.5	31.0	32.1	34.0	30.3	32.4	30.8	32.2	32.4	29.6
	71	32.7	29.3	29.3	33.2	28.8	35.0	35.3	37.8	30.0	30.8
Mean		33.3	31.4	31.9	33.3	29.5	32.0	32.3	32.9	31.1	30.2
S.E.		0.5	0.8	0.9	0.3	0.5	1.1	1.1	1.7	0.8	0.3
	48	33.6	34.1	39.0	35.2	32.5	33.9	32.4	31.2	31.1	28.7
Blackface	54	35.7	34.3	37.0	35.4	32.8	36.7	35.2	35.0	34.7	28.9
HbAB	79	33.9	34.6	34.0	34.6	31.5	25.5	32.1			
	93	33.9	33.9	34.2	31.9	32.6	35.6	33.5	37.1	35.6	30.6
Mean		34.3	34.2	36.0	34.3	32.4	32.9	33.3	34.4	33.8	29.4
S.E.		0.5	0.1	1.2	0.8	0.3	2.5	0.7	1.7	1.4	0.6
Blackface	53	36.2	36.1	35.9	36.8	37.0	30.6	37.5	36.9	31.1	25.0
HbB	85	35.5	37.4	36.7	34.6	35.4	37.2	37.1	32.0	28.2	27.3
Mean		35.9	36.8	36.3	35.7	36.2	33.9	37.3	34.5	29.7	26.1
S.E.		0.4	0.7	0.4	1.1	0.8	3.3	0.2	2.5	1.5	1.2

TABLE 9.

Serum iron concentrations (µg%) following infection with 350 H. contortus larvae/kg.

	Sheep No.	Days after infection 0	7	14	21	28	32
Blackface HbA	34	125	122	138	114	112	110
	51	144	140	110	82	106	100
	60	156	171	163	161	165	160
	71	125	121	113	96	110	80
Mean		138	139	131	113	123	113
S.E.		8	12	12	17	18	17
Blackface HbAB	48	139	133	119	68	76	70
	54	131	131	144	126	125	120
	79	159	140	113	114	*	*
	93	131	140	110	110	114	112
Mean		140	136	122	105	105	101
S.E.		7	2	8	13	15	16
Blackface HbB	53	127	133	123	115	121	118
	85	130	105	98	102	70	65
Mean		128	119	111	109	96	92
S.E.		2	14	13	7	26	26
Dorset HbAB	473	163	152	123	119	108	100
	485	177	160	173	68	71	69
	514	128	133	123	76	105	76
	520	165	157	135	117	127	116
Mean		158	151	139	95	103	90
S.E.		11	6	12	13	12	11
Dorset HbB	480	169	164	104	82	75	50
	489	167	183	154	141	12?	102
	501	150	164	117	134	100	90
	541	160	145	92	132	133	123
Mean		162	164	117	122	108	91
S.E.		4	8	13	14	13	15

* Died day 23

TABLE 10.

Total serum proteins (g%) following infection with 350 H. contortus larvae/kg.

	Sheep No.	Days after infection 0	7	14	21	28	32
Blackface HbA	34	6.50	6.75	6.60	5.70	5.50	5.30
	51	6.20	6.19	5.65	5.45	5.15	4.80
	60	6.40	6.70	6.45	6.30	5.30	5.30
	71	6.60	6.35	6.55	5.40	5.40	5.40
Mean		6.44	6.50	6.31	5.71	5.34	5.20
S.E.		0.09	0.14	0.22	0.21	0.07	0.14
Blackface HbAB	48	6.69	6.05	5.25	4.96	5.10	4.88
	54	5.80	5.94	5.50	5.28	4.91	4.80
	79	6.71	6.31	5.55	5.15	*	*
	93	6.30	5.88	5.15	5.50	5.11	4.70
Mean		6.38	6.05	5.36	5.22	5.04	4.79
S.E.		0.21	0.10	0.10	0.11	0.07	0.05
Blackface HbB	53	5.90	6.28	5.90	4.90	5.15	4.88
	85	6.18	6.15	6.20	5.75	4.75	4.60
Mean		6.04	6.22	6.05	5.33	4.95	4.74
S.E.		0.14	0.07	0.15	0.43	0.20	0.01
Dorset HbAB	473	6.00	5.55	5.50	4.66	4.65	4.70
	485	6.20	5.45	5.49	4.65	4.70	4.70
	514	6.31	6.20	5.55	4.30	4.20	4.00
	520	6.11	5.85	5.66	4.95	4.50	4.20
Mean		6.16	5.76	5.55	4.64	4.51	4.40
S.E.		0.07	0.17	0.04	0.13	0.11	0.18
Dorset HbB	480	5.80	6.05	5.62	5.25	4.30	3.80
	489	5.54	5.60	4.65	4.56	4.25	4.30
	501	6.30	6.25	6.00	5.00	4.45	4.00
	541	5.71	5.90	5.76	4.90	4.40	4.20
Mean		5.84	5.95	5.52	4.93	4.35	4.08
S.E.		0.16	0.14	0.30	0.14	0.05	0.11

* Died day 23

TABLE 11.

Serum albumin (g%) following infection with 350 *H. contortus* larvae/kg.

	Sheep No.	Days post-infection 0	7	14	21	28	32
Dorset HbAB	473	3.20	3.25	2.96	2.90	2.75	2.20
	485	3.30	3.30	3.05	2.88	2.35	1.80
	514	3.00	2.92	2.55	2.60	2.32	1.90
	520	3.70	3.39	3.11	2.79	2.40	1.90
Mean		3.30	3.22	2.92	2.79	2.46	1.95
S.E.		0.15	0.10	0.13	0.07	0.17	0.09
Dorset HbB	480	3.30	3.45	3.20	2.74	2.30	1.50
	489	3.00	3.29	3.00	2.60	1.91	1.82
	501	3.80	3.45	3.39	3.21	2.50	2.00
	541	3.30	2.95	3.05	2.98	2.65	2.10
Mean		3.35	3.24	3.16	2.88	2.34	1.86
S.E.		0.17	0.12	0.09	0.13	0.16	0.13
Blackface HbA	34	3.62	3.42	2.85	2.73	3.01	2.70
	51	3.50	3.51	3.45	3.05	3.15	3.00
	60	3.80	3.62	3.55	3.45	2.86	3.00
	71	3.45	3.35	2.75	3.01	3.00	2.70
Mean		3.58	3.48	3.15	3.06	3.01	2.85
S.E.		0.05	0.06	0.20	0.15	0.11	0.09
Blackface HbAB	48	3.60	3.70	3.00	2.75	2.45	2.30
	54	2.90	2.90	2.72	2.80	2.50	2.41
	79	3.48	3.42	3.25	2.99	*	*
	93	2.80	2.45	2.50	2.40	2.05	1.70
Mean		3.25	3.12	2.87	2.74	2.33	2.14
S.E.		0.15	0.28	0.16	0.12	0.14	0.22
Blackface HbB	53	3.69	3.55	3.25	2.75	2.75	2.20
	85	2.90	2.90	2.70	2.67	2.20	1.80
Mean		3.30	3.23	2.98	2.71	2.48	2.00
S.E.		0.35	0.33	0.28	0.04	0.08	0.20

* Died day 23

TABLE 12.

Serum globulins (g%) following infection with 350 H. contortus larvae/kg.

	Sheep No.	Days after infection 0	7	14	21	28	32
Blackface HbA	34	2.88	3.33	3.75	2.97	2.49	2.60
	51	2.70	2.68	2.20	2.40	2.00	1.80
	60	2.60	3.08	2.90	2.85	2.44	2.30
	71	3.15	3.00	2.75	2.38	2.40	2.70
Mean		2.83	3.02	2.90	2.65	2.33	2.35
S.E.		0.12	0.13	0.32	0.15	0.11	0.20
Blackface HbAB	48	3.09	2.35	2.25	2.21	2.65	2.58
	54	2.90	3.04	2.78	2.48	2.41	2.39
	79	3.23	2.89	2.30	2.16	*	*
	93	3.50	3.43	2.65	3.10	3.06	3.00
Mean		3.18	2.93	2.50	2.49	2.71	2.66
S.E.		0.13	0.22	0.13	0.22	0.19	0.18
Blackface HbB	53	2.21	2.73	2.65	2.15	2.40	2.68
	85	3.28	3.25	3.50	3.08	2.55	2.80
Mean		2.75	2.99	3.08	2.62	2.48	2.74
S.E.		0.54	0.26	0.43	0.47	0.08	0.06
Dorset HbAB	473	2.80	2.30	2.54	1.76	1.90	2.50
	485	2.90	2.51	2.44	1.77	2.35	2.90
	514	3.31	3.20	3.00	1.70	1.88	2.10
	520	2.41	2.46	2.55	2.16	2.10	2.30
Mean		2.89	2.62	2.63	1.85	2.06	2.45
S.E.		0.19	0.20	0.12	0.11	0.11	0.17
Dorset HbB	480	2.50	2.60	2.42	2.51	2.00	2.30
	489	2.54	2.31	2.65	1.96	2.34	2.48
	501	2.50	2.80	2.61	1.79	1.95	2.00
	541	2.41	2.95	2.71	1.92	1.75	2.10
Mean		2.49	2.67	2.60	2.05	2.01	2.22
S.E.		0.03	0.14	0.06	0.16	0.12	0.11

* Died on day 23

TABLE 13.

Percentage of Haemoglobin C in the blood following infection with 350 H. contortus larvae/kg.

	Sheep No.	Weeks after infection 0	1	2	3	4
Blackface HbA	34	2	3	4	9	13
	51	3	2	3	10	15
	60	2	1	3	8	10
	71	4	3	5	12	18
Mean		2.8	2.3	3.8	9.8	14.0
S.E.		0.5	0.5	0.5	0.9	1.7
Blackface HbAB	48	-	-	-	3	11
	54	-	-	-	4	6
	79	-	-	-	15	*
	93	-	-	-	2	13
Mean		-	-	-	6.0	10.0
S.E.		-	-	-	3.0	2.1
Dorset HbAB	473	-	-	-	5	8
	485	1	-	2	7	12
	514	-	-	-	4	7
	520	2	-	1	4	8
Mean		0.8	-	0.8	5.0	8.8
S.E.		0.5	-	0.5	0.7	1.1

* Died

Dorset and Blackface HbB negative throughout.

TABLE 14.

Faecal egg output ($X10^6$) of Scottish Blackface sheep infected with 350 H. contortus larvae/kg.

Days after infection	HbA Group					HbAB Group					HbB Group		
	34	51	60	71	Mean ±S.E.	48	54	79	93	Mean ±S.E.	53	85	Mean ±S.E.
16		All negative					All negative				0	0.01	0.01±0.01
17		All negative					All negative				0	0.60	0.30±0.30
18	0.30	0.30	0.60	0.70	0.50±0.10	0	0	0.03	0.03	0.02±0.01	1.10	0.90	1.00±0.10
19	1.50	1.20	0.40	2.80	1.50±0.50	0.50	0.50	0.20	0.02	0.30±0.10	2.90	2.20	2.60±0.30
20	2.70	3.10	1.10	6.10	3.30±1.00	0.50	1.90	1.30	0.50	1.10±0.30	4.90	2.00	3.50±1.50
21	1.00	2.80	1.00	6.40	2.80±1.30	2.20	2.50	3.30	1.00	2.30±0.50	9.80	2.20	6.00±3.80
22	3.20	3.10	8.50	5.50	5.10±1.30	4.20	3.90	*	3.20	3.80±0.30	10.20	7.50	8.90±1.40
23	6.70	5.90	8.90	4.30	6.50±1.00	5.50	16.60		5.70	9.30±3.70	11.00	7.80	9.40±1.60
24	5.90	9.10	13.20	8.50	9.20±1.50	8.50	15.20		8.80	10.80±2.20	11.00	8.30	9.70±1.40
25	6.70	9.60	19.40	12.40	12.00±2.70	12.80	13.00		8.30	11.40±1.50	13.60	10.00	11.80±1.80
26	4.90	9.40	18.30	13.20	11.50±2.80	16.90	17.60		10.70	15.10±2.20	13.70	18.80	16.20±2.60
27	5.60	8.40	16.60	13.70	11.10±2.50	14.50	17.80		11.10	14.60±1.90	14.60	18.90	16.80±2.10
28	5.20	10.60	16.70	14.80	11.80±2.50	20.70	12.90		11.10	14.90±3.00	14.10	14.10	14.10±0.00
29	5.20	14.70	11.10	11.90	10.70±2.00	24.60	14.40		10.30	16.40±4.30	15.40	19.30	17.40±1.90
30	6.70	14.70	16.60	12.50	12.70±2.00	24.50	16.70		10.40	17.20±4.10	15.70	19.70	17.70±2.00
31	6.80	14.00	13.40	11.40	11.40±1.60	22.30	12.30		10.70	15.10±3.60	15.80	16.30	16.10±0.30
32	6.70	10.60	14.20	11.80	10.80±1.60	25.40	14.40		10.70	16.80±4.40	15.40	17.30	16.40±0.90

* Died day 23

TABLE 15.

Faecal egg output ($X10^6$) of Finn Dorset sheep infected with 350 *H. contortus* larvae/kg.

Days after infection	HbAB Group					HbB Group				
	473	485	514	520	Mean ± S.E.	480	489	501	541	Mean ± S.E.
16	0	0	0	0.04	0.01 ± 0.01		All negative			
17	0	0.01	0.01	0.03	0.01 ± 0.01	0.06	0	0.03	0.02	0.03 ± 0.01
18	0.01	0.80	1.60	4.20	1.70 ± 0.90	1.70	0	0.04	2.10	1.00 ± 0.50
19	1.60	3.20	6.30	7.80	4.70 ± 1.40	3.60	0.30	8.90	4.50	4.30 ± 1.80
20	6.30	4.00	8.40	10.90	7.40 ± 1.50	5.50	1.20	12.60	16.70	9.00 ± 3.50
21	8.40	5.80	9.00	10.90	8.50 ± 1.10	5.70	2.40	12.70	16.70	9.40 ± 3.30
22	9.00	6.10	10.90	11.00	9.30 ± 1.20	8.50	3.70	13.20	14.70	10.00 ± 2.50
23	10.90	6.70	7.70	22.20	11.90 ± 3.60	13.00	4.50	17.60	12.80	12.00 ± 2.70
24	7.70	14.90	12.50	20.30	13.90 ± 2.60	16.00	7.40	25.30	10.20	14.70 ± 4.00
25	12.50	16.00	18.00	17.50	16.00 ± 1.20	16.50	7.00	39.60	24.50	21.90 ± 6.90
26	14.30	16.60	17.30	19.20	16.90 ± 1.00	26.20	8.00	30.50	29.30	23.50 ± 5.20
27	13.10	21.90	18.90	19.80	18.40 ± 1.90	26.70	12.80	29.70	29.80	24.80 ± 5.00
28	12.50	19.40	20.50	17.20	17.40 ± 1.80	24.80	16.90	39.10	29.60	27.60 ± 4.60
29	10.20	20.80	16.50	16.50	16.00 ± 2.20	29.40	22.40	35.20	25.40	28.10 ± 2.80
30	10.60	20.70	16.50	22.50	17.60 ± 2.60	30.20	18.90	30.60	32.10	28.00 ± 3.00
31	9.70	22.60	16.60	22.00	17.70 ± 3.00	37.30	18.60	35.30	32.60	31.00 ± 4.20
32	12.40	23.40	14.30	23.20	18.30 ± 2.90	35.80	17.40	39.10	32.60	31.20 ± 4.80

TABLE 16.

Bodyweights and blood volumes of Scottish Blackface sheep.

	Sheep No.	Wt. (kg)	RCV (ml)	RCV (ml/kg)	VP (ml)	VP (ml/kg)	BV (ml)	BV (ml/kg)
Before infection								
	34	25.9	489	18.9	1038	40.1	1527	59.0
Haemoglobin	51	25.0	478	19.1	968	38.7	1446	57.8
A	60	27.7	495	17.9	1052	38.0	1547	55.9
	71	23.6	448	19.0	910	38.6	1058	57.6
Mean		25.6	478	18.7	992	38.9	1470	57.6
S.E.		0.9	10	0.3	33	0.4	43	0.6
	48	26.8	438	16.3	931	34.7	1369	51.0
Haemoglobin	54	21.4	362	16.9	770	36.0	1132	52.9
AB	79	21.5	330	15.4	771	35.9	1101	51.3
	93	19.6	329	16.8	708	36.1	1037	52.9
Mean		22.3	365	16.4	795	35.6	1160	52.0
S.E.		1.6			48	0.3	72	
Haemoglobin	53	22.7	338	14.9	827	36.4	1165	51.3
B	85	21.0	365	17.4	812	38.7	1177	56.1
Mean		21.9	352	16.2	820	37.6	1172	53.8
S.E.		0.9	14	1.3	8	1.2	6	2.4
28 days after infection								
	34	21.4	345	16.1	1035	48.4	1380	64.5
Haemoglobin	51	24.5	296	12.1	871	35.6	1167	47.7
A	60	28.2	287	10.2	1018	36.1	1305	46.3
	71	25.0	194	7.8	885	35.4	1079	43.2
Mean		24.8	281	11.6	952	38.9	1233	50.5
S.E.		1.4	32	1.8	43	3.2	68	4.8
	48	26.7	198	7.4	902	33.8	1100	41.2
Haemoglobin	54	22.5	200	8.9	800	35.6	1000	44.5
AB	79	*	*	*	*	*	*	*
	93	18.2	127	7.0	665	36.5	792	43.5
Mean		22.4	175	7.8	789	35.3	964	43.1
S.E.		1.6	24	0.6	69	0.8	91	0.8
Haemoglobin	53	22.3	161	7.2	765	34.3	926	41.8
B	85	21.8	182	8.3	887	40.7	1069	49.0
Mean		22.1	172	7.8	826	37.5	998	45.4
S.E.		0.3	11	0.6	61	3.2	72	3.6

* Died day 23

TABLE 17.

Bodyweights and blood volumes of Finn Dorset Sheep.

	Sheep No.	Wt. (kg)	RCV (ml)	RCV (ml/kg)	VP (ml)	VP (ml/kg)	BV (ml)	BV (ml/kg)
Before infection								
Haemoglobin AB	473	25.5	492	19.2	1204	47.2	1696	66.4
	485	27.3	554	20.3	984	36.0	1538	56.3
	514	23.6	478	20.2	1011	42.8	1489	62.8
	520	26.4	498	18.9	1106	41.9	1604	60.8
Mean		25.7	506	19.7	1076	42.0	1582	61.6
S.E.		0.8	16.7	0.3	50	2.3	45	2.1
Haemoglobin B	480	25.9	521	20.1	1037	40.0	1558	60.1
	489	25.5	496	19.5	1104	43.3	1600	62.8
	501	29.1	488	16.8	1241	42.6	1729	59.4
	541	21.8	349	16.0	1011	46.3	1460	62.3
Mean		25.6	464	18.1	1098	43.1	1587	61.2
S.E.		1.5	39	0.1	52	1.3	56	0.8
28 days after infection								
Haemoglobin AB	473	25.0	247	9.9	988	39.5	1235	49.4
	485	25.0	168	6.7	1029	41.2	1197	47.9
	514	22.7	203	8.9	875	38.5	1078	47.4
	520	26.4	179	6.8	997	37.8	1176	44.6
Mean		24.8	199	8.1	972	39.3	1172	47.3
S.E.		0.7	18	0.8	34	0.7	34	1.0
Hamoglobin B	480	27.7	163	5.9	987	35.6	1150	41.5
	489	25.2	157	6.2	962	38.1	1119	44.3
	501	30.5	161	5.3	1165	38.2	1326	43.5
	541	22.7	182	8.0	858	37.8	1040	45.8
Mean		26.5	166	6.4	993	37.4	1159	43.8
S.E.		1.7	6	0.6	64	0.6	60	0.9

TABLE 18.

Influence of H. contortus on red cell survival. Apparent ^{51}Cr red cell half-life (hrs) at various stages of infection.

	Sheep No.	Initial 7 days of infection	21-28 days after infection
Blackface HbA	34	396	230
	51	463	165
	60	367	228
	71	372	173
Mean		400	199
S.E.		22	17
Blackface HbAB	48	352	132
	54	388	120
	79	377	*
	93	404	144
Mean		380	132
S.E.		11	7
Blackface HbB	53	348	168
	85	324	75
Mean		336	122
S.E.		12	47
Dorset HbAB	473	324	144
	485	360	96
	514	380	120
	520	380	124
Mean		361	121
S.E.		13	10
Dorset HbB	480	330	65
	489	333	132
	501	372	96
	540	360	100
Mean		349	98
S.E.		10	14

* Died day 23

TABLE 19.

Faecal blood clearance (ml/day) following infection with 350 *H. contortus* larvae/kg.

Days after infection	Blackface HbA				Mean	Blackface HbAB				Mean	Blackface HbB		Mean
	34	51	60	71	±S.E.	48	54	79	93	± S.E.	53	85	± S.E.
4	1.4	3.1	2.0	2.3	2.2± 0.4	1.6	0.2	1.5	3.9	1.8± 0.8	1.8	1.7	1.8± 0.1
6	2.1	2.5	2.0	2.0	2.2± 0.1	1.3	0.2	1.5	2.9	1.5± 0.6	2.2	1.6	1.9± 0.3
8	2.3	3.2	2.3	4.5	3.1± 0.5	2.4	2.6	1.7	1.2	2.0± 0.3	2.6	1.8	2.2± 0.4
10	3.5	4.4	5.6	7.5	5.3± 0.9	9.6	5.3	6.3	13.2	8.6± 1.8	6.9	5.4	6.2± 0.8
12	6.5	2.0	26.0	14.4	12.2± 5.3	21.9	8.9	10.2	50.3	22.8± 9.6	28.8	20.7	24.8± 4.1
14	24.5	54.5	69.0	51.9	50.0± 9.0	91.8	4.5	45.0	67.2	52.1±18.5	68.4	50.4	59.4± 9.0
16	36.9	37.5	81.3	102.0	64.4±16.3	128.6	54.0	85.5	51.8	80.0±17.9	98.1	76.8	87.5±10.7
18	41.0	46.2	66.0	85.5	59.7±10.2	122.6	85.1	90.0	71.6	92.3±10.8	98.4	145.8	122.0±23.7
20	68.0	78.2	83.7	87.0	79.2± 4.2	126.2	112.8	96.5	79.4	103.7±10.1	102.5	143.4	123.0±20.5
22	34.2	52.2	69.8	105.0	65.3±15.0	160.7	116.4	88.8	82.8	112.2±17.8	84.2	152.7	118.5±34.3
24	43.7	61.4	79.7	68.9	63.4± 7.6	144.6	109.5	*	73.2	109.1±20.6	89.1	163.2	126.2±37.0
26	34.8	54.6	108.4	89.1	71.7±16.6	189.0	99.5	*	81.5	123.3±33.2	123.8	175.1	149.0±25.7
28	42.3	56.4	87.9	91.5	69.5±12.0	165.0	90.9	*	77.0	111.0±27.3	123.8	182.9	153.4±30.0

	Finn Dorset HbAB				Mean	Finn Dorset HbB				Mean
	473	485	514	520	±S.E.	480	489	501	541	+S.E.
4	5.7	4.8	2.0	4.9	4.4± 0.8	1.3	3.3	3.1	2.9	2.7± 0.5
6	2.4	2.6	1.8	2.3	2.3± 0.2	1.9	2.3	2.7	2.0	2.4± 0.2
8	3.2	4.7	2.3	3.2	3.4± 0.5	1.8	1.2	3.1	3.4	2.4± 0.5
10	7.5	6.2	7.5	10.8	8.0± 1.0	8.1	13.1	9.2	8.3	9.6± 1.2
12	15.6	17.4	16.1	14.6	15.9± 0.6	15.6	44.4	21.9	20.0	25.5± 6.4
14	75.0	58.4	30.0	75.0	59.6±10.6	131.0	73.8	106.2	73.4	96.2±13.9
16	69.0	68.9	120.0	91.7	87.5±12.1	190.2	61.4	171.3	94.2	129.3±30.7
18	97.5	72.3	155.0	181.2	126.5±25.0	173.6	107.6	165.0	107.3	138.4±17.9
20	94.7	101.7	125.1	152.4	118.5±13.0	240.0	99.6	151.1	142.8	158.4±29.5
22	91.7	121.8	125.6	140.7	120.0±10.2	241.0	128.0	241.2	122.6	183.0±33.5
24	78.9	120.0	141.2	140.7	120.2±14.6	207.0	117.0	198.9	221.3	186.0±23.4
26	80.4	136.0	141.2	148.2	126.5±15.6	206.3	143.0	173.7	227.9	187.7±18.7
28	72.6	127.9	130.1	128.5	114.8±14.1	203.6	135.0	186.0	216.3	185.3±13.8

* Died on day 23

TABLE 20.

Faecal red cell clearances (ml/day) following infection with 350 *H. contortus* larvae/kg.

Days after infection	Blackface HbA				Mean	Blackface HbAB				Mean	Blackface HbB		Mean
	34	51	60	71	± S.E.	48	54	79	93	± S.E.	53	85	± S.E.
4	0.4	0.4	0.6	0.7	0.5±0.1	0.5	0.6	0.4	1.1	0.7±0.2	0.5	0.5	0.5±0.0
6	0.6	0.4	0.6	0.6	0.6±0.1	0.4	0.5	0.4	0.8	0.5±0.1	0.6	0.4	0.5±0.1
8	0.7	1.0	0.7	0.9	0.8±0.1	0.7	0.7	0.1	0.3	0.5±0.1	0.7	0.5	0.6±0.1
10	1.1	1.4	1.1	1.4	1.3±0.1	2.7	1.4	2.0	3.5	2.4±0.5	1.8	1.5	1.7±0.2
12	1.6	5.7	7.8	3.8	4.7±1.3	5.7	2.1	2.3	13.0	5.8±2.6	3.5	2.0	2.8±0.8
14	6.6	16.4	20.7	21.2	16.2±3.4	22.0	9.0	9.0	15.4	13.9±3.1	15.8	12.1	14.0±1.9
16	8.9	9.8	21.2	24.5	16.1±4.0	21.3	10.8	17.1	10.4	14.9±2.6	23.6	14.9	19.3±4.3
18	9.0	11.1	16.5	17.1	13.4±2.0	22.1	16.2	18.0	12.9	17.3±1.9	22.6	25.8	24.2±1.6
20	14.3	18.8	20.1	17.4	17.7±1.2	21.4	17.2	16.8	13.5	17.2±1.6	18.4	25.8	22.1±3.7
22	7.2	11.0	16.1	20.0	13.6±2.8	27.3	19.2	16.8	13.5	19.2±2.9	15.2	25.9	20.6±5.4
24	9.6	14.1	21.8	19.4	16.2±2.7	24.6	21.9	*	12.5	19.7±3.7	15.2	26.1	20.7±5.5
26	7.7	12.0	22.8	19.8	15.6±3.5	28.4	18.9		13.0	20.1±4.5	19.8	26.3	23.1±3.3
28	9.5	11.9	20.3	17.4	14.8±2.5	26.4	17.3		11.7	18.5±4.3	19.8	29.3	24.6±4.8

	Dorset HbAB				Mean	Dorset HbB				Mean
	473	485	514	520	± S.E.	480	489	501	541	± S.E.
4	1.6	1.2	0.6	1.6	1.3±0.2	0.4	1.0	1.0	0.8	0.8±0.1
6	0.7	0.9	0.4	0.7	0.7±0.1	0.6	0.8	0.8	0.6	0.7±0.1
8	0.9	1.6	0.7	0.9	1.0±0.2	0.6	0.3	0.8	1.0	0.7±0.1
10	2.0	2.0	2.3	3.2	2.4±0.3	2.3	3.0	2.9	2.4	2.7±0.2
12	3.8	5.2	4.5	4.2	4.5±0.3	4.2	10.2	6.2	5.4	6.5±1.3
14	18.0	15.8	8.1	20.3	15.6±2.7	28.8	17.7	27.6	19.8	23.6±2.8
16	15.9	15.1	26.4	24.8	20.6±2.9	41.9	13.5	32.6	23.6	27.9±6.1
18	21.4	15.9	27.9	36.0	25.4±4.3	36.5	19.4	28.1	21.5	26.4±3.9
20	18.0	18.3	22.8	29.0	22.1±2.6	43.2	17.0	22.7	24.3	26.9±5.7
22	17.4	19.7	18.1	22.5	19.5±1.1	36.2	19.2	33.8	22.1	27.8±4.2
24	15.0	19.2	24.0	22.5	20.3±2.0	31.1	17.6	33.6	35.4	29.4±4.0
26	15.3	20.4	24.0	23.7	20.9±1.6	33.0	21.5	24.3	36.5	28.8±2.7
28	13.8	19.2	19.5	20.6	18.3±1.5	32.6	18.9	27.9	30.3	27.5±3.0

* Died on day 23

TABLE 21.

Faecal ^{59}Fe blood clearances (ml/day) following infection with 350 *H. contortus* larvae/kg.

Days after infection	Blackface HbA				Mean	Blackface HbAB				Mean	Blackface HbB		Mean
	34	51	60	71	±S.E.	48	54	79	93	± S.E.	53	85	± S.E.
8	9.8	12.7	28.1	26.4	19.3±4.7	23.6	7.9	11.6	36.3	19.9±6.4	9.2	9.0	9.1±0.1
10	8.7	9.8	29.2	38.2	21.5±7.3	28.8	9.2	23.1	38.7	25.0±6.2	34.5	18.2	26.4±8.2
12	7.9	58.4	28.1	36.5	32.7±10.5	35.6	11.0	17.7	37.9	25.6±6.6	29.6	24.9	27.3±2.4
14	26.4	64.1	64.2	32.2	46.7±10.0	117.5	32.7	39.1	54.0	60.8±19.4	44.2	58.9	51.6±7.4
16	29.7	72.2	57.4	49.8	52.3±8.8	160.5	63.2	96.0	75.8	98.9±21.6	57.5	74.3	65.9±8.4
18	37.4	58.9	88.4	51.8	59.1±10.7	122.4	86.0	108.6	63.8	95.2±12.9	61.5	96.3	78.9±17.4
20	45.0	62.1	78.1	69.0	63.6±7.0	129.6	102.4	119.7	90.9	110.7±8.7	81.9	121.9	101.9±20.0
22	41.7	62.0	81.9	92.4	69.5±11.2	168.5	102.6	98.6	83.4	113.3±18.9	90.5	138.3	114.4±23.9
24	41.3	79.3	100.0	109.2	82.5±15.1	159.7	112.8	*	101.3	124.6±17.9	89.4	168.2	128.8±39.4
26	53.9	58.4	104.4	94.3	77.8±12.7	196.7	104.7		98.8	133.4±31.0	92.9	141.5	117.2±24.3
28	51.6	64.0	97.0	89.7	75.6±10.7	203.8	114.5		83.6	133.0±36.0	93.5	134.6	114.1±20.6

	Dorset HbAB				Mean	Dorset HbB				Mean
	473	485	514	520	± S.E.	480	489	501	541	± S.E.
8	26.3	13.9	9.1	14.1	15.9±3.7	13.8	28.5	9.1	9.7	15.3±4.5
10	26.8	22.6	19.7	23.2	23.1±1.5	21.9	20.0	26.8	30.6	24.8±2.4
12	25.2	42.3	52.3	58.8	44.7±7.3	59.6	18.5	74.2	30.1	45.6±12.9
14	61.5	62.9	81.9	95.3	75.4±8.1	192.5	86.9	170.3	53.4	125.8±33.2
16	82.3	69.5	109.2	137.1	99.5±15.0	258.4	95.4	145.1	132.5	157.9±35.1
18	74.4	92.1	102.9	171.3	110.2±21.2	164.0	92.1	133.7	138.9	157.2±37.1
20	90.4	127.1	91.5	149.7	114.7±14.5	242.1	137.9	188.7	119.1	172.0±27.6
22	91.4	132.5	110.9	157.8	123.0±14.3	231.8	108.9	164.4	178.8	171.0±25.3
24	92.7	134.4	119.4	167.1	128.4±15.5	221.1	137.6	185.4	164.1	177.1±17.6
26	94.8	131.4	122.1	158.6	126.7±13.2	269.4	205.4	193.5	178.5	211.7±20.0
28	110.4	132.5	130.9	157.5	130.3±10.1	230.4	161.7	206.4	174.6	193.2±15.5

* Died on day 23

TABLE 22.

Gastrointestinal iron losses (mg/day) following infection with 350 H. contortus larvae/kg.

Days after infection	Blackface HbA				Mean	Blackface HbAB				Mean	Blackface HbB		Mean
	34	51	60	71	± S.E.	48	54	79	93	± S.E.	53	85	± S.E.
4	0.6	1.0	0.6	0.7	0.7±0.1	0.6	0.1	0.5	1.2	0.6±0.2	0.6	0.6	0.6±0.0
6	0.7	0.8	0.7	0.6	0.7±0.1	0.5	0.1	0.6	0.4	0.4±0.1	0.7	0.6	0.7±0.1
8	0.8	1.1	1.8	0.9	1.2±0.2	0.8	0.9	0.7	0.4	0.7±0.1	0.8	0.8	0.8±0.0
10	1.2	1.5	1.8	2.3	1.7±0.2	3.5	1.8	1.8	3.8	2.5±0.7	2.3	1.8	2.1±0.3
12	1.8	0.8	7.8	3.8	3.6±1.5	6.9	2.4	2.9	13.8	6.5±2.6	8.9	6.2	7.6±1.4
14	6.3	16.1	19.8	13.1	13.8±2.9	23.9	1.1	11.4	18.6	13.8±4.9	21.0	15.2	18.1±2.9
16	8.6	10.8	23.4	23.9	16.7±4.0	33.0	14.4	16.5	11.7	18.9±4.8	24.9	22.7	23.8±1.1
18	9.2	11.6	17.7	20.0	14.7±2.5	24.9	18.8	16.5	15.8	19.0±2.1	21.8	32.7	27.3±5.5
20	15.4	16.7	21.3	19.5	18.3±1.3	23.1	22.2	16.5	15.1	19.2±2.0	20.6	30.2	25.4±4.8
22	7.9	11.9	20.4	23.9	16.0±3.7	29.6	23.4	13.4	16.7	20.8±3.6	16.8	32.1	24.5±7.7
24	10.2	14.4	19.7	20.3	16.2±2.4	27.0	23.7	*	14.7	21.8±3.7	21.2	31.7	26.5±5.2
26	7.5	12.8	24.6	20.3	16.4±3.8	35.4	21.9	*	16.1	24.5±5.7	23.5	28.7	26.1±2.6
28	9.2	12.8	20.0	17.4	14.9±2.4	32.6	20.0	*	14.7	22.4±5.3	23.11	27.5	25.3±2.2

	Dorset HbAB				Mean	Dorset HbB				Mean
	473	485	514	520	± S.E.	480	489	501	541	± S.E.
4	1.8	1.9	0.7	2.0	1.6±0.3	0.5	1.2	1.2	1.1	1.0±0.2
6	0.6	1.0	0.7	0.9	0.8±0.1	0.6	0.9	1.1	1.0	0.9±0.1
8	0.7	1.8	0.8	1.3	1.2±0.3	0.6	0.7	1.3	1.3	1.0±0.2
10	2.4	2.3	3.0	4.1	3.0±0.4	4.1	3.5	3.0	3.3	3.5±0.2
12	4.7	6.5	6.3	5.9	5.9±0.4	13.5	8.4	7.4	8.7	9.5±1.4
14	19.8	19.5	10.1	25.1	18.6±3.1	22.8	28.8	24.6	30.0	26.6±1.7
16	18.0	20.7	32.9	29.1	25.2±3.5	31.4	40.1	28.4	38.1	34.5±2.8
18	24.8	19.5	42.3	45.5	33.0±6.4	24.6	25.9	25.2	33.0	27.2±2.0
20	21.2	26.6	34.2	36.8	29.7±3.6	29.9	28.4	30.0	34.1	30.6±1.2
22	20.6	24.5	32.0	32.0	27.3±2.8	24.2	41.1	26.0	33.5	31.2±3.9
24	13.2	24.9	25.1	26.7	22.5±3.1	28.5	37.9	43.1	36.0	36.4±3.0
26	13.5	22.6	27.6	24.3	21.9±2.9	29.6	29.0	32.0	34.1	31.2±1.2
28	11.7	18.0	24.6	22.5	19.2±2.9	27.5	30.5	33.9	31.4	30.8±1.3

* Died on day 23

TABLE 23.

Faecal iron losses (mg/day) following infection with 350 H. contortus larvae/kg.

Days after infection	Blackface HbA				Mean ±S.E.	Blackface HbAB				Mean ±S.E.	Blackface HbB		Mean ±S.E.
	34	51	60	71		48	54	79	93		53	85	
8	3.1	4.2	9.4	8.8	6.4± 1.6	8.7	2.6	3.5	11.5	6.6± 2.1	3.1	3.0	3.1± 0.1
10	2.8	3.4	9.8	11.2	6.8± 2.2	9.1	3.1	6.6	11.5	7.6± 1.8	10.6	6.1	8.4± 2.3
12	2.0	18.5	8.5	10.4	9.9± 3.4	10.1	3.7	4.9	10.5	7.3± 1.8	8.9	7.5	8.2± 0.7
14	6.8	18.8	18.4	8.1	13.0± 3.2	30.6	10.4	8.2	13.5	15.7± 5.1	12.5	16.7	14.6± 2.1
16	7.6	19.3	15.5	12.5	13.7± 2.5	37.5	16.5	17.6	17.2	22.2± 5.1	14.4	21.0	17.7± 3.3
18	8.4	14.6	23.9	12.1	14.8± 3.3	24.9	19.0	18.5	13.6	19.0± 2.3	13.6	21.6	17.6± 4.0
20	10.2	13.3	19.3	15.7	14.6± 1.9	23.8	19.8	19.2	17.3	20.0± 1.4	16.4	25.7	21.1± 4.7
22	9.7	13.3	20.2	21.0	15.8± 2.6	30.9	19.2	14.8	15.9	20.2± 3.7	17.8	29.1	23.5± 5.6
24	9.6	18.0	24.7	24.8	19.3± 3.6	28.2	22.6	*	21.3	24.0± 2.1	17.6	28.7	23.2± 5.6
26	12.6	13.9	24.4	21.4	18.1± 2.9	36.8	23.1		18.8	26.2± 5.4	17.4	22.7	20.1± 2.7
28	11.2	14.5	22.0	17.1	16.2± 2.3	37.4	25.2		15.9	26.2± 6.2	17.5	21.6	19.6± 2.1

	Dorset HbAB				Mean ±S.E.	Dorset HbB				Mean ±S.E.
	473	485	514	520		480	489	501	541	
8	8.0	5.1	3.3	5.5	5.5± 1.0	5.1	9.0	3.6	3.9	5.4± 1.2
10	7.8	8.3	7.2	8.8	8.0± 0.3	7.2	6.2	10.1	11.3	8.7± 1.2
12	6.6	14.1	19.3	19.6	14.9± 3.0	19.7	6.1	22.8	10.4	14.8± 3.9
14	15.4	19.5	25.7	30.2	22.7± 3.3	53.4	25.3	46.1	15.6	35.1± 8.8
16	21.2	20.0	31.3	38.9	27.9± 4.5	55.2	25.2	36.3	35.8	38.1± 6.2
18	18.9	24.9	28.2	42.9	28.7± 5.1	56.4	22.7	29.0	33.9	35.5± 7.3
20	20.2	26.3	20.8	34.0	25.3± 3.2	42.8	28.6	32.1	25.1	32.2± 3.8
22	15.3	22.1	24.9	29.5	23.0± 3.0	38.7	22.6	31.3	34.6	31.8± 3.4
24	15.5	22.4	21.1	31.2	22.6± 3.2	36.9	28.0	35.3	31.8	33.0± 2.0
26	15.2	18.4	23.2	26.0	20.7± 2.4	44.9	41.8	31.7	29.8	37.1± 3.7
28	17.7	14.1	19.8	25.8	19.4± 2.5	33.8	25.4	33.8	27.4	30.1± 2.2

* Died on day 23

TABLE 24.

Albumin pools before infection with H. contortus.

	Sheep No.	CA g	CA g/kg	EA g	EA g/kg	TA g	TA g/kg	EA/CA
	34	37.6	1.45	57.5	2.22	95.1	3.67	1.53
Blackface	51	33.9	1.36	52.3	2.09	86.2	3.45	1.54
HbA	60	39.9	1.44	48.5	1.75	87.4	3.19	1.22
	71	31.3	1.32	43.8	1.86	75.1	3.18	1.41
Mean		35.7	1.39	50.5	1.98	86.0	3.37	1.43
S.E.		1.9	0.03	2.9	0.11	4.1	0.11	0.07
	48	33.5	1.25	47.7	1.78	81.2	3.03	1.42
Blackface	54	21.6	1.01	43.9	2.05	65.5	3.06	2.03
HbAB	79	26.9	1.25	49.9	2.32	76.8	3.57	1.86
	93	19.8	1.01	28.0	1.43	47.8	2.44	1.42
Mean		25.5	1.13	42.4	1.90	67.9	3.03	1.68
S.E.		3.1	0.07	5.0	0.19	7.5	0.23	0.16
Blackface	53	30.4	1.34	46.1	2.03	76.5	3.37	1.52
HbB	85	23.5	1.12	41.8	1.99	65.3	3.11	1.78
Mean		27.0	1.23	44.0	2.01	71.0	3.24	1.65
S.E.		3.5	0.11	2.2	0.02	5.6	0.13	0.13
	473	38.5	1.51	53.3	2.09	91.8	3.60	1.39
Dorset	485	32.5	1.19	54.6	2.00	87.1	3.19	1.68
HbAB	514	34.1	1.29	58.1	2.20	92.2	3.49	1.47
	520	36.6	1.55	53.8	2.28	90.4	3.83	1.71
Mean		35.4	1.39	55.0	2.14	90.4	3.53	1.56
S.E.		1.3	0.09	1.1	0.06	1.2	0.13	0.08
	480	34.2	1.32	46.1	1.78	80.3	3.10	1.78
Dorset	489	33.2	1.30	39.8	1.56	73.0	2.86	1.20
HbB	501	47.1	1.62	51.2	1.76	98.3	3.38	1.09
	541	33.4	1.53	58.0	2.66	91.4	4.19	1.74
Mean		37.0	1.44	48.8	1.94	85.8	3.38	1.45
S.E.		3.4	0.08	3.9	0.25	5.6	0.29	0.18

TABLE 25.

Albumin pools 28 days after infection with H. contortus.

	Sheep No.	CA g	CA g/kg	EA g	EA g/kg	TA g	TA g/kg	EA/CA
Blackface HbA	34	31.1	1.45	43.9	2.05	75.0	3.50	1.41
	51	27.4	1.12	43.9	1.79	71.3	2.91	1.60
	60	29.1	1.03	38.9	1.38	68.0	2.41	1.34
	71	26.6	1.06	51.5	2.06	78.1	3.12	1.94
Mean		28.6	1.17	44.6	1.82	73.1	2.99	1.57
S.E.		1.0	0.10	2.6	0.16	2.2	0.23	0.13
Blackface HbAB	48	22.1	0.83	35.1	1.31	57.2	2.14	1.58
	54	20.0	0.89	29.5	1.31	49.5	2.20	1.47
	79	*	*	*	*	*	*	*
	93	13.6	0.75	18.8	1.03	32.1	1.78	1.37
Mean		18.6	0.82	27.8	1.22	46.3	2.04	1.47
S.E.		2.6	0.04	4.8	0.09	7.4	0.13	0.06
Blackface HbB	53	21.0	0.94	27.4	1.23	48.4	2.17	1.31
	85	19.5	0.89	19.0	0.87	38.5	1.76	0.98
Mean		20.3	0.92	23.2	1.05	43.5	1.97	1.14
S.E.		0.8	0.03	4.2	0.18	5.0	0.21	0.17
Dorset HbAB	473	27.2	1.09	40.5	1.62	67.7	2.71	1.49
	485	24.2	0.97	34.5	1.38	58.7	2.35	1.42
	514	20.3	0.89	28.6	1.26	48.9	2.15	1.42
	520	23.9	0.91	34.0	1.29	57.9	2.20	1.42
Mean		23.9	0.97	34.4	1.39	58.3	2.36	1.43
S.E.		1.4	0.05	2.4	0.08	3.8	0.13	0.01
Dorset HbB	480	22.7	0.82	28.8	1.04	51.5	1.86	1.27
	489	18.4	0.73	27.7	1.10	46.1	1.83	1.51
	501	29.1	0.95	29.3	0.96	58.4	1.91	1.01
	541	22.7	1.00	29.5	1.30	52.2	2.30	1.30
Mean		23.2	0.88	28.8	1.10	52.0	1.98	1.27
S.E.		2.2	0.06	0.4	0.07	2.5	0.11	0.10

* Died on day 23

TABLE 26.

Fractional catabolic rate of albumin F(CA) following infection with 350 H. contortus larvae/kg.

Days after infection	Blackface HbA 34	51	60	71	Mean ± S.E.	Blackface HbAB 48	54	79	93	Mean ± S.E.	Blackface HbB 53	85	Mean ± S.E.
4	.071	.058	.063	.057	.062±.003	.041	.067	.065	.055	.057±.006	.057	.033	.045±.012
6	.060	.059	.065	.067	.063±.002	.048	.020	.038	.038	.036±.006	.076	.031	.054±.023
8	.084	.104	.115	.100	.101±.006	.099	.107	.094	.090	.098±.004	.081	.088	.085±.004
10	.077	.088	.092	.086	.085±.003	.061	.088	.062	.033	.061±.011	.052	.068	.060±.008
12	.082	.094	.098	.080	.089±.004	.072	.100	.098	.074	.086±.008	.056	.048	.052±.004
14	.082	.101	.112	.102	.099±.006	.074	.100	.107	.092	.093±.007	.103	.051	.077±.026
16	.094	.116	.104	.078	.098±.008	.118	.092	.110	.071	.098±.010	.087	.094	.091±.004
18	.090	.102	.121	.110	.106±.007	.127	.120	.102	.071	.105±.013	.106	.113	.110±.004
20	.100	.103	.106	.112	.105±.003	.134	.124	.110	.094	.116±.009	.094	.162	.128±.034
22	.113	.121	.106	.096	.109±.005	.111	.106	*	.144	.120±.012	.104	.181	.143±.039
24	.106	.105	.120	.118	.112±.004	.170	.123		.125	.139±.015	.096	.177	.137±.041
26	.113	.110	.122	.131	.119±.005	.160	.109		.100	.123±.019	.113	.172	.143±.030
28	.108	.108	.113	.123	.113±.004	.212	.126		.115	.151±.031	.137	.212	.175±.038

	Dorset HbAB 473	485	514	520	Mean ± S.E.	Dorset HbB 480	489	501	541	Mean ± S.E.
4	.057	.072	.086	.036	.063±.011	.047	.038	.046	.046	.044±.002
6	.066	.063	.035	.082	.062±.010	.079	.057	.071	.083	.073±.005
8	.071	.102	.093	.078	.086±.007	.088	.076	.084	.070	.080±.004
10	.060	.070	.050	.049	.057±.005	.059	.071	.055	.056	.060±.004
12	.062	.080	.046	.052	.060±.007	.048	.080	.076	.070	.069±.007
14	.080	.061	.131	.112	.096±.016	.124	.091	.120	.106	.110±.007
16	.081	.124	.134	.086	.106±.013	.115	.098	.126	.134	.118±.008
18	.090	.094	.130	.092	.102±.009	.161	.101	.140	.167	.142±.015
20	.098	.133	.133	.110	.119±.009	.171	.102	.151	.155	.145±.015
22	.160	.141	.183	.190	.121±.010	.224	.208	.259	.280	.243±.016
24	.162	.186	.241	.206	.199±.020	.245	.198	.247	.249	.235±.012
26	.162	.205	.245	.186	.200±.018	.245	.231	.299	.217	.248±.018
28	.192	.197	.208	.254	.213±.014	.242	.229	.216	.198	.221±.009

* Died on day 23

TABLE 27.

Faecal ^{125}I "clearances" (ml/day) following infection with 350 *H. contortus* larvae/kg.

Days after infection	Blackface HbA				Mean	Blackface HbAB				Mean	Blackface HbB		Mean
	34	51	60	71	±S.E.	48	54	79	93	± S.E.	53	85	± S.E.
4	10.8	13.1	19.3	12.7	14.0±1.8	13.1	16.2	15.3	8.2	13.2±1.8	16.9	15.8	16.4±0.6
6	19.8	20.1	19.5	18.0	19.4±0.5	19.7	10.1	19.0	12.8	15.4±2.4	14.7	15.2	15.0±0.3
8	15.3	14.5	13.3	18.0	15.3±1.0	24.6	7.0	11.3	16.4	14.8±3.8	16.8	17.8	17.3±0.5
10	9.2	14.7	28.6	14.5	16.8±4.2	18.5	9.1	17.7	22.1	16.9±2.8	22.3	22.6	22.5±0.2
12	13.6	23.0	22.4	18.8	19.5±2.2	30.2	14.7	12.9	24.6	20.6±4.1	21.6	29.2	25.4±3.8
14	22.7	25.9	19.3	18.3	21.6±1.7	29.5	18.9	14.1	21.9	21.1±3.2	24.4	30.8	27.6±3.2
16	26.5	30.4	20.1	26.2	25.8±2.1	37.2	28.0	18.1	27.8	27.8±3.9	30.6	47.6	39.1±8.5
18	23.5	28.1		32.3	28.0±2.5	42.2	20.2	14.3	30.6	26.8±6.1	21.7	49.7	35.7±14.0
20	30.5	30.6	32.3	30.5	31.0±0.4	42.2	40.2	17.2	27.4	31.8±5.9	27.1	52.3	39.7±12.6
22	20.6	35.4	36.1	29.7	30.5±3.6	53.2	41.5	*	23.8	39.5±8.5	33.5	56.8	45.2±11.7
24	26.5	34.6	29.7	34.1	31.2±1.9	55.2	36.8		29.1	40.4±7.7	35.2	47.9	41.6±6.4
26	26.4	30.5	32.4	38.9	32.1±2.6	51.1	36.5		26.8	38.1±7.1	32.4	51.6	42.0±9.6
28	27.3	24.8	30.5	29.8	28.1±1.3	55.0	43.8		27.4	42.1±8.0	27.8	45.3	36.6±8.8

	Dorset HbAB				Mean	Dorset HbB				Mean
	473	485	514	520	± S.E.	480	489	501	541	± S.E.
4	19.6	10.3	13.0	10.8	13.4±2.1	16.8	12.8	17.4	10.8	14.5±1.6
6	13.9	19.2	21.2	19.8	18.5±1.6	23.7	10.9	17.6	17.2	17.4±2.6
8	17.0	26.9	28.6	15.3	22.0±3.4	19.2	19.0	21.9	14.7	18.7±1.5
10	18.7	23.0	37.0	22.7	25.4±4.0	26.9	21.3	26.9	26.5	25.4±1.4
12	21.3	25.7	42.5	26.5	29.0±4.6	25.7	28.3	41.9	40.3	34.1±4.1
14	18.9	32.6	41.9	37.8	32.8±5.0	36.6	24.2	48.5	49.7	39.8±6.0
16	24.4	41.8	48.5	39.1	38.5±5.1	43.5	27.0	52.2	56.4	44.8±6.5
18	28.3	54.5	56.8	53.8	48.4±6.7	64.7	23.8	66.8	67.5	55.7±10.6
20	27.0	57.6	52.6	54.2	47.9±7.0	77.6	35.7	52.6	72.8	59.7±9.7
22	29.8	49.7	57.7	66.8	51.0±7.9	68.4	38.1	77.7	91.4	68.9±11.3
24	31.3	61.5	52.2	57.5	49.1±6.4	75.4	38.5	82.2	87.8	71.0±11.1
26	30.8	61.4	58.7	60.7	52.9±7.4	78.2	44.1	77.0	89.3	72.2±9.7
28	34.3	73.7	57.1	54.6	54.9±8.1	75.2	45.3	72.1	84.7	69.3±8.4

* Died on day 23

TABLE 28.

Albumin catabolism during first week of infection.

	Sheep No.	Serum albumin (g%)	Apparent $t_{\frac{1}{2}}$ (hrs)	F(CA) /day	Absolute amount catabolised (g/day)	Faecal ^{125}I clearance (ml/day)
	34	3.62	576	0.071	0.103	15.3
Blackface	51	3.50	444	0.058	0.079	15.9
HbA	60	3.80	408	0.068	0.098	17.4
	71	3.45	648	0.075	0.099	16.2
Mean		3.59	519	0.068	0.095	16.2
S.E.		0.08	56	0.004	0.005	0.4
	48	3.60	552	0.063	0.079	19.1
Blackface	54	2.90	492	0.065	0.066	11.1
HbAB	79	3.48	456	0.066	0.083	15.2
	93	2.80	400	0.061	0.062	12.5
Mean		3.19	475	0.064	0.073	14.5
S.E.		0.20	32	0.001	0.005	1.8
Blackface	53	3.69	532	0.071	0.095	16.1
HbB	85	2.90	492	0.051	0.057	16.3
Mean		3.30	512	0.061	0.076	16.2
S.E.		0.40	20	0.010	0.019	0.10
	473	3.20	600	0.065	0.098	18.8
Dorset	485	3.30	488	0.079	0.094	16.8
HbAB	514	3.00	605	0.071	0.092	20.9
	520	3.70	549	0.065	0.101	15.3
Mean		3.30	561	0.070	0.096	18.0
S.E.		0.15	27	0.003	0.002	1.2
	480	3.30	400	0.071	0.094	19.9
Dorset	489	3.00	492	0.057	0.074	14.2
HbB	501	3.80	624	0.065	0.106	19.0
	541	3.30	588	0.066	0.101	14.2
Mean		3.35	526	0.065	0.094	16.8
S.E.		0.17	50	0.003	0.007	1.5

TABLE 29.

Albumin catabolism 21-28 days after infection.

	Sheep No.	albumin (g%)	Apparent $t_{\frac{1}{2}}$ (hrs)	F(CA) /day	Absolute amount catabolised (g/day)	Faecal ^{125}I clearance (ml/day)
	34	3.01	389	0.110	0.160	25.2
Blackface	51	3.15	312	0.111	0.124	31.3
HbA	60	2.86	324	0.115	0.118	32.2
	71	3.00	336	0.117	0.124	33.1
Mean		3.01	340	0.113	0.132	30.5
S.E.		0.06	17	0.002	0.010	1.8
	48	2.45	264	0.163	0.135	53.6
Blackface	54	2.50	300	0.116	0.103	39.7
HbAB	79	*	*	*	*	*
	93	2.05	342	0.121	0.091	26.8
Mean		2.33	302	0.133	0.110	40.0
S.E.		0.14	23	0.015	0.013	7.7
Blackface	53	2.75	295	0.113	0.106	32.2
HbB	85	2.20	226	0.186	0.166	50.4
Mean		2.48	261	0.150	0.136	41.3
S.E.		0.28	35	0.040	0.030	9.1
	473	2.75	336	0.172	0.187	31.5
Dorset	485	2.35	218	0.182	0.177	61.6
HbAB	514	2.32	262	0.219	0.195	56.4
	520	2.40	246	0.209	0.190	59.9
Mean		2.46	266	0.196	0.187	52.4
S.E.		0.10	25	0.011	0.004	7.0
	480	2.30	216	0.239	0.196	74.3
Dorset	499	1.91	248	0.216	0.158	41.5
HbB	501	2.50	184	0.255	0.242	77.3
	541	2.65	167	0.233	0.233	88.3
Mean		2.34	203	0.236	0.207	70.4
S.E.		0.16	18	0.008	0.019	10.1

* Died on day 23

APPENDIX 1

(B) The Influence of Haemoglobin Type on the Response of Scottish Blackface Sheep to Infection with 1400 *H. contortus* Larvae/kg Bodyweight.

TABLE 30.

Packed cell volumes (%) of Scottish Blackface sheep infected with 1400 H. contortus larvae/kg.

	Sheep No.	Days before infection 28	21	14	7	Days after infection 0	3	6	9	12	16	19	24
	966	34.0	34.0	30.0	31.0	29.0	28.0	26.0	24.0	19.0	14.0	13.0	13.0
	977	37.0	40.0	34.0	34.0	31.0	28.0	29.0	28.0	14.0	12.0	12.0	13.0
Blackface HbA	987	40.0	39.0	35.0	31.0	30.0	28.0	25.0	21.0	16.0	10.0	8.0	*
	988	32.0	35.0	28.0	29.0	29.0	27.0	24.0	25.0	17.0	14.0	10.0	8.0
	990	35.0	36.0	31.0	33.0	30.0	30.0	31.0	28.0	21.0	18.0	14.0	13.0
Mean		35.6	36.8	31.6	31.6	29.8	28.2	27.0	25.2	17.4	13.6	11.4	11.8
S.E.		1.4	1.2	1.3	0.9	0.4	0.5	1.3	1.3	1.2	1.3	1.1	1.3
	970	33.0	35.0	28.0	28.0	25.0	26.0	23.0	24.0	18.0	15.0	13.0	14.0
	973	30.0	32.0	25.0	27.0	28.0	26.0	27.0	24.0	20.0	15.0	15.0	12.0
Blackface HbAB	978	33.0	34.0	28.0	28.0	26.0	24.0	24.0	26.0	23.0	19.0	16.0	17.0
	979	36.0	39.0	30.0	32.0	30.0	26.0	26.0	30.0	20.0	13.0	10.0	9.0
	984	36.0	36.0	30.0	30.0	27.0	27.0	26.0	24.0	15.0	11.0	9.0	7.0
Mean		33.6	35.2	28.2	29.0	27.2	25.8	25.2	25.6	19.2	14.6	12.6	11.8
S.E.		1.1	1.2	0.9	0.9	0.9	0.5	0.7	1.2	1.3	1.3	1.4	1.8
	1	32.0	37.0	31.0	30.0	25.0	26.0	27.0	23.0	13.0	12.0	8.0	8.0
	2	41.0	39.0	34.0	32.0	28.0	29.0	32.0	29.0	23.0	12.0	11.0	10.0
Blackface HbB	3	32.0	36.0	29.0	28.0	29.0	23.0	26.0	27.0	17.0	13.0	12.0	14.0
	6	31.0	33.0	30.0	28.0	28.0	24.0	22.0	24.0	21.0	18.0	18.0	17.0
	8	37.0	34.0	30.0	31.0	30.0	26.0	24.0	26.0	17.0	13.0	12.0	10.0
Mean		34.6	35.8	30.8	29.8	28.0	25.6	26.2	25.6	18.2	13.6	12.2	11.8
S.E.		1.9	1.1	0.9	0.8	0.8	1.0	1.7	1.1	1.7	1.1	1.6	1.6

* Died on day 23

TABLE 31.

Red cell counts ($X10^6$) of Scottish Blackface sheep infected with 1400 H. contortus larvae/kg.

	Sheep No.	Days before infection 28	21	14	7	Days after infection 0	3	6	9	12	16	19	24
	966	9.5	9.8	8.1	9.7	8.3	8.1	7.8	6.9	5.1	3.2	2.3	2.6
	977	12.3	11.1	9.3	10.0	7.9	7.7	8.0	7.5	5.6	3.0	2.6	2.6
Blackface HbA	987	12.1	11.1	8.1	9.6	7.7	7.7	6.9	5.9	4.2	2.1	1.6	*
	988	9.2	9.2	7.2	8.2	7.7	7.7	6.7	6.8	4.6	2.8	2.1	2.0
	990	10.7	9.4	9.0	9.7	8.6	8.5	9.6	7.7	6.0	3.8	2.4	2.5
Mean		10.8	10.1	8.3	9.4	8.1	8.0	7.8	6.9	5.1	3.0	2.2	2.4
S.E.		0.6	0.4	0.4	0.3	0.2	0.2	0.6	0.3	0.3	0.3	0.2	0.1
	970	9.5	9.8	7.3	7.7	7.0	6.8	7.7	6.2	5.0	3.4	2.9	3.1
	973	10.4	8.4	7.7	7.8	7.4	7.5	7.3	6.3	4.7	3.1	3.0	2.4
Blackface HbAB	978	10.5	9.7	7.7	8.4	7.7	6.8	7.2	7.6	5.8	3.5	3.1	3.3
	979	11.2	10.8	6.7	8.8	7.5	7.2	7.7	8.4	6.8	2.4	2.1	2.5
	984	10.3	11.2	7.5	9.7	8.0	8.4	8.0	6.2	4.2	2.8	1.6	1.4
Mean		10.4	10.0	7.4	8.5	7.5	7.3	7.6	7.0	5.3	3.04	2.5	2.5
S.E.		0.3	0.5	0.2	0.4	0.2	0.3	0.1	0.5	0.5	0.2	0.3	0.3
	1	9.2	9.9	9.7	9.1	8.3	7.9	8.5	6.9	4.4	2.9	1.3	1.5
	2	12.0	11.4	9.8	9.7	8.8	9.2	10.2	8.8	6.5	2.8	2.1	2.0
Blackface HbB	3	9.8	11.0	7.7	8.3	7.8	8.4	8.1	7.9	4.9	3.1	2.3	2.6
	6	8.3	8.7	8.1	8.5	7.2	7.3	6.6	6.5	5.6	4.2	4.0	4.1
	8	12.0	10.0	8.7	9.0	8.1	8.1	7.4	7.3	5.0	2.8	2.3	2.2
Mean		10.2	10.2	8.8	8.9	8.0	8.2	8.2	7.4	5.3	3.2	2.4	2.5
S.E.		0.8	0.5	0.4	0.3	0.3	0.3	0.6	0.4	0.4	0.3	0.4	0.4

* Died on day 23

TABLE 32.

Blood haemoglobin concentrations (g%) of Scottish Blackface sheep infected with 1400 H. contortus larvae/kg.

	Sheep No.	Days before infection				Days after infection							
		28	21	14	7	0	3	6	9	12	16	19	24
	966	11.3	12.6	9.7	10.3	10.0	9.5	9.9	9.1	6.6	4.9	4.9	4.3
	977	14.1	14.9	11.9	11.7	10.9	10.2	11.3	10.3	7.1	2.5	4.9	5.7
Blackface HbA	987	14.1	15.8	11.7	11.7	10.9	10.3	10.0	8.0	6.6	3.4	2.8	*
	988	12.2	14.0	9.7	10.3	9.7	9.9	10.0	9.4	7.1	4.6	4.2	3.1
	990	12.5	13.2	10.6	11.1	10.7	10.9	11.3	9.1	8.3	6.3	5.7	5.1
Mean		12.8	14.1	10.7	11.0	10.4	10.1	10.5	9.2	7.1	4.9	4.5	4.6
S.E.		0.6	0.6	0.5	0.3	0.3	0.2	0.3	0.4	0.3	0.5	0.5	0.5
	970	11.9	12.9	9.1	9.2	9.1	9.5	9.7	8.3	6.9	5.7	4.9	5.7
	973	10.6	13.7	10.3	10.3	10.0	9.5	10.6	8.9	7.7	5.2	5.2	4.6
Blackface HbAB	978	12.2	13.2	9.9	10.2	10.0	9.2	9.4	10.3	8.3	7.7	6.3	6.9
	979	13.8	13.7	10.6	10.9	9.9	9.5	10.6	10.6	7.7	4.9	4.2	2.8
	984	13.1	14.0	10.3	11.4	10.0	10.0	10.3	7.7	6.3	4.3	3.9	2.5
Mean		12.3	13.5	10.0	10.4	9.8	9.5	10.1	9.2	7.4	5.6	4.9	4.5
S.E.		0.6	0.2	0.3	0.4	0.2	0.1	0.2	0.6	0.4	0.3	0.5	0.8
	1	11.6	13.5	10.7	10.9	9.4	10.0	10.9	8.9	5.5	4.3	3.0	3.1
	2	14.7	14.9	12.7	11.7	10.3	10.6	12.3	11.4	9.4	4.6	4.3	3.4
Blackface HbB	3	11.9	14.3	10.0	9.4	9.0	10.9	9.7	10.4	6.9	4.9	4.9	6.3
	6	11.3	13.2	10.6	10.0	9.7	9.5	8.9	8.9	8.6	7.4	7.2	7.1
	8	13.5	13.2	11.2	10.6	9.1	10.0	9.4	9.7	7.1	4.9	4.3	4.3
Mean		12.6	13.8	11.0	10.5	9.5	10.2	10.2	9.9	7.5	5.2	4.7	4.8
S.E.		0.7	0.4	0.5	0.4	0.2	0.3	0.6	0.5	0.7	0.6	0.7	0.8

* Died on day 23

TABLE 33.

Mean corpuscular volumes (μ^3) of Scottish Blackface sheep infected with 1400 *H. contortus* larvae/kg.

	Sheep No.	Days before infection 28	21	14	7	Days after infection 0	3	6	9	12	16	19	24
	966	35.6	34.6	37.0	31.4	34.3	34.0	33.2	34.7	36.4	43.8	56.5	50.0
	977	30.1	35.7	36.3	34.1	39.2	36.3	36.3	37.5	33.7	46.7	46.2	50.0
Blackface HbA	987	33.2	34.7	43.4	32.2	38.8	35.6	36.0	35.7	38.1	47.6	50.0	*
	988	34.7	38.1	38.1	35.5	36.9	35.0	35.9	36.7	37.0	50.0	47.6	40.0
	990	32.8	37.8	34.0	34.0	34.4	35.1	32.2	36.6	34.2	47.4	58.3	52.0
Mean		33.3	36.2	37.8	33.4	36.7	35.2	34.7	36.2	35.9	47.1	51.7	48.0
S.E.		1.0	0.8	1.6	0.7	1.1	0.4	0.9	0.5	0.9	1.0	2.4	2.7
	970	34.7	35.3	37.7	35.6	35.9	38.2	29.9	38.0	36.0	44.1	44.9	45.2
	973	28.8	38.0	32.6	34.6	37.8	34.7	37.0	37.9	41.1	48.4	50.0	50.0
Blackface HbAB	978	31.3	35.0	36.6	33.4	33.0	35.1	33.2	33.6	38.5	54.3	51.6	51.5
	979	32.1	36.0	44.9	35.7	39.4	35.5	33.7	35.0	28.6	54.1	47.6	36.0
	984	34.9	31.8	39.8	31.0	33.8	32.1	32.5	37.7	35.4	42.9	56.2	50.0
Mean		32.4	35.2	38.3	34.1	36.0	35.1	33.3	36.4	35.9	48.8	50.1	46.5
S.E.		1.1	1.1	2.1	0.9	1.2	1.0	1.2	0.9	2.1	2.4	1.9	2.8
	1	34.9	36.9	32.1	32.8	30.3	32.8	31.8	32.8	29.8	41.4	61.5	53.3
	2	34.2	34.1	34.8	32.9	32.0	31.0	31.2	32.9	34.8	42.9	52.4	50.0
Blackface HbB	3	32.7	32.6	36.8	33.8	37.0	26.9	31.9	34.2	34.6	41.9	54.5	53.8
	6	37.4	37.8	36.2	33.0	38.8	32.9	33.3	36.8	37.8	42.9	45.0	41.5
	8	30.9	33.4	33.4	34.0	36.9	32.0	32.4	35.1	33.9	36.4	52.2	45.5
Mean		34.0	35.0	34.7	33.3	35.0	31.1	32.1	34.4	34.2	41.1	53.1	48.8
S.E.		1.1	1.0	0.9	0.3	1.6	1.1	0.4	0.8	1.3	1.2	2.6	2.4

* Died on day 23

TABLE 34.

Mean corpuscular haemoglobin concentrations (%) of Scottish Blackface sheep infected with 1400 *H. contortus* larvae/kg.

	Sheep No.	Days before infection 28	21	14	7	Days after infection 0	3	6	9	12	16	19	24
	966	33.2	37.1	32.3	33.8	35.1	34.4	34.1	37.9	35.7	36.3	37.7	33.1
	977	38.1	37.7	35.0	34.4	35.2	36.4	39.0	36.8	37.4	37.1	40.1	43.8
Blackface HbA	987	35.3	41.0	33.4	37.7	36.3	37.5	40.0	38.1	41.3	35.8	37.3	*
	988	38.1	40.0	35.3	35.5	34.0	36.7	41.7	37.6	41.8	34.1	41.5	38.8
	990	35.7	37.2	34.8	33.6	36.3	36.3	36.5	32.5	40.5	36.0	40.7	39.2
Mean		36.1	38.6	34.2	35.0	35.4	36.3	39.1	36.6	39.3	35.9	39.6	38.7
S.E.		1.0	0.8	0.6	0.8	0.5	0.5	0.9	1.1	1.2	0.5	0.9	2.0
	970	36.1	37.4	33.1	33.5	36.4	36.3	42.2	35.3	38.3	38.0	37.7	42.2
	973	35.3	42.8	41.2	38.1	35.7	36.3	39.3	37.1	39.5	34.7	34.3	38.3
Blackface HbAB	978	37.0	38.8	35.4	36.3	39.2	38.1	39.2	40.4	36.9	41.6	39.4	40.6
	979	38.3	35.1	35.3	34.6	33.6	37.1	40.8	35.9	39.5	37.7	41.5	31.3
	984	36.4	39.4	34.3	38.0	37.0	37.0	39.6	32.8	42.0	37.4	42.8	35.7
Mean		35.6	38.7	35.9	36.1	36.4	37.0	40.2	36.3	39.2	37.9	39.1	37.6
S.E.		0.5	1.3	1.4	0.9	0.9	0.3	0.6	1.3	0.9	1.1	1.5	2.0
	1	36.3	36.8	34.5	36.3	37.6	38.5	40.4	39.6	41.9	35.8	37.5	38.8
	2	35.9	38.2	35.1	36.6	36.8	37.2	38.4	39.3	41.8	38.3	39.1	34.0
Blackface HbB	3	37.2	39.7	35.1	33.6	31.4	38.4	37.3	38.5	40.6	37.7	40.8	45.0
	6	36.5	40.0	35.9	35.7	34.6	39.4	40.5	37.1	41.0	41.1	40.9	43.0
	8	36.5	39.4	38.0	34.8	30.3	38.5	39.2	38.0	41.8	37.7	35.8	45.3
Mean		36.5	38.8	35.7	35.4	34.1	40.4	39.2	38.5	41.4	38.1	38.8	41.2
S.E.		0.2	0.6	0.6	0.6	1.5	2.1	0.6	0.5	0.3	0.9	1.0	2.2

* Died on day 23

TABLE 35.

Serum iron concentration (µg%).

	Sheep No.	Days before infection 28	21	14	7	Days after infection 0	7	14	21	24
	977	198	161	131	139	121	91	133	59	67
	988	123	140	167	108	146	126	139	57	67
Blackface HbA	990	174	151	150	161	149	142	130	56	55
	966	161	150	172	174	139	158	104	76	52
	987	147	152	185	139	136	126	83	53	*
Mean		161	151	161	144	138	129	118	60	60
S.E.		14	4	10	12	5	12	24	5	4
	978	137	122	157	136	143	120	152	97	79
	979	187	165	187	171	185	160	130	50	79
Blackface HbAB	973	130	142	187	147	144	135	143	78	82
	984	135	174	185	196	147	147	142	31	52
	970	138	156	169	142	156	156	110	61	52
Mean		145	152	177	158	155	144	135	63	69
S.E.		12	10	7	12	9	8	16	12	7
	6	137	167	167'	157	126	153	148	74	49
	1	188	199	196	172	159	128	159	35	45
Blackface HbB	2	146	158	141	137	142	150	122	56	79
	8	149	188	188	151	158	147	128	57	64
	3	202	165	177	187	165	156	141	33	37
Mean		164	175	174	161	150	147	140	51	55
S.E.		15	8	10	10	8	5	15	8	8

* Died on day 23

TABLE 36.

Total serum proteins (g%) of Scottish Blackface sheep infected with 1400 H. contortus larvae/kg.

	Sheep No.	Days before infection 28	21	14	7	Days after infection 0	7	14	21	24
	966	5.79	5.20	5.64	5.96	5.97	5.83	4.64	3.91	3.50
	977	6.10	5.60	6.06	6.09	5.25	5.01	4.10	4.02	4.48
Blackface HbA	987	6.93	5.48	6.46	6.33	6.15	5.58	4.90	3.50	*
	988	6.53	6.30	6.45	6.35	6.43	6.05	5.14	4.65	3.69
	990	6.76	6.15	6.63	6.90	6.56	6.18	5.47	4.07	4.14
Mean		6.42	5.75	6.25	6.33	6.07	5.73	4.85	4.03	3.95
S.E.		0.21	0.21	0.18	0.16	0.23	0.21	0.24	0.19	0.20
	970	5.75	5.54	5.81	5.86	5.88	5.47	4.88	4.22	3.86
	973	6.12	5.91	6.36	6.16	6.14	5.45	4.85	4.20	4.05
Blackface HbAB	978	6.40	6.30	6.57	6.57	6.44	6.21	5.80	5.24	5.11
	979	6.24	6.15	6.50	6.26	5.91	5.34	4.89	3.97	3.69
	984	7.02	6.75	6.91	6.72	6.55	5.91	4.78	4.21	3.86
Mean		6.31	6.13	6.43	6.31	6.18	5.68	5.04	4.37	4.11
S.E.		0.21	0.20	0.18	0.16	0.14	0.17	0.20	0.23	0.26
	1	6.16	6.16	6.18	6.02	5.93	5.62	4.43	3.80	3.41
	2	6.54	6.10	6.45	6.50	6.24	6.03	5.24	4.59	4.33
Blackface HbB	3	6.60	6.84	6.81	6.83	7.07	6.51	5.23	4.41	4.90
	6	6.50	6.48	6.78	6.83	5.99	5.61	5.03	5.03	5.44
	8	6.23	6.03	6.50	6.63	5.95	5.51	5.13	3.93	4.14
Mean		6.41	6.32	6.54	6.56	6.24	5.86	5.01	4.35	4.44
S.E.		0.09	0.16	0.12	0.15	0.22	0.19	0.16	0.23	0.35

* Died on day 23

TABLE 37.

Serum albumin (g%) of Scottish Blackface sheep infected with 1400 H. contortus larvae/kg.

	Sheep No.	Days before infection 28	21	14	7	Days after infection 0	7	14	21	24
	966	3.28	3.39	3.08	3.06	3.56	3.15	2.25	2.04	1.56
	977	3.78	3.45	3.47	3.09	3.37	2.92	2.7	2.03	2.05
Blackface HbA	987	3.31	2.99	2.81	2.76	3.20	2.96	2.36	1.17	*
	988	2.79	2.91	2.79	2.63	2.77	2.66	2.62	1.72	1.70
	990	3.55	3.51	3.30	3.07	3.88	3.69	3.05	1.88	2.26
Mean		3.34	3.25	3.09	2.92	3.36	3.08	2.61	1.77	1.89
S.E.		0.17	0.13	0.14	0.10	0.19	0.18	0.15	0.16	0.15
	970	3.81	3.89	3.56	3.20	3.63	3.33	3.49	2.38	2.14
	973	3.12	3.02	3.38	2.66	3.22	2.91	2.57	1.87	1.82
Blackface HbAB	978	3.55	3.79	3.32	3.18	3.48	2.96	3.00	2.57	2.76
	979	3.57	3.51	3.43	3.03	3.21	2.87	2.54	1.70	1.52
	984	3.25	3.20	3.43	3.47	3.54	3.41	2.89	1.92	1.23
Mean		3.46	3.48	3.42	3.11	3.42	3.10	2.90	2.09	1.89
S.E.		0.12	0.17	0.04	0.14	0.09	0.11	0.18	0.17	0.27
	1	3.53	3.48	3.58	3.37	3.65	3.25	2.76	1.50	1.61
	2	3.04	2.92	3.21	2.90	3.62	3.22	2.57	2.12	1.68
Blackface HbB	3	4.27	3.64	3.57	3.35	3.35	2.92	2.51	1.76	1.44
	6	3.18	3.26	3.22	3.03	3.57	3.33	3.17	2.51	2.76
	8	3.59	3.35	3.60	3.28	3.98	3.71	3.45	2.44	2.19
Mean		3.52	3.33	3.44	3.19	3.63	3.29	2.89	2.07	1.94
S.E.		0.22	0.12	0.09	0.10	0.11	0.13	0.18	0.20	0.25

* Died on day 23

TABLE 38.

Serum globulin (g%) of Scottish Blackface sheep infected with 1400 H. contortus larvae/kg.

	Sheep No.	Days before infection 28	21	14	7	Days after infection 0	7	14	21	24
	966	2.51	1.81	2.56	2.90	2.41	2.68	2.39	1.87	1.94
	977	2.32	2.65	2.59	3.00	1.88	2.09	1.34	1.99	2.43
Blackface HbA	987	3.62	2.49	3.65	3.57	2.95	2.62	2.54	2.33	*
	988	3.74	3.39	3.66	3.72	3.66	3.39	2.52	2.93	1.99
	990	3.21	2.64	3.33	3.83	2.68	2.49	2.42	2.19	1.88
Mean		3.08	2.60	3.16	3.40	2.72	2.65	2.24	2.26	2.06
S.E.		0.29	0.26	0.25	0.20	0.30	0.21	0.23	0.19	0.11
	970	1.94	1.65	2.25	2.66	2.52	2.14	1.39	1.84	1.72
	973	3.00	2.89	2.98	2.50	2.92	2.54	2.28	2.33	2.23
Blackface HbAB	978	2.85	2.51	3.25	3.39	2.69	3.25	2.80	2.67	2.35
	979	2.67	2.63	3.07	3.23	2.70	2.47	2.35	2.27	2.17
	984	3.77	3.55	3.48	3.25	3.01	2.50	1.89	2.29	1.63
Mean		2.85	2.65	3.01	3.01	2.82	2.85	2.14	2.28	2.02
S.E.		0.30	0.32	0.21	0.18	0.10	0.19	0.24	0.14	0.15
	1	2.63	2.68	2.60	2.65	2.28	2.37	1.67	2.30	1.80
	2	3.50	3.18	3.24	3.60	2.62	2.81	2.67	2.47	2.65
Blackface HbB	3	2.33	3.20	3.24	3.48	3.72	3.59	2.72	2.65	3.46
	6	3.32	3.22	3.56	3.80	2.42	2.28	1.86	2.52	2.68
	8	2.64	2.68	2.90	3.35	1.97	1.80	1.68	1.49	1.95
Mean		2.88	2.99	3.11	3.38	2.60	2.57	2.12	2.29	2.51
S.E.		0.23	0.13	0.17	0.20	0.31	0.31	0.24	0.21	0.30

* Died on day 23

TABLE 39.

Percentage of HbC in the blood following infection with 1400 H. contortus larvae/kg.

	Sheep No.	Days after infection 0	7	14	21	24
	977	2	2	4	9	18
	988	0	1	2	16	34
Blackface HbA	990	1	3	3	12	23
	966	2	0	2	18	28
	987	3	2	4	22	*
Mean		1.6	1.6	3.0	15.4	25.8
S.E.		0.5	0.5	0.5	2.3	3.4
	978	-	-	1	-	4
	979	-	-	-	5	27
Blackface HbAB	973	-	-	2	3	12
	984	-	-	-	12	23
	970	-	-	-	4	9
Mean		0	0	0.6	4.8	15.0
S.E.		0	0	0.4	2.0	4.3

* Died

TABLE 40.

Body weights and blood volumes before and after infection with Haemonchus contortus.

	Sheep No.	Before infection: Wt. (kg)	RCV (ml)	RCV (ml/kg)	Vp (ml)	Vp (ml/kg)	BV (ml)	BV (ml/kg)	24 days after infection: Wt. (kg)	RCV (ml)	RCV (ml/kg)	Vp (ml)	Vp (ml/kg)	BV (ml)	BV (ml/kg)
	966	36.4	656	18.0	1439	39.5	2095	57.5	36.4	252	7.0	1687	46.3	1939	53.3
	977	31.6	576	18.2	1121	35.5	1697	53.7	30.0	210	7.0	1410	47.0	1621	54.0
Blackface HbA	987	36.9	790	21.4	1647	44.6	2437	67.0							
	988	31.4	574	18.3	1324	42.2	1898	60.5	30.2	139	4.5	1598	53.0	1737	57.5
	990	36.6	631	17.2	1328	36.3	1959	53.5	35.5	228	6.4	1529	43.1	1757	49.5
Mean		34.6	645	18.6	1372	39.6	2017	58.4	33.0	207	6.2	1556	47.4	1764	53.5
S.E.		1.3	39	0.7	86	1.7	123	2.5	1.7	24	0.6	58	2.1	66	1.6
	970	30.7	498	16.2	1212	39.5	1710	55.7	30.5	212	6.9	1302	42.7	1514	49.6
	973	29.1	492	16.9	1202	41.3	1694	58.2	29.3	192	6.5	1409	48.1	1601	54.6
Blackface HbAB	978	29.6	485	16.4	1172	39.6	1657	56.0	29.8	267	9.0	1302	43.7	1569	52.7
	979	32.8	597	18.2	1228	37.4	1825	55.6	31.6	154	4.3	1555	49.8	1709	54.1
	984	31.6	683	21.6	1454	46.0	2137	67.6	29.8	122	4.1	1620	54.4	1742	58.5
Mean		30.8	551	17.9	1254	40.8	1805	58.7	30.2	189	6.2	1438	47.7	1627	53.9
S.E.		0.7	39	1.0	51	1.4	88	2.3	0.4	25	0.9	65	2.1	43	1.4
	1	33.4	562	16.8	1281	38.4	1843	55.2	33.6	151	4.5	1734	51.6	1885	56.1
	2	31.9	638	20.0	1388	43.5	2026	63.5	29.3	164	5.6	1480	50.5	1644	56.1
Blackface HbB	3	32.4	550	17.0	1256	38.8	1806	55.8	30.0	218	7.2	1340	44.7	1558	51.9
	6	33.2	566	17.0	1358	40.9	1924	57.9	34.5	322	9.3	1570	45.5	1892	54.8
	8	32.1	614	19.1	1212	37.8	1826	56.9	29.8	158	5.3	1426	47.9	1584	53.2
Mean		32.6	586	18.0	1299	39.9	1885	57.9	31.4	203	6.4	1510	48.0	1713	54.4
S.E.		0.3	17	0.7	33	1.0	41	1.5	1.1	32	0.9	67	1.4	73	0.8

TABLE 41.

Faecal blood clearance (ml/day) of Scottish Blackface sheep infected with 1400 H. contortus larvae/kg.

	Sheep No.	Days after infection										
		-28-0	4	6	8	10	12	14	16	18	20	22
Blackface HbA	966		1.9	4.8	29.0	49.0	120.2	246.8	307.0	443.3	475.0	459.0
	977		1.5	5.3	29.3	62.1	141.0	241.0	271.0	291.0	241.0	200.0
	987		3.3	5.5	83.2	90.4	274.0	641.0	595.0	625.0	550.0	*
	988		3.8	5.7	26.3	48.7	152.0	230.0	317.0	704.0	505.0	328.0
	990		4.9	8.5	44.9	69.1	89.4	152.1	139.2	129.2	135.0	136.3
Mean		2.2	3.1	6.0	42.5	63.9	155.3	302.0	325.8	438.5	381.2	280.8
S.E.		0.1	0.6	0.7	10.7	7.7	31.5	86.4	74.4	105.5	81.5	71.5
Blackface HbAB	970		1.6	3.8	24.7	33.9	71.7	125.7	131.9	122.8	129.7	100.3
	973		2.1	4.4	28.6	42.8	99.0	188.5	258.2	302.0	378.0	618.0
	978		1.2	2.2	7.5	7.3	17.3	41.4	65.7	97.0	129.1	121.3
	979		2.1	4.2	25.0	48.8	43.2	238.4	338.5	366.4	402.1	279.0
	984		4.0	4.8	30.4	67.1	147.6	235.0	250.0	207.2	225.2	176.0
Mean		2.5	2.2	3.9	23.2	40.0	75.8	165.8	208.9	219.1	252.8	258.9
S.E.		0.4	0.5	0.5	4.1	9.8	22.6	37.1	48.7	51.4	58.8	95.0
Blackface HbB	1		7.0	9.8	52.0	95.3	318.0	377.6	398.0	397.0	426.0	354.0
	2		2.9	2.9	13.8	52.0	102.0	227.0	397.0	497.0	236.0	353.0
	6		2.6	4.6	31.2	36.0	45.5	56.8	80.0	130.3	151.3	145.7
	8		2.6	5.1	39.5	58.3	120.8	172.1	242.4	271.0	216.2	179.3
Mean		2.2	3.8	5.6	34.1	60.5	146.6	208.4	279.4	323.8	257.4	258.0
S.E.		0.2	1.1	1.5	8.0	12.7	59.3	66.6	75.8	79.4	59.0	55.6

* Died on day 23

TABLE 42.

Faecal red cell clearances (ml/day) of Scottish Blackface sheep infected with 1400 *H. contortus* larvae/kg.

	Sheep No.	Days after infection										
		-28-0	4	6	8	10	12	14	16	18	20	22
Blackface HbA	966		0.5	1.2	7.3	11.4	24.1	40.7	45.5	61.0	60.1	47.8
	977		0.4	1.4	7.9	15.8	29.6	39.3	40.7	39.8	31.0	23.9
	987		0.9	1.5	14.9	25.2	54.0	71.4	62.3	54.0	37.3	*
	988		0.9	1.4	6.7	11.8	28.9	33.3	44.7	61.3	50.2	37.4
	990		1.4	2.3	11.4	18.5	22.1	31.2	25.0	18.7	16.6	13.4
Mean		0.7	0.8	1.6	9.6	16.5	31.7	43.2	43.6	47.0	39.0	30.6
S.E.		0.1	0.2	0.2	1.5	2.5	5.7	7.3	6.0	8.1	7.5	7.5
Blackface HbAB	970		0.4	0.9	5.4	6.9	13.7	21.8	20.7	17.4	16.6	11.4
	973		0.7	1.4	8.0	10.9	18.9	27.4	30.0	28.8	24.1	27.7
	978		0.3	0.6	2.0	1.9	4.6	10.8	15.7	20.8	24.8	20.6
	979		0.6	1.1	6.5	12.5	10.3	38.7	43.2	40.3	38.5	19.7
	984		1.0	1.3	7.6	16.3	33.4	39.7	31.5	26.3	27.2	19.0
Mean		0.7	0.6	1.1	5.9	9.7	16.2	27.7	28.2	26.7	26.2	19.7
S.E.		0.1	0.1	0.1	1.1	2.5	4.9	5.4	4.8	3.9	3.5	2.6
Blackface HbB	1		1.9	2.6	12.8	22.1	57.5	61.4	61.6	41.1	37.3	25.3
	2		0.7	0.7	3.1	7.4	23.0	42.3	52.2	65.9	28.0	33.6
	6		0.7	1.2	8.4	9.7	12.3	15.2	18.6	26.4	27.0	25.3
	8		0.6	1.1	3.1	12.6	22.7	27.2	35.1	42.3	37.1	31.1
Mean		0.7	1.0	1.4	6.9	13.0	28.9	36.5	41.9	43.9	32.4	28.8
S.E.		0.1	0.3	0.4	2.3	3.2	9.9	10.0	9.5	8.2	2.8	2.1

* Died on day 23

TABLE 43.

Ferrokinetic indices 28 days before infection.

	Sheep No.	Serum iron (µg %)	Plasma ^{59}Fe $t_{\frac{1}{2}}$ (min)	Plasma iron turnover rate		
				mg/day	mg/day/100 ml blood	mg/kg/day
Blackface HbA	966	161	177	12.8	0.60	0.38
	977	198	210	9.8	0.39	0.32
	987	147	200	11.4	0.44	0.33
	988	123	112	14.1	0.75	0.45
	990	174	163	13.4	0.69	0.37
Mean		161	172	12.3	0.57	0.37
S.E.		13	18	0.8	0.07	0.02
Blackface HbAB	970	138	156	10.4	0.59	0.35
	973	130	151	10.2	0.60	0.36
	978	137	126	10.6	0.73	0.37
	979	187	188	11.6	0.67	0.36
	984	135	158	12.8	0.55	0.43
Mean		145	156	11.1	0.63	0.37
S.E.		10	10	0.5	0.03	0.01
Blackface HbB	1	188	273	8.5	0.47	0.26
	2	146	77	24.6	1.12	0.78
	3	202	168	14.3	0.82	0.42
	6	137	177	9.9	0.53	0.31
	8	149	144	12.3	0.65	0.39
Mean		164	168	14.0	0.72	0.43
S.E.		13	32	3.0	0.12	0.08

TABLE 44.

Albumin pools before and after infection with Haemonchus contortus.

	Sheep No.	Before infection CA (g)	CA (gm/kg)	EA (g)	EA (g/kg)	TA (g)	TA (g/kg)	EA/CA	24 days after infection CA (g)	CA (g/kg)
	966	39.8	1.26	82.8	2.62	122.6	3.88	2.08	26.2	0.72
	977	37.1	1.18	65.0	2.07	102.1	3.25	1.75	28.8	0.96
Blackface HbA	987	50.5	1.38	91.5	2.50	142.0	3.88	1.81		
	988	48.0	1.32	93.5	2.57	141.5	3.89	1.95	27.5	0.91
	990	53.9	1.46	103.0	2.79	156.9	4.25	1.91	34.4	0.97
Mean		45.9	1.32	87.2	2.51	133.0	3.83	1.90	29.2	0.89
S.E.		3.2	0.05	6.4	0.12	9.5	0.16	0.06	1.8	0.06
	970	45.1	1.47	77.1	2.51	122.2	3.98	1.11	27.8	0.91
	973	38.1	1.31	62.9	2.16	101.0	3.47	1.65	25.8	0.88
Blackface HbAB	978	41.1	1.39	68.6	2.31	109.7	3.71	1.67	36.1	1.21
	979	42.3	1.26	67.9	2.07	110.2	3.33	1.64	23.7	0.75
	984	49.6	1.57	80.2	2.54	129.8	4.11	1.62	20.0	0.67
Mean		43.2	1.40	71.3	2.32	114.5	3.72	1.66	26.7	0.88
S.E.		1.9	0.06	3.2	0.09	5.1	0.15	0.11	2.7	0.09
	1	46.1	1.38	70.8	2.12	116.9	3.50	1.54	27.9	0.83
	2	46.6	1.46	89.3	2.80	135.9	4.26	1.92	24.9	0.85
Blackface HbB	3	47.3	1.46	92.0	2.84	139.3	4.30	1.95	19.2	0.64
	6	46.1	1.39	87.3	2.63	133.4	4.02	1.89	43.5	1.26
	8	46.2	1.44	87.0	2.71	133.2	4.15	1.88	31.3	1.05
Mean		46.5	1.43	85.3	2.62	131.8	4.05	1.84	29.4	0.93
S.E.		0.2	0.02	3.7	0.13	3.9	0.15	0.08	4.1	0.11

TABLE 45.

Fractional catabolic rate of albumin F(CA) in Scottish Blackface sheep infected with 1400 H. contortus larvae/kg.

	Sheep No.	Days after infection										
		-28-0	4	6	8	10	12	14	16	18	20	22
Blackface HbA	966		.045	.052	.084	.085	.085	.157	.212	.306	.313	.334
	977		.076	.067	.092	.088	.088	.133	.214	.435	.423	.305
	987		.081	.067	.056	.115	.133	.179	.271	.326	.263	*
	988		.082	.080	.098	.097	.134	.158	.225	.375	.433	.530
	990		.090	.082	.088	.099	.160	.204	.251	.353	.349	.224
Mean		.070	.075	.070	.084	.097	.120	.166	.235	.359	.356	.348
S.E.		.010	.007	.005	.007	.005	.015	.012	.011	.022	.032	.065
Blackface HbAB	970		.061	.030	.081	.079	.095	.077	.124	.171	.309	.340
	973		.066	.082	.103	.080	.135	.161	.202	.247	.309	.407
	978		.037	.039	.050	.161	.148	.144	.058	.082	.050	.103
	979		.083	.081	.112	.092	.121	.095	.279	.488	.652	.550
	984		.070	.073	.104	.123	.129	.306	.466	.364	.323	.286
Mean		.050	.063	.061	.090	.107	.126	.157	.226	.270	.329	.337
S.E.		.010	.008	.011	.011	.016	.009	.040	.071	.071	.010	.073
Blackface HbB	1		.072	.049	.130	.115	.138	.177	.093	.471	.428	.524
	2		.081	.046	.056	.102	.161	.084	.202	.245	.102	.298
	6		.049	.058	.074	.077	.068	.058	.080	.141	.129	.141
	8		.061	.056	.111	.080	.102	.102	.246	.473	.391	.376
Mean		.060	.066	.052	.093	.094	.117	.105	.155	.333	.263	.335
S.E.		.010	.007	.003	.017	.009	.020	.025	.040	.083	.085	.080

* Died on day 23

TABLE 46.

Faecal ^{125}I "clearances" (ml/day) of Scottish Blackface sheep infected with 1400 H. contortus larvae/kg.

	Sheep No.	Days after infection										
		-28-0	4	6	8	10	12	14	16	18	20	22
Blackface HbA	966		13.8	15.7	22.6	25.3	42.4	68.8	84.8	132.0	175.0	179.0
	977		5.5	13.8	13.3	20.8	28.1	44.0	57.0	77.4	68.2	66.5
	987		18.2	21.3	30.0	25.1	25.3	38.6	71.3	71.3	67.6	*
	988		40.7	37.1	33.5	45.8	52.7	59.7	97.7	117.8	142.6	90.8
	990		8.2	10.4	15.8	25.4	31.9	23.3	25.5	68.5	72.9	41.6
Mean		16.3	17.3	19.7	23.0	28.5	36.1	46.9	67.3	93.4	105.3	94.5
S.E.		4.0	6.3	4.7	3.9	4.4	5.1	8.0	12.5	13.1	22.5	29.9
Blackface HbAB	970		6.9	9.7	15.3	14.7	10.8	15.7	17.2	29.6	46.3	48.0
	973		16.6	19.0	26.2	29.3	46.2	55.2	92.0	128.5	171.4	218.6
	978		6.9	8.8	8.4	16.3	15.3	20.4	18.1	24.9	33.9	29.5
	979		13.1	15.5	15.0	24.2	49.7	49.1	57.6	91.0	140.2	96.7
	984		15.0	15.1	21.8	36.7	33.0	32.9	41.4	43.4	41.5	38.7
Mean		10.3	11.7	13.6	17.3	24.2	31.0	34.7	76.2	63.5	86.7	86.3
S.E.		1.0	2.0	1.9	3.1	4.1	7.9	7.7	26.8	20.0	28.7	35.1
Blackface HbB	1		8.4	18.1	27.9	29.9	48.0	65.9	81.3	82.2	81.5	57.0
	2		27.4	11.2	17.8	36.5	39.4	52.8	153.8	115.5	89.7	96.6
	6		15.6	15.6	29.9	20.0	38.7	37.6	35.1	44.3	44.1	43.0
	8		11.3	9.8	17.7	18.9	24.4	27.8	52.6	57.4	49.7	40.3
Mean		15.3	15.7	13.7	23.3	26.3	37.6	46.0	80.7	74.9	66.3	59.2
S.E.		3.0	4.2	1.9	3.2	4.2	4.9	8.4	26.2	15.0	11.0	13.0

* Died on day 23

APPENDIX 2

The Influence of Haemoglobin Type and Breed on the Response of Sheep to Primary and Secondary Infections with *H. contortus*.

TABLE 1.
Packed cell volumes (%).

Days after infection	Blackface HbA 56	57	91	Mean ±S.E.	Blackface HbAB 80	82	90	Mean ±S.E.	Dorset HbAB 482	521	538	Mean ±S.E.	Dorset HbB 477	547	549	Mean ±S.E.
0	27.0	28.0	32.0	29.0±1.5	29.0	25.0	27.0	27.0±1.2	31.0	31.0	33.0	31.7±0.7	27.0	33.0	33.0	31.0±2.0
4	27.0	28.0	31.0	28.7±1.2	30.0	25.0	26.0	27.0±1.5	30.0	34.0	34.0	32.7±1.3	28.0	33.0	32.0	31.0±1.5
7	28.0	27.0	32.0	29.0±1.5	30.0	27.0	26.0	27.7±1.2	30.0	33.0	34.0	32.3±1.2	25.0	32.0	33.0	30.0±2.5
11	26.5	25.0	29.0	26.8±1.2	26.0	25.0	23.0	24.7±0.9	25.0	30.0	30.0	28.3±1.7	25.0	28.0	31.0	28.0±1.7
14	24.5	24.5	27.0	25.3±0.8	25.0	22.0	21.0	22.7±1.2	24.0	27.0	25.0	25.3±0.9	21.0	25.0	26.0	24.0±1.5
18	21.5	22.0	22.0	21.8±0.2	20.0	20.0	19.0	19.7±0.3	20.0	19.0	20.0	19.7±0.3	18.0	17.0	22.0	19.0±1.5
21	20.0	21.0	21.0	20.7±0.3	19.0	20.0	17.0	18.7±0.9	19.0	17.0	17.0	17.7±0.7	17.0	17.0	18.0	17.3±0.3
25	21.0	23.0	21.0	21.7±0.7	20.0	20.0	16.0	18.7±1.3	18.0	15.0	18.0	17.0±1.0	17.0	12.0	15.0	14.7±1.5
28	20.0	22.0	22.0	21.3±0.7	19.0	20.0	17.0	18.7±0.9	18.0	16.0	16.0	16.7±0.7	16.0	12.0	15.5	14.5±1.3
32	20.0	21.5	19.0	20.2±0.7	18.0	19.0	15.0	17.3±1.2	16.0	15.0	14.0	15.0±0.6	14.0	10.0	10.0	11.3±1.3
35	20.0	18.0	18.0	18.7±0.7	17.0	16.0	15.0	16.0±0.6	15.0	12.0	13.0	13.3±0.9	14.0	10.0	9.0	11.0±1.5
Days after treatment																
4	20.0	22.0	20.0	20.7±0.7	20.0	19.0	18.0	19.0±0.6	17.0	14.0	14.0	15.0±1.0	18.0	12.0	13.0	14.3±1.9
7	21.0	24.0	20.0	21.7±1.2	22.0	22.0	19.0	21.0±1.0	18.0	15.0	17.0	16.7±0.9	17.0	14.0	15.0	15.3±0.9
11	23.0	26.0	20.0	23.0±1.7	23.0	21.0	20.0	21.3±0.9	19.0	20.0	19.0	19.3±0.3	22.0	17.0	16.0	18.3±1.9
14	23.0	25.0	20.0	22.7±1.5	23.0	23.0	23.0	23.0±0.0	21.0	19.0	18.5	19.5±0.5	24.0	18.5	19.0	20.5±1.8
18	23.5	27.0	26.0	25.5±1.0	24.0	22.5	23.0	23.2±0.4	21.0	21.0	21.0	21.0±0.0	23.0	18.5	20.0	20.5±1.3
Days after reinfection																
4	21.0	25.0	29.0	25.0±2.3	22.0	22.0	22.0	22.0±0.0	21.5	20.0	22.0	21.2±0.6	24.0	19.0	21.0	21.3±1.5
7	23.0	25.0	29.0	25.7±1.8	23.0	22.0	22.5	22.5±0.3	21.0	22.5	23.0	22.2±0.6	21.0	20.0	20.0	20.3±0.3
11	21.0	23.0	29.0	24.3±2.4	23.0	21.0	21.5	21.8±0.6	22.0	23.0	23.0	22.7±0.3	21.0	20.0	20.0	20.3±0.3
14	20.5	21.0	27.0	22.8±2.1	21.0	18.0	18.0	19.0±1.0	21.0	22.0	21.0	21.3±0.3	20.0	15.5	19.0	18.2±1.4
18	18.5	20.5	24.5	21.2±1.8	19.5	17.0	16.5	17.7±0.9	18.0	19.0	18.0	18.3±0.3	17.0	13.0	16.5	15.5±1.3
21	17.0	20.0	22.0	19.7±1.5	18.0	17.0	15.0	16.7±0.9	15.0	17.0	16.0	16.0±0.6	14.5	10.0	13.0	12.5±1.3
25	17.0	19.0	22.0	19.3±1.5	18.0	17.5	13.5	16.3±1.4	14.0	*	13.5	13.8±0.3	15.0	10.0	14.0	13.0±1.5
28	17.0	18.5	20.0	18.5±0.9	15.5	14.0	12.5	14.0±0.9	12.0		12.0	12.0±0.0	12.5	6.0	12.0	10.2±2.1
32	17.0	18.0	20.0	18.3±0.9	15.0	14.0	12.0	13.0±0.9	12.0		11.5	11.8±0.3	12.0	6.0	10.0	9.3±1.8

* Died on day 23

TABLE 2.
Haemoglobin concentrations (g%).

Days after infection	Blackface HbA 56	57	91	Mean ±S.E.	Blackface HbAB 80	82	92	Mean ±S.E.	Dorset HbAB 482	521	538	Mean ±S.E.	Dorset HbB 477	547	549	Mean ±S.E.
0	10.6	9.5	12.3	10.8±0.8	11.0	11.2	10.6	10.9±0.2	11.5	13.5	12.0	12.3±0.6	13.5	11.7	12.0	12.4±0.6
4	8.3	9.3	10.3	9.3±0.6	10.3	9.7	9.3	9.8±0.3	10.1	11.5	11.4	11.0±0.5	10.0	11.9	11.8	11.2±0.6
7	9.0	9.3	10.4	9.6±0.4	10.1	9.1	9.3	9.5±0.3	10.1	11.9	11.8	11.3±0.6	8.9	14.5	12.7	12.0±1.7
11	8.8	8.9	9.7	9.1±0.3	9.5	9.2	8.7	9.1±0.2	9.6	10.8	11.2	10.5±0.5	8.9	10.5	11.9	10.4±0.9
14	9.1	8.2	9.5	8.9±0.7	9.4	8.6	7.9	8.6±0.4	8.7	9.5	9.5	9.2±0.3	7.8	8.8	9.9	8.8±0.6
18	6.3	7.1	7.4	6.9±0.3	7.1	7.5	6.9	7.2±0.2	7.6	6.8	7.4	7.3±0.2	6.1	6.4	7.6	6.7±0.5
21	6.6	6.8	6.7	6.7±0.6	6.7	6.8	5.6	6.4±0.4	6.4	6.1	5.9	6.1±0.2	5.9	5.7	6.2	5.9±0.2
25	6.4	7.2	6.6	6.7±0.2	6.4	7.3	5.6	6.4±0.5	6.3	5.0	5.7	5.7±0.4	6.2	4.2	5.0	5.1±0.6
28	7.2	7.6	7.0	7.3±0.2	6.0	7.1	5.3	6.1±0.5	6.1	5.2	5.4	5.6±0.3	5.0	3.5	4.3	4.3±0.4
32	5.7	6.1	6.4	6.1±0.2	4.9	6.2	4.7	5.3±0.5	4.7	3.8	3.9	4.1±0.3	4.2	2.3	2.9	3.1±0.6
35	5.7	5.8	5.1	5.5±0.2	5.6	5.4	4.3	5.1±0.4	4.4	3.1	3.2	3.6±0.4	3.7	1.9	2.4	2.7±0.5
Days after treatment																
4	6.3	6.8	5.7	6.3±0.3	6.6	6.3	5.3	6.1±0.4	4.9	4.0	4.1	4.3±0.3	5.1	2.7	3.4	3.7±0.7
7	6.5	7.2	6.3	6.7±0.3	7.1	6.8	6.8	6.9±0.1	5.5	4.7	5.0	5.1±0.2	6.0	4.0	4.3	4.8±0.6
11	7.2	8.2	8.1	7.8±0.3	7.7	7.6	7.4	7.6±0.1	6.2	5.8	5.8	5.9±0.2	7.1	5.0	4.9	5.7±0.7
14	7.3	8.5	8.1	8.0±0.4	8.5	7.6	7.6	7.9±0.3	6.8	6.4	5.8	6.3±0.3	7.5	5.3	5.5	6.1±0.7
18	7.3	8.8	8.5	8.2±0.5	7.6	7.9	7.8	7.8±0.1	6.8	6.6	6.7	6.7±0.1	7.6	5.8	6.6	6.7±0.5
Days after reinfection																
4	7.3	8.5	8.2	8.0±0.4	7.8	7.4	7.1	7.4±0.2	7.0	6.5	6.9	6.8±0.2	7.1	6.1	6.5	6.6±0.3
7	7.3	8.6	9.3	8.4±0.6	7.9	7.7	7.3	7.6±0.2	5.7	7.0	7.7	6.8±0.6	7.4	6.4	6.7	6.8±0.3
11	7.1	7.9	9.2	8.1±0.6	7.6	7.4	7.4	7.5±0.1	7.2	7.2	7.5	7.3±0.1	7.1	6.5	6.6	6.7±0.2
14	7.0	7.8	9.2	8.0±0.6	7.8	6.8	6.2	7.3±0.3	7.5	7.5	7.5	7.5±0.0	6.8	5.8	6.2	6.3±0.3
18	6.0	6.7	8.8	7.2±0.8	7.5	6.3	5.5	6.4±0.6	6.5	7.0	6.3	6.6±0.2	5.4	4.2	5.4	5.0±0.4
21	5.2	7.1	7.9	6.7±0.8	6.7	5.7	5.2	5.9±0.4	5.2	6.1	5.4	5.6±0.3	4.6	3.2	4.5	4.1±0.5
25	5.7	6.5	6.4	6.2±0.3	5.7	5.2	4.7	5.2±0.3	4.3	*	4.7	4.5±0.2	4.8	3.2	4.2	4.0±0.5
28	5.3	6.1	6.3	5.9±0.3	5.1	4.8	4.1	4.7±0.3	4.0		4.1	4.1±0.1	3.4	1.6	3.1	2.7±0.6
32	5.4	5.5	5.9	5.6±0.2	5.0	4.8	4.0	4.6±0.3	4.1		4.2	4.2±0.1	3.1	1.3	2.7	2.4±0.6

* Died on day 23.

TABLE 3.

Red cell counts ($x10^6$)

Days after infection	Blackface HbA			Mean ±S.E.	Blackface HbAB			Mean ±S.E.	Dorset HbAB			Mean ±S.E.	Dorset HbB			Mean ±S.E.
	56	57	91		80	82	92		482	521	538		477	547	549	
0	8.0	8.0	8.6	8.2±0.2	8.2	8.0	8.1	8.1±0.1	9.0	9.0	10.0	10.0±0.1	8.0	8.9	10.2	9.0±0.6
4	7.5	8.7	9.7	8.6±0.6	9.6	8.7	8.3	8.9±0.4	9.9	10.2	10.0	10.0±0.1	9.3	11.0	11.2	10.5±0.6
7	8.0	8.5	10.1	8.9±0.6	9.3	8.8	8.5	8.9±0.2	9.5	9.7	10.4	9.9±0.3	8.0	10.1	9.9	9.3±0.7
11	7.5	8.0	9.3	8.3±0.5	8.2	8.0	7.6	7.9±0.2	10.7	9.6	9.2	9.8±0.5	7.7	9.1	10.7	9.2±0.9
14	7.5	7.2	8.3	7.7±0.3	8.8	6.9	6.9	7.5±0.6	7.0	8.2	7.5	7.6±0.4	6.4	7.5	8.4	7.4±0.6
18	5.7	5.9	6.2	5.9±0.2	6.7	5.9	4.9	5.8±0.5	4.9	5.7	5.9	5.5±0.3	4.7	6.2	8.5	6.5±1.1
21	5.1	6.0	5.5	5.5±0.3	5.7	5.5	4.6	5.3±0.3	5.0	5.0	4.8	4.9±0.1	4.5	4.5	4.9	4.6±0.1
25	5.2	5.9	5.1	5.4±0.3	5.5	5.6	3.8	5.0±0.6	4.5	3.9	4.4	4.3±0.2	4.5	3.2	4.3	4.0±0.4
28	5.6	5.4	5.5	5.5±0.1	4.9	5.0	3.4	4.4±0.5	4.1	3.2	3.5	3.6±0.3	4.0	2.6	3.1	3.2±0.4
32	5.7	5.6	4.5	5.3±0.4	4.5	4.7	3.4	4.2±0.4	3.8	3.2	3.3	3.4±0.2	3.7	2.0	2.3	2.7±0.5
35	4.8	4.6	4.0	4.5±0.2	4.2	3.6	3.6	3.8±0.2	3.4	2.8	3.0	3.1±0.2	3.2	2.3	2.4	2.6±0.3
Days after treatment																
4	5.2	5.8	5.3	5.4±0.2	5.4	4.7	4.9	5.0±0.2	4.9	3.9	2.9	3.9±0.6	4.7	2.3	2.6	3.2±0.8
7	6.0	6.6	6.5	6.4±0.2	6.0	6.0	6.4	6.1±0.1	6.0	4.5	4.9	5.1±0.5	5.2	4.2	4.5	4.6±0.3
11	6.2	6.7	6.9	6.6±0.2	6.5	6.8	6.2	6.5±0.2	5.6	5.9	5.5	5.7±0.1	6.1	3.8	5.3	5.1±0.7
14	7.1	7.3	7.9	7.4±0.2	7.3	6.9	6.6	6.9±0.2	6.0	5.7	4.4	5.4±0.5	7.0	4.5	4.5	5.3±0.8
18	7.0	7.2	9.2	7.8±0.7	6.8	7.5	6.7	7.0±0.3	5.9	5.9	5.2	5.7±0.2	6.3	4.5	5.7	5.5±0.5
Days after reinfection																
4	5.3	7.1	8.0	6.8±0.8	6.9	6.0	6.2	6.4±0.3	6.0	5.2	5.0	5.4±0.3	5.3	4.6	5.7	5.2±0.3
7	5.7	7.1	7.6	6.8±0.6	6.7	6.7	6.7	6.7±0.0	5.4	6.5	6.5	6.1±0.4	6.6	5.7	6.3	6.2±0.3
11	5.7	6.8	7.4	6.6±0.5	6.0	6.2	5.9	6.0±0.1	6.3	5.9	5.9	6.0±0.1	5.3	5.7	6.7	5.6±0.1
14	6.0	7.0	6.8	6.6±0.3	7.3	6.7	5.2	6.4±0.6	6.0	6.4	6.8	6.4±0.2	6.8	7.1	7.7	7.2±0.3
18	5.8	6.3	6.7	6.3±0.3	6.0	5.3	5.0	5.4±0.3	5.3	5.8	4.7	5.3±0.3	5.1	3.6	5.0	4.6±0.5
21	4.3	5.7	5.2	5.1±0.4	5.2	4.7	4.2	4.7±0.3	4.8	4.9	4.3	4.7±0.2	4.2	3.2	4.5	4.0±0.4
25	4.2	4.8	5.7	4.9±0.4	4.4	4.6	2.9	4.0±0.5	3.5	*	2.9	3.2±0.3	3.3	2.1	3.0	2.8±0.4
28	4.3	4.1	5.5	4.6±0.4	3.6	3.2	2.5	3.1±0.3	2.8		2.6	2.7±0.1	3.0	1.2	2.6	2.3±0.6
32	4.0	4.3	5.0	4.4±0.3	3.2	2.9	2.3	2.8±0.3	3.2		2.9	3.1±0.2	3.1	1.5	2.0	2.2±0.5

*Died on day 23

TABLE 4.

Mean corpuscular volumes (μ^3).

Days after infection	Blackface HbA 56	57	91	Mean ±S.E.	Blackface HbAB 80	82	92	Mean ±S.E.	Dorset HbAB 482	521	538	Mean ±S.E.	Dorset HbB 477	547	549	Mean ±S.E.
0	33.6	35.1	37.3	35.3±1.1	35.4	31.4	33.3	33.4±1.2	34.4	34.4	33.0	33.9±0.5	33.6	37.0	32.0	34.2±1.5
4	36.0	32.2	32.1	33.4±1.3	31.3	28.9	31.3	30.5±0.8	30.4	33.3	33.9	32.5±1.1	30.1	30.1	28.6	29.6±0.5
7	35.1	31.7	31.5	32.8±1.2	32.2	30.5	30.7	31.1±0.5	31.5	34.0	32.8	32.8±0.7	31.3	31.8	33.5	32.2±0.7
11	35.1	31.4	30.0	32.2±1.5	31.8	30.5	30.6	31.0±0.4	32.3	31.3	32.5	32.0±0.4	32.6	30.8	28.9	30.8±1.1
14	32.7	34.1	32.6	33.1±0.5	28.5	32.1	30.4	30.3±1.0	34.3	32.9	33.2	33.5±0.4	32.7	33.3	31.0	32.3±0.7
18	32.9	37.3	35.5	35.2±1.3	29.7	33.9	36.6	33.4±2.0	41.2	33.1	33.9	36.1±2.6	38.0	37.3	33.7	36.3±1.3
21	39.1	35.7	38.2	37.7±1.0	31.5	36.1	37.0	34.9±1.7	37.8	33.7	35.8	35.8±1.2	37.6	37.4	36.7	37.2±0.3
25	40.2	39.1	41.0	40.1±0.6	36.1	35.6	35.7	35.8±0.2	40.0	38.3	41.3	39.9±0.9	38.1	38.4	35.1	37.2±1.1
28	38.0	41.0	40.0	39.7±0.9	39.2	40.1	47.0	42.1±2.5	44.2	47.0	51.0	47.4±2.0	40.0	46.1	52.0	46.0±3.5
32	38.2	39.1	42.0	39.8±1.2	40.0	40.2	44.4	41.5±1.4	42.0	47.0	42.0	43.7±1.7	38.4	50.0	43.0	43.8±3.4
35	42.0	39.0	45.0	42.0±1.7	40.0	44.0	42.0	42.0±1.2	44.0	43.0	43.0	43.3±0.3	44.0	43.0	38.0	41.7±1.9
Days after treatment																
4	38.5	37.9	37.7	38.0±0.2	37.0	40.4	36.7	38.0±1.2	34.7	35.9	48.3	39.6±4.3	38.2	52.2	50.0	46.8±4.3
7	35.0	36.4	36.8	36.1±0.5	36.7	36.7	30.0	34.5±2.2	30.0	33.3	34.7	32.7±1.4	32.7	33.3	33.3	33.1±0.2
11	37.1	38.8	39.0	38.3±0.6	35.4	30.9	32.3	32.9±1.3	33.9	33.9	34.6	34.1±0.2	36.1	44.7	30.2	37.0±4.2
14	32.4	34.2	35.3	33.9±0.8	31.5	33.3	34.5	33.1±0.9	35.0	33.3	42.0	36.8±2.7	34.3	41.1	42.2	39.2±2.5
18	33.6	37.5	38.3	36.5±1.5	35.3	30.0	34.3	33.2±1.6	35.6	35.6	40.4	37.2±1.6	36.5	41.1	35.1	37.6±1.8
Days after reinfection																
4	39.6	35.2	36.3	37.0±1.3	31.9	36.7	35.5	34.7±1.4	35.8	38.5	44.0	39.4±2.4	45.3	41.3	36.8	41.3±2.5
7	40.4	35.2	38.2	38.0±1.5	34.3	32.8	33.6	33.6±0.4	38.9	34.6	35.4	36.3±1.3	31.8	35.1	37.7	34.9±1.7
11	36.8	33.8	39.2	36.6±1.6	38.3	33.9	36.4	36.2±1.3	34.9	39.0	39.0	37.6±1.4	39.6	35.1	30.0	34.9±2.7
14	34.2	30.0	39.7	34.6±2.8	28.8	26.9	34.6	30.1±2.3	26.3	26.2	23.9	25.5±0.8	29.4	21.8	24.7	25.3±2.2
18	31.9	32.5	36.6	33.7±1.5	32.5	32.1	33.0	32.5±0.3	34.0	32.8	38.3	35.0±1.7	33.3	36.1	33.0	34.1±1.0
21	39.5	35.1	42.0	38.9±2.0	34.6	36.2	35.7	35.5±0.5	31.3	34.7	37.2	34.4±1.7	34.5	31.3	28.9	31.6±1.6
25	40.0	39.6	39.3	39.6±0.2	40.9	38.1	46.6	41.9±2.5	40.0	*	46.6	43.3±3.3	45.6	47.6	46.7	46.6±0.6
28	40.0	45.1	36.1	40.4±2.6	43.1	43.8	50.0	45.6±2.2	42.9		46.2	44.6±1.7	41.7	50.0	46.2	46.0±2.4
32	42.5	41.9	40.0	41.5±0.8	46.9	48.2	52.3	49.1±1.6	37.5		39.7	38.6±1.1	38.7	40.0	50.0	42.9±3.6

* Died on day 23

TABLE 5.

Mean corpuscular haemoglobin concentrations (%).

Days after infection	Blackface HbA 56	57	91	Mean ±S.E.	Blackface HbAB 80	82	92	Mean ±S.E.	Dorset HbAB 482	521	538	Mean ±S.E.	Dorset HbB 477	547	549	Mean ±S.E.
0	39.3	33.9	38.4	37.2±1.7	37.9	44.8	39.3	40.7±2.1	37.1	43.5	36.4	39.0±2.3	50.0	35.5	37.5	41.0±4.5
4	30.7	33.2	33.2	32.4±0.8	34.3	38.3	35.6	36.1±1.2	32.6	33.8	33.5	33.3±0.4	35.7	36.1	36.9	36.2±0.4
7	32.1	34.4	32.5	33.0±0.7	33.7	33.7	25.8	31.1±2.6	33.7	36.1	34.7	34.8±0.7	35.6	45.3	38.5	39.8±2.9
11	33.2	35.6	33.4	34.1±0.8	36.5	36.8	37.8	37.0±0.4	38.4	36.0	37.3	37.2±0.7	35.6	37.5	38.4	37.2±0.8
14	37.1	33.5	35.2	35.3±1.0	37.6	30.7	37.6	35.3±2.3	36.3	35.2	38.0	36.5±0.8	37.1	35.2	36.1	36.1±0.5
18	29.3	32.3	33.6	31.7±1.3	35.5	37.5	38.3	37.1±0.8	38.0	35.8	37.0	37.9±0.6	33.9	37.6	34.5	35.3±1.1
21	33.0	32.4	31.9	32.4±0.3	35.3	34.0	32.9	34.1±0.7	33.7	35.9	34.7	34.9±0.6	34.7	33.5	34.4	34.2±0.4
25	30.5	31.3	31.4	31.1±0.3	32.0	36.5	35.0	34.5±1.3	35.0	33.3	31.7	33.3±1.0	36.5	35.0	33.3	34.9±0.9
28	36.0	34.5	31.8	34.1±1.2	31.6	35.5	31.2	32.8±1.4	33.9	32.5	33.8	33.4±0.5	21.3	29.2	27.7	26.1±2.4
32	28.5	28.4	33.7	30.2±1.8	27.2	32.6	31.3	30.4±1.6	29.4	25.3	27.9	27.5±1.2	30.0	23.0	29.0	27.3±2.2
35	30.8	32.2	28.3	30.4±1.1	32.9	33.8	28.7	31.8±1.6	29.3	25.8	24.6	26.6±1.4	26.4	19.0	26.7	24.0±2.5
Days after treatment																
4	31.5	30.9	28.5	30.3±0.9	33.0	33.2	29.4	31.9±1.2	28.8	28.6	29.3	28.9±0.2	28.3	22.5	26.2	25.7±1.7
7	31.0	30.0	31.5	30.8±0.4	32.3	30.9	35.8	33.0±1.5	30.6	31.3	29.4	30.4±0.6	35.3	28.6	28.7	30.9±2.2
11	31.3	31.5	40.5	34.4±3.0	33.5	36.2	37.0	35.6±1.1	32.6	29.0	30.5	30.7±1.0	30.9	29.4	30.6	30.3±0.5
14	31.7	34.0	40.5	35.4±2.6	37.0	33.0	33.0	34.3±1.3	32.4	33.7	31.4	32.5±0.7	31.3	28.6	28.9	29.6±0.9
18	31.1	32.6	32.7	32.1±0.5	31.7	35.1	33.9	35.6±1.0	32.4	31.4	31.9	31.9±0.3	33.0	31.4	33.0	32.5±0.5
Days after reinfection																
4	34.8	34.0	28.3	32.4±2.0	35.5	33.6	32.3	33.8±0.9	32.6	32.5	31.4	32.2±0.4	30.0	32.1	31.0	31.0±0.6
7	31.7	34.4	32.1	32.7±0.8	34.3	35.0	32.4	33.9±0.8	27.1	31.1	33.5	30.6±1.9	35.2	32.0	33.5	33.6±0.9
11	33.8	34.3	31.7	33.3±0.8	33.0	35.2	34.4	34.2±0.6	32.7	31.3	32.6	32.2±0.5	33.8	32.5	33.0	33.1±0.4
14	34.2	37.1	34.1	35.1±1.0	37.1	37.8	34.4	36.4±1.0	35.7	34.1	35.7	35.2±0.5	34.0	37.4	32.6	34.7±1.4
18	38.7	32.7	35.9	35.8±1.7	38.5	37.1	33.3	36.3±1.6	36.1	36.8	35.0	36.0±0.5	31.8	32.3	33.2	32.4±0.4
21	30.6	35.5	35.9	34.0±1.7	37.2	33.5	34.7	35.1±1.1	34.7	35.9	33.8	34.8±0.6	32.0	32.0	35.2	33.1±0.7
25	33.5	34.2	29.1	32.3±1.6	31.7	29.7	28.7	30.0±0.9	32.6	*	35.0	33.8±1.2	32.0	32.0	30.0	31.3±0.7
28	31.2	33.0	31.5	31.9±0.6	32.9	34.3	33.0	33.4±0.5	33.0		34.2	33.6±0.6	27.2	27.0	26.2	26.8±0.3
32	31.3	30.6	29.5	30.6±0.7	33.3	34.3	33.3	33.6±0.3	34.2		36.5	35.4±1.2	25.9	22.3	27.0	25.1±1.4

* Died on day 23

TABLE 6.

Serum iron concentrations (µg%).

Days after infection	Blackface HbA			Mean ±S.E.	Blackface HbAB			Mean ± S.E.	Dorset HbAB			Mean ±S.E.	Dorset HbB			Mean ±S.E.
	56	57	91		80	82	92		482	521	538		477	547	549	
0	165	135	162	154±10	144	96	138	126±15	142	163	119	141±13	140	174	179	164±12
7	156	156	147	153± 3	131	104	133	123± 9	154	136	100	130±16	169	156	159	161± 4
14	142	149	142	144± 2	127	87	144	119±17	142	149	113	135±11	140	167	173	160±10
21	126	121	129	125± 2	104	110	130	115± 8	118	111	90	106± 8	104	125	135	121± 9
28	114	131	131	125± 6	95	90	91	92± 2	82	67	80	76± 7	87	78	90	85± 4
35	127	124	141	130± 5	109	77	84	90±10	60	50	53	54± 3	84	88	80	84± 2
Days after treatment																
7	120	90	123	111±11	64	67	70	67± 2	48	56	86	63±12	88	64	76	76± 7
14	140	118	117	125± 8	125	132	93	117±12	115	102	78	98±11	107	90	73	90±10
18	125	107	125	119± 6	145	121	115	127± 9	98	123	87	103±11	92	110	90	97± 6
Days after reinfection																
7	103	108	128	113± 8	120	82	123	108±13	73	100	92	88± 8	95	90	105	97± 4
14	140	130	146	139± 5	135	113	164	137±15	119	104	104	109± 5	106	91	113	103± 7
21	100	140	127	122±12	132	111	149	131±11	115	*	102	108± 4	103	100	108	104± 2
28	83	106	120	103±11	94	111	120	108± 8	68		91	80± 7	74	65	88	76± 7

* Died

TABLE 7.

Total serum proteins (g%).

Days after infection	Blackface HbA 56	57	91	Mean ±S.E.	Blackface HbAB 80	82	92	Mean ±S.E.	Dorset HbAB 482	521	538	Mean ±S.E.	Dorset HbB 477	547	549	Mean ±S.E.
0	6.3	5.9	6.0	6.1±0.12	5.6	5.9	5.4	5.6±0.15	6.1	5.4	5.9	5.8±0.21	5.3	5.5	5.3	5.4±0.07
7	5.5	5.7	5.4	5.5±0.09	5.9	5.7	5.5	5.7±0.12	5.9	5.9	5.9	5.9±0.00	5.0	5.6	5.1	5.2±0.19
14	6.4	5.6	5.4	5.8±0.31	5.8	5.2	5.2	5.4±0.20	4.9	6.0	5.5	5.5±0.32	4.9	5.9	5.0	5.3±0.32
21	5.7	4.8	5.0	5.2±0.27	5.2	5.4	5.1	5.2±0.09	5.0	5.6	4.2	4.9±0.41	5.0	4.1	4.4	4.5±0.27
28	5.4	5.7	5.3	5.5±0.12	5.4	5.6	4.7	5.2±0.27	5.2	4.5	4.4	4.7±0.25	4.9	4.0	4.2	4.4±0.27
35	5.4	5.5	5.6	5.5±0.06	5.2	5.5	5.0	5.2±0.15	4.5	4.5	4.5	4.5±0.00	4.7	3.4	4.2	4.1±0.38
Days after treatment																
7	6.3	6.0	6.0	6.1±0.10	5.7	6.0	6.1	5.9±0.12	4.9	5.6	5.4	5.3±0.21	5.3	4.5	5.3	5.0±0.27
14	6.4	6.3	6.2	6.3±0.06	6.8	6.0	6.3	6.4±0.23	5.5	5.8	4.7	5.3±0.33	6.0	5.7	5.9	5.9±0.09
18	6.3	6.0	5.8	6.0±0.15	5.6	6.2	6.1	6.0±0.19	5.4	5.9	5.9	5.7±0.17	5.7	5.0	5.9	5.5±0.27
Days after reinfection																
7	5.8	6.3	6.3	6.1±0.17	6.1	6.2	6.0	6.1±0.06	5.6	5.7	5.7	5.7±0.03	5.4	5.7	5.8	5.6±0.12
14	6.1	5.9	5.7	5.9±0.12	5.7	6.0	5.6	5.8±0.12	5.5	5.9	6.0	5.8±0.15	5.1	5.6	6.1	5.6±0.29
21	5.5	5.5	5.5	5.5±0.00	5.5	5.9	5.5	5.6±0.13	5.5	5.1	5.3	5.3±0.12	4.9	5.0	5.3	5.1±0.12
28	5.3	5.9	5.6	5.6±0.17	5.2	5.7	5.3	5.4±0.15	3.9	*	5.1	4.5±0.60	3.9	4.2	5.1	4.4±0.36
32	5.0	5.7	4.5	5.1±0.35	4.6	5.5	4.7	4.9±0.29	4.1		4.4	4.3±0.15	4.3	3.6	4.1	4.0±0.21

* Died on day 23

TABLE 8.

Serum albumin (g%).

Days after infection	Blackface HbA			Mean ±S.E.	Blackface HbAB			Mean S.E.	Dorset HbAB			Mean ±S.E.	Dorset HbB			Mean ±S.E.
	56	57	91		80	82	92		482	521	538		477	547	549	
0	3.3	3.1	3.2	3.2±0.06	2.6	3.2	2.9	2.9±0.17	3.1	3.5	3.5	3.4±0.13	2.9	3.4	3.8	3.4±0.26
7	3.3	3.3	3.0	3.2±0.10	2.6	3.1	3.0	2.9±0.15	2.7	3.1	3.0	2.9±0.07	3.0	3.6	3.6	3.4±0.20
14	3.1	3.0	2.7	2.9±0.12	2.4	3.0	2.9	2.8±0.19	3.1	2.9	2.9	3.0±0.07	2.9	3.4	3.7	3.3±0.23
21	2.9	3.0	2.6	2.8±0.10	2.4	2.9	2.7	2.7±0.15	2.9	2.8	2.8	2.8±0.03	2.7	3.3	3.4	3.1±0.22
28	2.4	2.6	2.4	2.5±0.07	2.2	2.3	2.3	2.3±0.03	2.4	1.7	2.1	2.1±0.20	2.0	1.6	2.3	2.0±0.15
35	2.9	2.5	2.5	2.6±0.13	2.0	2.5	2.5	2.3±0.12	2.0	1.8	2.0	1.9±0.07	2.3	1.8	2.0	2.0±0.15
Days after treatment																
7	2.8	2.8	2.4	2.7±0.13	2.6	2.7	2.9	2.7±0.09	2.3	2.3	2.3	2.3±0.00	2.6	2.6	2.3	2.5±0.10
14	3.5	3.4	3.2	3.4±0.09	2.8	2.9	3.3	3.0±0.15	2.9	2.6	2.5	2.7±0.12	3.0	2.3	2.9	2.7±0.22
18	3.6	3.0	3.1	3.2±0.26	2.5	2.9	3.2	2.9±0.20	2.6	2.6	2.7	2.6±0.03	2.9	2.1	2.9	2.6±0.27
Days after reinfection																
7	3.0	3.1	3.1	3.1±0.03	2.2	2.5	3.1	2.6±0.27	2.3	2.6	2.7	2.5±0.12	2.4	2.0	2.3	2.2±0.12
14	2.9	3.2	3.4	3.2±0.15	2.6	2.5	3.2	2.8±0.22	2.7	3.0	3.0	2.9±0.10	2.8	2.3	2.9	2.7±0.19
21	3.0	3.0	3.0	3.0±0.00	2.3	2.5	2.9	2.6±0.18	2.4	2.6	2.5	2.5±0.06	2.7	2.3	2.6	2.5±0.12
28	3.1	2.7	3.1	3.0±0.13	2.6	2.5	3.2	2.8±0.22	2.4	*	2.6	2.5±0.10	2.4	2.4	3.0	2.6±0.20
32	3.1	3.2	3.4	3.2±0.09	2.8	2.6	2.9	2.8±0.08	2.7		2.9	2.8±0.10	2.9	2.4	2.9	2.7±0.17

* Died on day 23

TABLE 9.

Serum globulins (g%).

Days after infection	Blackface HbA			Mean ±S.E.	Blackface HbAB			Mean ±S.E.	Dorset HbAB			Mean ±S.E.	Dorset HbB			Mean ±S.E.
	56	57	91		80	82	92		482	521	538		477	547	549	
0	3.0	2.8	2.8	2.9±0.07	3.0	2.7	2.5	2.7±0.15	3.0	2.0	2.5	2.5±0.29	2.4	2.1	1.5	2.0±0.26
7	2.7	2.5	2.4	2.5±0.09	3.3	2.6	2.5	2.8±0.25	3.2	2.8	2.6	2.9±0.18	2.0	2.0	1.5	1.8±0.17
14	3.3	2.6	2.7	2.9±0.22	3.4	2.2	2.3	2.6±0.38	2.0	5.0	2.5	3.2±0.92	2.0	2.5	1.4	2.0±0.32
21	2.8	2.7	2.4	2.6±0.12	2.8	2.4	2.4	2.5±0.13	2.1	2.3	1.4	1.9±0.27	2.3	1.3	1.0	1.5±0.39
28	3.0	3.1	2.9	3.0±0.06	3.2	3.2	2.4	2.9±0.27	2.9	2.8	2.3	2.7±0.19	2.9	2.4	1.9	2.4±0.29
35	3.0	3.0	3.1	3.0±0.03	3.2	2.9	2.6	2.9±0.17	2.5	2.7	2.4	2.5±0.09	2.4	1.7	2.1	2.1±0.20
Days after treatment																
7	3.0	3.0	3.1	3.0±0.03	3.2	2.9	2.6	2.9±0.17	2.5	2.7	2.4	2.5±0.09	2.4	1.7	2.1	2.1±0.20
14	3.5	3.3	3.6	3.5±0.09	3.1	3.3	3.2	3.2±0.06	2.5	3.3	3.1	3.0±0.24	2.8	1.9	3.1	2.6±0.36
18	2.7	3.0	2.7	2.8±0.23	3.1	3.3	2.8	3.0±0.15	2.8	3.3	3.2	3.1±0.15	3.8	2.9	3.0	3.2±0.28
Days after reinfection																
7	2.8	3.2	3.2	3.1±0.13	3.9	3.7	2.9	3.5±0.31	3.2	3.2	3.0	3.1±0.07	2.0	3.7	3.6	3.1±0.55
14	3.0	2.7	2.3	2.6±0.20	3.2	3.5	2.4	3.0±0.33	2.8	2.9	3.0	2.9±0.06	2.3	3.3	3.3	3.0±0.33
21	2.5	2.5	2.5	2.5±0.00	3.1	3.4	2.7	3.0±0.20	3.1	2.4	2.8	2.8±0.20	2.2	2.7	2.6	2.5±0.15
28	2.2	3.2	2.6	2.7±0.29	2.6	3.3	2.0	2.6±0.38	1.5	*	2.5	2.0±0.50	1.5	1.7	2.1	1.8±0.18
32	1.9	2.4	1.2	1.8±0.35	1.9	2.9	1.8	2.2±0.35	1.4	*	1.8	1.6±0.20	1.4	1.2	1.2	1.3±0.07

* Died on day 23

TABLE 10.

Faecal egg counts ($x10^3$) of Scottish Blackface sheep.

Days after infection	Blackface HbA 56	57	91	Mean ±S.E.	Blackface HbAB 80	82	92	Mean ±S.E.
14	-	-	-		-	-	-	
15	-	-	-		-	-	-	
16	-	-	-		-	-	0.1	0.03±0.03
17	-	-	-		-	-	-	
18	1.5	1.3	2.2	1.7±0.3	1.7	0.1	1.3	1.0±0.5
19	3.0	5.4	2.7	3.7±0.9	5.0	1.4	3.3	3.2±1.0
20	4.7	4.6	5.4	4.9±0.3	9.2	1.3	6.8	5.8±2.3
21	6.0	7.4	6.8	6.7±0.4	18.2	2.7	9.3	10.1±4.5
22	7.1	8.1	12.5	9.2±1.7	18.2	3.1	9.6	10.3±4.4
23	5.7	8.1	8.6	7.5±0.9	16.1	2.8	13.3	10.7±4.0
24	5.8	6.6	12.2	8.2±2.0	14.2	3.3	8.1	8.5±3.2
25	5.0	7.2	13.2	8.5±2.5	14.5	3.7	9.5	9.2±3.1
26	4.5	8.0	13.2	8.6±2.5	15.9	4.2	11.1	10.4±3.4
27	6.7	9.1	17.3	11.0±3.2	18.6	4.1	17.3	13.3±4.6
28	7.4	10.2	18.6	12.1±3.4	17.3	4.9	11.3	11.2±3.6
29	11.1	12.4	19.0	14.2±2.5	23.1	6.9	17.7	15.9±4.8
30	12.5	13.6	18.4	14.8±1 8	18.5	7.4	20.5	15.5±4.1
31	11.8	9.6	13.9	11.8±1.2	26.5	8.4	11.9	15.6±5.5
32	6.5	11.3	21.8	13.2±4.5	28.7	12.1	12.2	17.7±5.5
33	14.2	14.5	26.5	18.4±4.1	32.5	17.9	27.4	25.9±4.3
34	22.8	16.4	19.1	19.4±1.9	32.6	17.7	29.3	26.5±4.5
35	29.9	14.8	18.1	20.9±4.6	33.5	15.5	26.4	25.1±5.2
Days after treatment								
1	12.2	20.5	11.7	14.8±2.9	26.1	11.9	28.7	22.2±5.2
2	-	-	-		0.5	-	0.3	0.3±0.2
3	-	-	-		-	-	-	
7	-	-	-		-	-	-	
14	-	-	-		-	-	-	
18	-	-	-		-	-	-	
Days after reinfection								
14	-	-	-		-	-		
15	-	-	-		-	-		
16	-	-	-		-	-		
17	-	-	-		-	-		
18	-	-	-		-	-		
19	-	-	-		-	-		
20	-	-	-		-	-		
21	-	-	-		-	-		
22	-	-	-		-	-		
23	-	-	-		-	-	-	
24	0.6	-	-	0.2±0.2	0.2	0.1	-	0.1±0.05
25	0.9	0.6	0.3	0.6±0.2	0.5	0.3	-	0.3±0.15
26	2.0	1.1	0.3	1.1±0.5	2.2	0.8	0.3	1.1±0.6
27	3.5	3.1	1.4	2.7±0.6	3.7	2.7	1.2	2.5±0.7
28	6.5	5.7	3.0	5.1±1.1	8.7	4.1	3.4	5.4±1.7
29	7.5	6.1	3.7	5.8±1.1	11.5	6.7	3.3	7.2±2.4

TABLE 11.

Faecal egg counts ($x10^3$) of Finn Dorset sheep.

Days after infection	Dorset HbAB 482	521	538	Mean S.E.	Dorset HbB 477	547	549	Mean S.E.
14	-	-	-		0.1	-	-	0.03±0.03
15	-	0.1	-	0.03±0.03	0.1	-	-	0.03±0.03
16	-	-	0.1	0.03±0.03	-	0.1	0.1	0.07±0.03
17	-	-	-		-	10.3	-	3.4± 3.4
18	1.0	3.2	5.1	3.1± 1.2	1.2	11.2	6.9	6.4± 2.9
19	2.9	5.1	17.4	8.5± 4.5	2.3	23.1	13.4	12.9± 6.0
20	4.9	12.3	18.5	11.9± 3.9	5.2	24.2	16.4	15.3± 5.5
21	6.9	10.6	26.6	14.7± 6.1	5.4	32.2	33.2	23.6± 9.1
22	8.3	9.0	24.7	14.0± 5.4	7.9	19.7	21.8	16.5± 4.3
23	8.3	14.5	28.3	17.0± 5.9	4.7	37.0	27.8	23.2± 9.6
24	10.6	20.8	29.2	20.2± 5.4	17.0	26.3	27.2	23.5± 3.3
25	11.5	21.2	29.0	20.6± 5.1	9.0	24.5	28.2	20.6± 5.9
26	12.6	21.7	28.5	20.9± 4.6	10.8	12.7	29.2	17.6± 5.8
27	9.1	24.9	38.6	24.2± 8.5	30.5	44.1	23.5	32.7± 6.1
28	12.7	43.7	48.0	34.8±11.1	13.6	59.6	43.1	38.8±13.5
29	11.2	31.8	51.1	31.4±11.5	21.8	44.6	33.2	33.2± 6.6
30	11.6	39.6	57.5	36.2±13.4	18.8	57.6	49.3	41.9±11.8
31	10.6	30.7	52.9	31.4±12.2	20.6	46.8	62.8	43.4±12.3
32	11.3	22.4	27.5	20.4± 4.8	20.0	57.4	35.1	37.5±10.9
33	14.9	30.2	47.0	30.7± 9.3	15.8	59.2	41.4	38.8±12.6
34	21.7	34.4	55.2	37.1± 9.8	21.0	50.8	31.6	34.5± 8.7
35	23.2	27.2	47.4	32.6± 7.5	19.6	36.1	29.7	28.5± 4.8
Days after treatment								
1	18.1	19.4	32.6	23.4± 4.6	17.4	34.3	25.4	24.7± 5.8
2	0.2	0.1	0.7		1.3	0.4	1.4	1.0± 0.3
3	-	-	-		-	-	-	
7	-	-	-		-	-	-	
14	-	-	-		-	-	-	
18	-		-		-	-	-	
Days after reinfection								
14	-	-	-		-	0.1	0.1	0.07±0.03
15	-	-	-		-	0.1	-	0.03±0.03
16	-	-	-		-	0.1	-	0.03±0.03
17	-	-	-		-	0.1	-	0.03±0.03
18	-	-	-		-	0.2	-	0.07±0.07
19	-	-	-		-	1.3	-	0.4± 0.4
20	-	-	-		0.1	2.0	-	0.7± 0.7
21	-	0.1	0.2	0.1±0.06	-	3.5	0.1	1.2± 1.2
22	0.2	0.1	0.7	0.3± 0.2	0.1	4.9	0.2	1.7± 1.6
23	0.2	*	2.2	1.2± 1.0	1.0	4.5	1.5	2.3± 1.1
24	0.3		2.7	1.5± 1.2	3.4	5.5	3.3	4.1± 0.7
25	0.5		3.9	2.2± 1.7	5.6	7.1	6.7	6.5± 0.5
26	1.1		5.4	3.3± 2.2	7.2	5.9	11.8	8.3± 1.8
27	2.2		6.4	4.3± 2.1	9.9	7.1	12.0	9.7± 1.4
28	3.5		10.4	7.0± 3.5	10.5	9.7	13.6	11.3± 1.2
29	2.0		13.1	7.6± 5.6	12.3	9.3	15.2	12.3± 1.7

* Died on day 23

TABLE 12.

Packed cell volumes (%) of challenge controls.

Days after infection	Blackface HbA 68	73	95	Mean ±S.E.	Blackface HbAB 69	72	Mean ±S.E.
0	32	31	30	31.0±0.6	31	29	30.0±1.0
4	30	32	31	31.0±0.6	32	29	30.5±1.5
7	30	31	30	30.3±0.3	32	30	31.0±1.0
11	27	27	25	26.3±0.7	29	28	28.5±0.5
14	25	23	21	23.0±1.2	24	22	23.0±1.0
18	20	18	17	18.3±0.9	20	18	19.0±1.0
21	17	16	15	16.0±0.6	21	16	18.5±2.5
25	17	17	16	16.7±0.3	18	14	16.0±2.0
28	18	15	16	16.3±0.9	16	15	15.5±0.5
32	18	17	17	17.3±0.3	16	16	16.0±0.0

Faecal egg counts ($x10^3$) of challenge controls.

14	-	-	-		-	-	
15	-	-	-		-	-	
16	0.8	0.1	0.4	0.4±0.2	0.6	0.9	0.8±0.2
17	1.2	1.8	1.1	1.4±0.2	2.4	1.3	1.9±0.6
18	3.4	5.3	1.8	3.5±1.0	8.3	4.8	6.6±1.8
19	4.2	6.2	4.2	4.9±0.7	12.4	5.1	8.9±3.7
20	3.8	8.4	9.4	7.2±1.7	12.8	9.3	11.1±1.8
21	7.6	9.8	5.3	7.6±1.3	17.6	12.4	15.0±2.6
22	9.8	12.7	12.6	11.7±1.0	19.4	9.4	14.4±5.0
23	12.2	13.2	14.8	13.4±0.8	21.3	15.6	18.5±2.9
24	14.6	12.7	15.8	14.4±0.9	24.6	16.3	20.5±4.2
25	18.5	14.8	21.4	18.2±1.9	18.7	19.7	19.2±0.5
26	14.8	19.9	23.8	19.5±2.6	25.3	24.8	25.1±0.3
27	15.6	21.8	21.7	19.7±2.1	27.6	22.6	25.1±2.5
28	19.4	22.3	27.6	23.1±2.4	31.4	27.6	29.5±1.9
29	19.8	25.6	24.3	23.2±1.8	28.6	22.3	25.5±3.2

TABLE 13.

Body weight and blood volumes.

	Sheep No.	Before infection Wt. (kg)	RCV (ml)	RCV (ml/kg)	VP (ml)	VP (ml/kg)	BV (ml)	BV (ml/kg)	After treatment Wt. (kg)	RCV (ml)	RCV (ml/kg)	VP (ml)	VP (ml/kg)	BV (ml)	BV (ml/kg)
	56	21	368	17.2	995	47.4	1363	64.6	21	324	15.4	728	34.7	1052	50.1
Blackface HbA	57	24	389	16.5	1000	41.7	1389	58.2	23	335	14.6	914	39.7	1249	54.3
	91	23	490	21.1	952	41.4	1442	62.5	23	402	17.5	810	35.2	1212	52.7
Mean		22.7	416	18.3	982	43.5	1398	61.8	22.3	354	15.8	817	36.5	1171	52.4
S.E.		0.9	38	1.4	15	2.0	23	1.9	0.7	24	0.9	53	1.6	61	1.2
	80	20	474	23.7	881	44.1	1355	57.8	19	285	15.0	775	40.8	1060	55.8
Blackface HbAB	82	22	403	18.3	940	42.7	1343	61.0	21	302	14.4	851	40.5	1153	54.9
	92	21	418	19.9	818	39.0	1236	58.9	20	286	14.3	702	35.1	988	49.4
Mean		21.0	432	20.6	880	41.9	1311	59.2	20.0	291	14.6	776	38.8	1067	53.4
S.E.		0.6	22	1.6	35	1.5	38	0.9	0.6	6	0.2	43	1.9	48	2.0
	482	22	499	22.7	1092	49.6	1591	72.3	22	348	15.8	833	37.9	1181	53.7
Dorset HbAB	521	27	572	21.2	1138	43.8	1710	65.0	26	391	15.0	1208	46.5	1599	61.5
	538	32	701	21.9	1273	39.8	1974	61.7	30	297	9.9	1208	40.3	1505	50.2
Mean		27.0	591	21.9	1168	44.4	1758	66.3	26.0	345	13.6	1083	41.6	1428	55.1
S.E.		2.9	59	0.4	54	2.9	113	3.1	2.3	27	1.8	125	2.6	127	3.3
	477	29	475	16.6	1221	42.1	1696	58.7	28	374	13.3	1064	34.4	1438	47.7
Dorset HbB	547	25	615	24.6	1048	41.9	1663	66.5	25	298	11.9	1144	45.8	1442	57.7
	549	28	708	25.1	1275	45.5	1983	70.6	27	333	12.3	1142	42.3	1475	54.6
Mean		27.3	599	22.1	1181	43.2	1780	65.3	27.7	335	12.5	1117	40.8	1452	53.3
S.E.		1.2	68	2.8	69	1.2	102	3.5	0.9	22	0.4	26	3.4	12	3.0

TABLE 14.
Body weight and blood volumes.

	Sheep No.	28 days after reinfection: Wt. (kg)	RCV (ml)	RCV (ml/kg)	VP (ml)	VP (ml/kg)	BV (ml)	BV (ml/kg)	28 days after infection: Wt. (kg)	RCV (ml)	RCV (ml/kg)	VP (ml)	VP (ml/kg)	BV (ml)	BV (ml/kg)
	56	19	194	10.2	702	37.0	896	47.2	21.5	270	12.6	878	40.8	1148	53.4
Blackface HbA	57	24	262	10.9	894	37.3	1156	48.2	24.0	298	12.4	987	41.1	1285	53.5
	91	24	278	11.6	736	30.7	1014	42.3	24.0	356	14.8	908	37.8	1264	52.6
Mean		22.3	245	10.9	777	35.0	1022	45.9	23.2	308	13.3	924	39.9	1232	53.2
S.E.		1.7	26	0.4	59	2.2	75	1.8	0.8	25	0.8	33	1.1	43	0.3
	80	20	187	9.4	737	36.9	924	46.3	19.5	255	13.1	876	44.9	1131	58.0
Blackface HbAB	82	22	194	8.8	770	35.0	964	43.8	22.0	277	12.6	908	41.3	1185	53.9
	92	19	186	9.8	676	35.6	862	45.4	20.0	217	10.8	816	40.8	1033	51.6
Mean		20.3	189	9.3	728	35.8	917	45.2	20.5	250	12.2	867	42.3	1116	54.5
S.E.		0.9	3	0.3	28	0.6	30	0.7	0.8	18	0.7	27	1.3	45	1.9
	482	22	165	7.5	826	37.6	991	45.1	23.5	285	12.1	1070	45.5	1355	57.6
Dorset HbAB	521	*							28.0	311	11.1	1368	48.9	1679	60.0
	538	31	207	6.7	1045	33.7	1252	40.4	32.0	252	7.9	1352	42.3	1604	51.2
Mean		26.5	186	7.1	936	35.7	1122	42.8	27.8	283	10.4	1263	45.6	1546	56.3
S.E.		4.5	21	0.4	110	2.0	131	2.4	2.5	17	1.3	97	1.9	98	2.6
	477	29	196	6.8	940	32.4	1136	39.2	30.0	280	9.3	1207	40.2	1487	49.5
Dorset HbB	547	27	122	4.5	1192	44.2	1314	48.7	27.0	201	7.4	1276	47.3	1477	54.7
	549	30	221	7.4	1007	33.6	1228	41.0	28.5	289	10.1	1302	45.7	1591	55.8
Mean		28.9	180	6.2	1046	36.7	1226	42.9	28.5	257	8.9	1262	44.4	1518	53.3
S.E.		0.9	30	0.9	75	3.8	51	2.9	0.9	28	0.8	28	2.2	37	1.9

* Died on day 23

TABLE 15.

Faecal blood clearance (ml/day).

Days after infection	Blackface HbA 56	57	91	Mean ±S.E.	Blackface HbAB 80	82	92	Mean ±S.E.	Dorset HbAB 482	521	538	Mean ±S.E.	Dorset HbB 477	547	549	Mean ±S.E.
32	51.0	80.0	134.0	88.3±24.3	115.0	63.0	110.0	96.0±16.6	142.0	242.0	220.0	201.0±30.0	262.0	324.0	220.0	269.0±30.0
33	52.0	86.0	90.0	76.0±12.1	102.0	77.0	121.0	100.0±12.7	145.0	208.0	257.0	203.0±32.4	204.0	361.0	281.0	282.0±45.0
34	36.5	88.0	90.0	71.5±17.5	102.0	56.0	102.0	86.7±15.3	117.5	211.0	253.0	194.0±40.0	187.0	306.0	244.0	245.0±34.0
35	42.3	96.7	100.0	79.7±18.7	108.0	72.0	102.0	94.0±11.1	116.0	228.0	276.0	207.0±47.0	171.0	268.0	251.0	230.0±29.9
Days after treatment																
4	24.0	40.6	38.6	34.4± 5.2	35.0	43.2	58.3	45.5± 6.8	54.0	99.0	107.0	86.7±16.5	70.0	93.5	85.0	82.8± 6.9
6	2.0	1.4	1.3	1.6± 0.2	1.8	1.0	2.0	1.6± 0.3	3.3	3.0	13.5	6.6± 3.5	8.4	4.7	8.3	7.1± 1.2
8	1.4	0.7	1.1	1.1± 0.2	1.1	0.6	1.0	0.9± 0.2	2.0	1.0	2.2	1.7± 0.4	3.4	2.8	3.7	3.3± 0.3
10	2.6	0.6	1.8	1.7± 0.6	0.8	0.7	1.0	0.8± 0.1	0.7	1.2	1.9	1.3± 0.3	2.5	5.2	2.3	3.3± 1.0
12	1.9	1.5	2.1	1.8± 0.2	1.3	1.5	1.2	1.3± 0.1	2.2	0.8	3.7	2.2± 0.8	3.7	1.9	7.8	4.5± 1.8
14	2.4	1.1	2.5	2.0± 0.5	0.7	0.4	1.6	0.9± 0.4	1.6	0.8	2.9	1.8± 0.6	1.0	5.3	3.1	3.1± 1.2
16	2.4	0.7	4.8	2.6± 1.2	1.2	0.2	0.2	0.5± 0.3	1.0	1.0	3.8	1.9± 1.0	1.3	3.3	1.8	2.1± 0.6
18	1.7	0.5	5.7	2.6± 1.6	0.8	1.6	0.3	0.9± 0.4	0.7	2.1	3.7	2.2± 0.9	0.4	0.7	2.8	1.3± 0.8
Days after reinfection																
4	2.5	1.5	0.4	1.5± 0.6	0.4	0.7	0.5	0.5± 0.1	0.6	1.4	0.8	0.9± 0.2	1.0	0.8	1.4	1.1± 0.2
6	2.2	1.1	0.6	1.3± 0.5	0.4	0.9	0.6	0.6± 0.2	0.6	1.3	0.9	0.9± 0.2	1.7	0.7	1.1	1.2± 0.3
8	1.1	0.9	0.5	0.8± 0.2	0.5	0.7	0.5	0.6± 0.0	0.7	1.3	0.9	1.0± 0.2	1.2	0.9	1.3	1.1± 0.1
10	1.7	3.7	2.8	2.7± 0.6	2.5	3.8	3.3	3.2± 0.4	4.1	3.3	3.1	3.5± 0.3	4.6	5.6	3.8	4.7± 0.5
12	5.9	5.9	5.4	5.7± 0.2	3.8	5.9	7.8	5.8± 1.2	6.0	2.2	4.7	4.3± 1.1	5.8	8.0	9.5	7.8± 1.1
14	18.8	23.2	20.3	20.8± 1.3	15.7	25.1	33.1	24.6± 5.0	24.4	7.8	23.0	18.4± 5.3	27.9	61.0	22.7	37.2±12.0
16	32.9	45.6	41.5	40.0± 3.7	25.1	52.7	68.0	48.6±12.6	43.9	47.1	67.5	52.8± 7.4	65.0	116.6	55.0	78.9±19.1
18	38.9	33.5	56.0	42.8± 6.8	37.5	56.7	79.0	57.7±12.0	46.4	57.0	75.6	59.6± 8.5	71.4	121.0	72.6	88.3±16.3
20	80.3	35.3	74.8	63.5±14.2	52.1	62.1	74.0	62.7± 6.3	70.6	91.5	131.5	97.9±17.9	116.7	132.0	94.3	114.3±11.0
22	59.8	40.1	75.2	58.4±10.2	55.0	82.0	53.6	63.5± 9.2	70.9	67.1	108.5	82.2±13.2	112.6	115.4	101.0	
24	57.5	43.7	52.9	51.4± 4.1	60.0	**	53.6	56.8± 3.2	82.7	*	111.0	96.9±14.2	79.4	103.0	91.0	
26	62.4	59.8	47.5	56.6± 4.6	54.0		54.6	54.3± 0.3	54.3		97.0	75.7±21.4	95.7	118.4	75.0	96.4±12.5
28	67.0	67.0	48.7	60.9± 6.1	64.0		66.7	65.4± 1.4	63.1		104.0	83.6±20.5	109.3	121.0	108.0	112.8± 4.1

* Died on day 23 ** Missing value

TABLE 16.

Faecal red cell clearance (ml/day).

Days after infection	Blackface HbA 56	57	91	Mean ±S.E.	Blackface HbAB 80	82	92	Mean ±S.E.	Dorset HbAB 482	521	538	Mean ±S.E.	Dorset HbB 477	547	549	Mean ±S.E.
32	10.2	11.1	26.7	16.0±5.4	21.8	13.3	17.5	17.5±2.5	24.1	36.3	30.8	30.4±3.5	39.3	32.4	24.1	31.9±4.4
33	10.4	16.6	17.1	14.7±2.2	18.5	14.6	18.2	17.1±1.3	23.1	31.0	36.0	30.0±3.8	28.5	36.0	37.0	33.8±2.7
34	7.3	17.5	17.1	14.0±3.3	13.0	10.7	16.4	13.4±1.7	17.6	27.5	32.9	26.0±4.5	23.6	30.6	24.4	26.2±2.2
35	8.5	19.3	19.0	15.6±3.6	15.0	12.3	15.3	14.2±1.0	18.6	27.4	35.8	27.3±5.0	25.6	24.1	20.1	23.3±1.6
Days after treatment																
4	4.6	8.1	7.7	6.8±1.1	6.3	7.4	10.0	7.9±1.1	8.1	11.9	13.9	11.3±1.7	11.2	10.2	9.4	10.3±0.5
6	0.4	0.3	0.3	0.3±0.3	0.2	0.4	0.4	0.3±0.1	0.6	0.4	1.9	1.0±0.5	1.5	0.6	1.2	1.1±0.3
8	0.3	0.2	0.2	0.2±0.0	0.1	0.2	0.3	0.2±0.0	0.4	0.1	0.3	0.3±0.0	0.6	0.4	0.5	0.5±0.1
10	0.5	0.4	0.4	0.4±0.0	0.2	0.3	0.2	0.2±0.0	0.1	0.2	0.3	0.2±0.0	0.4	0.7	0.3	0.5±0.1
12	0.5	0.3	0.5	0.4±0.1	0.4	0.3	0.3	0.3±0.0	0.4	0.1	0.6	0.4±0.2	0.8	0.3	1.2	0.8±0.3
14	0.6	0.2	0.7	0.5±0.2	0.8	0.4	0.2	0.5±0.2	0.3	0.2	0.6	0.4±0.1	0.2	0.9	0.5	0.5±0.2
16	0.5	0.2	1.3	0.7±0.3	0.5	0.6	0.2	0.4±0.1	0.2	0.2	0.8	0.4±0.2	0.3	0.6	0.3	0.4±0.1
18	0.4	0.1	1.4	0.6±0.4	0.4	0.6	0.2	0.4±0.1	0.1	0.4	0.7	0.4±0.2	0.1	0.1	0.5	0.2±0.1
Days after reinfection																
4	0.2	0.3	0.1	0.2±0.0	0.1	0.2	0.1	0.1±0.0	0.1	0.3	0.2	0.2±0.0	0.3	0.2	0.2	0.2±0.0
6	0.2	0.3	0.1	0.2±0.0	0.1	0.2	0.1	0.1±0.0	0.1	0.3	0.2	0.2±0.0	0.3	0.2	0.2	0.2±0.0
8	0.2	0.3	0.1	0.2±0.0	0.1	0.2	0.1	0.1±0.0	0.1	0.3	0.2	0.2±0.0	0.3	0.2	0.3	0.3±0.0
10	1.0	0.9	0.8	0.9±0.0	0.6	0.8	0.8	0.7±0.0	0.9	0.6	0.7	0.7±0.0	1.0	1.1	0.8	1.0±0.0
12	1.3	1.4	1.5	1.4±0.0	0.9	1.2	3.1	1.7±0.7	1.3	0.5	1.1	1.0±0.2	1.2	5.9	1.9	3.0±1.5
14	3.9	5.3	5.9	5.0±0.6	3.6	5.3	9.9	6.3±1.9	5.4	0.8	5.3	3.8±1.5	5.9	13.4	4.5	7.9±2.8
16	6.9	10.5	11.2	9.5±1.3	5.0	10.0	16.9	10.6±3.5	10.1	6.6	14.2	10.3±2.2	13.0	26.1	8.6	15.9±5.3
18	7.8	7.4	15.1	10.1±2.5	8.2	10.8	11.2	10.1±0.9	9.7	9.5	12.9	10.7±1.1	12.8	17.4	14.9	15.0±1.3
20	14.5	7.0	14.9	12.1±2.6	10.4	10.6	11.8	10.9±0.4	13.4	10.5	23.7	15.9±4.0	19.8	15.7	15.3	16.9±1.4
22	10.8	8.8	12.5	10.7±1.1	10.4	7.2	10.0	9.2±1.0	12.8	15.2	17.4	15.1±1.3	19.1	16.1	14.5	16.6±1.5
24	9.8	8.7	9.4	9.3±0.3	11.8	9.1	15.0	12.0±1.7	13.2	*	18.9	16.1±2.9	11.9	11.7	12.0	11.9±0.0
26	10.6	12.0	10.2	10.9±0.6	9.2	9.0	13.5	10.6±1.5	8.1		14.6	11.4±3.3	12.4	11.6	11.4	11.8±0.3
28	10.1	13.2	9.7	11.0±1.1	11.6	9.0	10.4	10.3±0.8	8.8		14.5	11.7±2.9	16.4	15.4	15.0	15.6±0.4

* Died on day 23

TABLE 17.

Ferrokinetic indices before infection.

	Sheep No.	Plasma ^{59}Fe $t_{\frac{1}{2}}$ (min)	Plasma Iron Turnover Rate mg/day	mg/kg/day	mg/kg/100 ml blood
	56	185	8.9	0.42	0.67
Blackface HbA	57	157	8.6	0.36	0.62
	91	165	9.3	0.40	0.61
Mean		169	8.9	0.39	0.63
S.E.		8	0.2	0.02	0.02
	80	153	8.3	0.29	0.58
Blackface HbAB	82	110	8.2	0.37	0.58
	92	162	7.0	0.33	0.51
Mean		142	7.8	0.33	0.56
S.E.		16	0.4	0.02	0.02
	482	243	6.4	0.29	0.42
Dorset HbAB	521	235	8.2	0.30	0.54
	538	165	9.2	0.29	0.61
Mean		214	7.9	0.29	0.52
S.E.		25	0.8	0.00	0.06
	477	126	13.5	0.47	0.99
Dorset HbB	547	244	7.5	0.30	0.50
	549	180	12.6	0.45	0.84
Mean		183	11.2	0.41	0.78
S.E.		34	1.9	0.05	0.15

TABLE 18.

Ferrokinetic indices 28 days after infection.

	Sheep No.	Plasma ^{59}Fe $t_{\frac{1}{2}}$ (min)	Plasma Iron Turnover Rate mg/day	mg/kg/day	mg/kg/100 ml blood
	56	65	15.4	0.71	1.23
Blackface HbA	57	55	23.5	0.98	1.83
	91	50	23.7	0.99	1.84
Mean		57	20.9	0.89	1.63
S.E.		4	2.7	0.09	0.20
	80	47	17.7	0.91	1.40
Blackface HbAB	82	43	19.0	0.86	1.51
	92	30	24.7	1.24	2.07
Mean		40	20.5	1.00	1.66
S.E.		5	2.2	0.12	0.21
	482	28	31.2	1.33	2.56
Dorset HbAB	521	20	45.7	1.63	3.66
	538	25	32.4	1.01	2.42
Mean		24	36.4	1.32	2.88
S.E.		2	4.7	0.18	0.39
	477	18	44.2	1.47	3.71
Dorset HbB	547	20	36.9	1.21	2.42
	549	22	41.3	1.60	4.01
Mean		20	40.8	1.43	3.38
S.E.		1	2.1	0.12	0.49

TABLE 19.

Ferrokinetic indices before reinfection.

	Sheep No.	Plasma ^{59}Fe $t_{\frac{1}{2}}$ (min)	Plasma Iron Turnover Rate mg/day	mg/kg/day	mg/kg/100 ml blood
	56	93	9.8	0.47	0.78
Blackface HbA	57	120	8.1	0.36	0.60
	91	90	11.2	0.40	0.81
Mean		101	9.7	0.41	0.73
S.E.		10	0.9	0.03	0.07
	80	100	11.2	0.59	0.87
Blackface HbAB	82	103	10.0	0.48	0.77
	92	115	7.0	0.35	0.54
Mean		106	9.4	0.47	0.73
S.E.		5	1.2	0.07	0.10
	482	114	7.1	0.31	0.56
Dorset HbAB	521	109	10.4	0.40	0.83
	538	112	9.4	0.31	0.75
Mean		112	9.0	0.34	0.71
S.E.		2	1.0	0.03	0.08
	477	73	13.4	0.48	1.04
Dorset HbB	547	90	14.0	0.56	1.13
	549	86	11.9	0.44	0.95
Mean		83	13.1	0.49	1.04
S.E.		5	0.6	0.04	0.05

TABLE 20.

Ferrokinetic indices 28 days after reinfection.

	Sheep No.	Plasma ^{59}Fe $t_{\frac{1}{2}}$ (min)	Plasma Iron Turnover Rate mg/day	mg/kg/day	mg/kg/100 ml blood
	56	45	16.6	0.89	1.42
Blackface HbA	57	39	24.2	1.00	1.96
	91	23	38.3	1.60	2.99
Mean		36	26.4	1.16	2.12
S.E.		7	6.4	0.22	0.46
	80	28	24.7	1.24	2.02
Blackface HbAB	82	28	30.5	1.39	2.53
	92	22	36.8	1.94	3.20
Mean		26	30.7	1.52	2.58
S.E.		2	3.5	0.21	0.34
	482	30	18.7	0.85	1.61
Dorset HbAB	521	*	*	*	*
	538	25	38.0	1.23	3.11
Mean		28	28.4	1.04	2.36
S.E.		3	9.7	0.19	0.75
	477	16	43.3	1.49	3.67
Dorset HbB	547	23	33.6	1.24	3.00
	549	25	35.4	1.18	3.04
Mean		21	37.4	1.30	3.24
S.E.		3	3.0	0.09	0.22

* Died on day 23

TABLE 21.

Albumin pools before infection.

	Sheep No.	CA g	CA g/kg	EA g	EA g/kg	TA g	TA g/kg	EA/CA
	56	32.8	1.56	53.6	2.55	86.4	4.11	1.63
Blackface HbA	57	31.0	1.29	43.7	1.82	74.7	3.11	1.41
	91	30.5	1.32	45.3	1.97	75.8	3.29	1.49
Mean		31.4	1.39	47.5	2.11	79.0	3.50	1.51
S.E.		0.7	0.09	3.1	0.22	3.7	0.31	0.06
	80	22.9	1.15	45.8	2.30	68.7	3.45	2.00
Blackface HbAB	82	30.1	1.37	54.6	2.48	84.7	3.85	1.81
	92	23.7	1.13	40.3	1.92	64.0	3.05	1.70
Mean		25.6	1.22	46.9	2.23	72.5	3.45	1.84
S.E.		2.3	0.08	4.2	0.17	6.3	0.23	0.09
	482	33.9	1.54	55.6	2.43	89.5	3.97	1.58
Dorset HbAB	521	39.8	1.48	62.1	2.30	101.9	3.78	1.55
	538	44.6	1.39	61.4	1.92	106.0	3.31	1.38
Mean		39.4	1.47	59.0	2.22	99.1	3.69	1.50
S.E.		3.1	0.04	2.7	0.15	5.0	0.20	0.06
	477	35.4	1.22	54.5	1.88	89.9	3.10	1.54
Dorset HbB	547	35.6	1.43	50.8	2.03	86.4	3.46	1.42
	549	48.5	1.73	60.8	2.17	109.3	3.90	1.25
Mean		39.8	1.46	55.4	2.03	95.2	3.49	1.40
S.E.		4.3	0.15	2.9	0.08	7.1	0.23	0.08

TABLE 22.

Albumin pools 28 days after infection.

	Sheep No.	CA g	CA g/kg	EA g	EA g/kg	TA g	TA g/kg	EA/CA
	56	29.7	1.38	42.5	1.97	72.2	3.35	1.43
Blackface HbA	57	26.2	1.09	31.7	1.32	57.9	2.41	1.21
	91	21.6	0.90	27.2	1.13	48.8	2.03	1.26
Mean		25.8	1.12	33.8	1.47	59.6	2.60	1.30
S.E.		2.4	0.14	4.5	0.25	6.8	0.39	0.07
	80	19.1	0.98	28.7	1.47	47.8	2.45	1.50
Blackface HbAB	82	20.7	0.94	32.1	1.46	52.8	2.40	1.55
	92	19.2	0.96	25.3	1.27	44.5	2.23	1.32
Mean		19.7	0.96	28.7	1.40	48.3	2.36	1.46
S.E.		0.5	0.01	2.0	0.07	2.4	0.07	0.07
	482	25.9	1.10	32.9	1.40	58.8	2.50	1.27
Dorset HbAB	521	23.5	0.84	28.2	1.01	51.7	1.85	1.20
	538	28.2	0.88	33.0	1.03	61.2	1.91	1.17
Mean		25.9	0.94	31.4	1.15	57.2	2.09	1.21
S.E.		1.4	0.08	1.6	0.13	2.9	0.21	0.03
	477	24.0	0.80	25.4	0.85	49.4	1.65	1.06
Dorset HbB	547	20.8	0.77	20.0	0.74	40.8	1.51	0.96
	549	29.9	1.05	30.5	1.07	60.4	2.12	1.02
Mean		24.9	0.87	25.3	0.89	50.2	1.76	1.01
S.E.		2.7	0.09	3.0	0.10	5.7	0.18	0.03

TABLE 23.

Albumin pools before reinfection.

	Sheep No.	CA g	CA g/kg	EA g	EA g/kg	TA g	TA g/kg	EA/CA
	56	23.2	1.11	47.9	2.28	71.1	3.39	2.05
Blackface HbA	57	27.4	1.19	35.0	1.52	62.4	2.71	1.28
	91	25.1	1.09	31.1	1.35	56.2	2.44	1.24
Mean		25.2	1.13	38.0	1.72	63.2	2.85	1.52
S.E.		1.2	0.03	5.1	0.29	4.3	0.28	0.26
	80	19.4	1.02	35.0	1.84	54.4	2.86	1.80
Blackface HbAB	82	24.6	1.17	37.8	1.80	62.4	2.97	1.54
	92	22.5	1.13	27.0	1.35	49.5	2.48	1.19
Mean		22.2	1.11	33.3	1.66	55.4	2.77	1.51
S.E.		1.5	0.05	3.2	0.16	3.8	0.15	0.18
	482	21.7	0.99	41.4	1.88	63.1	2.97	1.90
Dorset HbAB	521	31.4	1.21	47.3	1.82	78.7	3.03	1.50
	538	32.6	1.09	42.3	1.41	74.9	2.50	1.29
Mean		28.6	1.10	43.7	1.70	72.2	2.83	1.56
S.E.		3.5	0.06	1.8	0.15	4.7	0.17	0.18
	477	30.9	1.11	38.6	1.38	69.5	2.49	1.24
Dorset HbB	547	24.0	0.96	40.5	1.62	64.5	2.58	1.69
	549	33.1	1.23	41.0	1.52	74.1	2.75	1.24
Mean		29.3	1.10	40.0	1.51	69.4	2.61	1.39
S.E.		2.7	0.08	0.7	0.07	2.8	0.08	0.15

TABLE 24.

Albumin pools 28 days after reinfection.

	Sheep No.	CA g	CA g/kg	EA g	EA g/kg	TA g	TA g/kg	EA/CA
	56	22.6	1.19	35.7	1.88	58.3	3.07	1.58
Blackface HbA	57	24.7	1.03	27.6	1.15	52.3	2.18	1.12
	91	25.1	1.05	29.0	1.21	54.1	2.26	1.15
Mean		24.1	1.09	30.8	1.41	54.9	2.50	1.28
S.E.		0.8	0.05	2.5	0.23	1.8	0.28	0.15
	80	19.2	0.96	28.6	1.43	47.8	2.39	1.49
Blackface Hb AB	82	19.3	0.88	26.6	1.21	45.9	2.09	1.38
	92	21.6	1.13	18.8	0.99	40.4	2.12	0.88
Mean		20.0	0.99	24.7	1.21	44.7	2.20	1.25
S.E.		0.8	0.07	3.0	0.13	2.2	0.10	0.19
	482	19.8	0.90	34.3	1.56	54.1	2.46	1.73
Dorset HbAB	521	*	*	*	*	*	*	*
	538	27.2	0.88	29.8	0.96	57.0	1.84	1.09
Mean		23.5	0.89	32.1	1.26	55.6	2.15	1.41
S.E.		3.7	0.01	2.3	0.30	1.5	0.31	0.32
	477	22.6	0.78	28.4	0.98	51.0	1.76	1.26
Dorset HbB	547	28.6	1.06	33.2	1.23	61.8	2.29	1.16
	549	30.2	1.00	32.7	1.09	62.9	2.09	1.09
Mean		27.1	0.95	31.4	1.10	58.6	2.05	1.17
S.E.		2.3	0.09	1.5	0.07	3.8	0.16	0.05

* Died on day 23

TABLE 25.

Fractional catabolic rate of albumin F(CA).

Days after infection	Blackface HbA 56	57	91	Mean ±S.E.	Blackface HbAB 80	82	92	Mean ±S.E.	Dorset HbAB 482	521	538	Mean ±S.E.	Dorset HbB 477	547	549	Mean ±S.E.
32	.092	.121	.168	.127±.022	.273	.082	.092	.149±.062	.100	.170	.248	.173±.043	.226	.200	.238	.221±.011
33	.095	.141	.176	.137±.023	.297	.125	.131	.184±.056	.137	.265	.265	.222±.043	.184	.218	.241	.214±.017
34	.093	.147	.148	.129±.018	.246	.098	.117	.154±.047	.109	.242	.197	.183±.039	.213	.256	.223	.231±.013
35	.120	.155	.167	.147±.014	.210	.160	.140	.170±.021	.161	.218	.189	.189±.017	.182	.203	.209	.198±.008
Days after treatment																
4	.092	.033	.079	.085±.004	.130	.086	.086	.101±.015	.082	.159	.100	.114±.023	.070	.079	.105	.085±.011
6	.071	.062	.091	.075±.009	.090	.063	.100	.084±.011	.116	.125	.121	.121±.003	.077	.090	.105	.091±.008
8	.086	.062	.063	.070±.008	.077	.054	.101	.077±.014	.085	.103	.082	.090±.007	.088	.067	.105	.087±.011
10	.096	.022	.082	.067±.023	.068	.073	.096	.079±.009	.079	.087	.063	.076±.007	.066	.084	.102	.084±.010
12	.062	.059	.060	.060±.000	.106	.059	.058	.074±.016	.058	.080	.057	.065±.008	.040	.117	.065	.074±.023
14	.071	.041	.060	.057±.009	.064	.040	.055	.053±.007	.055	.059	.114	.076±.019	.056	.102	.088	.082±.013
16	.070	.042	.059	.057±.008	.074	.056	.061	.064±.005	.028	.055	.075	.053±.014	.050	.072	.091	.071±.012
18	.049	.049	.048	.049±.000	.058	.056	.068	.061±.003	.047	.066	.090	.068±.012	.046	.067	.080	.064±.010
Days after reinfection																
4	.058	.045	.065	.056±.006	.038	.066	.061	.055±.009	.046	.047	.053	.049±.002	.052	.049	.055	.052±.002
6	.084	.053	.070	.069±.009	.040	.063	.067	.057±.008	.057	.053	.054	.055±.001	.046	.060	.065	.057±.006
8	.079	.061	.067	.069±.005	.055	.084	.079	.073±.009	.087	.057	.070	.071±.009	.102	.070	.069	.080±.011
10	.047	.030	.061	.046±.009	.053	.052	.075	.060±.007	.073	.069	.084	.075±.005	.098	.090	.056	.081±.013
12	.100	.065	.072	.079±.010	.056	.052	.082	.063±.009	.051	.040	.066	.052±.008	.067	.080	.058	.068±.006
14	.124	.060	.078	.087±.019	.095	.079	.104	.093±.007	.100	.053	.060	.071±.015	.087	.086	.052	.075±.012
16	.113	.075	.123	.104±.015	.072	.059	.134	.088±.023	.098	.063	.111	.091±.014	.119	.135	.109	.121±.008
18	.112	.099	.095	.102±.005	.050	.086	.178	.105±.038	.113	.093	.113	.106±.007	.126	.155	.098	.126±.017
20	.088	.103	.082	.091±.006	.068	.121	.102	.097±.016	.112	.094	.079	.095±.010	.128	.200	.089	.139±.033
22	.092	.084	.082	.086±.003	.091	.121	.122	.111±.010	.096	.084	.111	.097±.008	.148	.246	.092	.162±.045
24	.104	.056	.084	.081±.014	.064	.122	.140	.109±.023	.095	*	.115	.105±.010	.156	.152	.102	.137±.018
26	.096	.074	.111	.094±.011	.088	.085	.156	.110±.023	.132		.118	.125±.007	.141	.157	.119	.139±.011
28	.107	.094	.107	.103±.004	.080	.167	.140	.109±.017	.105		.120	.113±.008	.156	.201	.159	.172±.015

* Died on day 23

TABLE 26.

Faecal ^{125}I "clearances" (ml/day).

Days after infection	Blackface HbA			Mean ±S.E.	Blackface HbAB			Mean ±S.E.	Dorset HbAB			Mean ±S.E.	Dorset HbB			Mean ±S.E.
	56	57	91		80	82	92		482	521	538		477	547	549	
32	36.6	56.3	53.4	48.8± 6.1	90.0	51.0	67.4	69.5±11.3	83.4	157.2	135.2	125.3±21.9	144.1	71.1	79.3	98.2±23.1
33	47.2	87.7	84.6	73.2±13.0	60.0	58.1	62.4	60.2± 1.2	106.4	125.7	97.8	110.0± 8.3	84.5	59.7	93.7	79.3±10.2
34	55.5	91.9	61.8	69.7±11.2	71.9	55.5	64.5	64.0± 4.7	94.1	84.8	157.0	112.0±22.7	69.1	70.4	126.8	88.8±19.0
35	55.1	66.1	55.4	58.9± 3.6	82.6	69.8	67.2	73.2± 4.8	94.2	77.1	143.0	104.8±19.7	70.8	129.8	135.2	111.9±20.6
Days after treatment																
4	22.8	47.3	49.2	39.8± 8.5	58.1	22.5	34.6	38.4±10.5	49.7	106.2	54.8	70.2±18.0	34.2	74.0	37.3	48.5±12.8
6	23.1	36.7	30.9	30.2± 3.9	17.0	20.0	27.3	21.4± 3.1	25.3	84.3	32.7	47.4±18.6	50.6	60.0	45.7	52.1± 4.2
8	19.6	25.8	32.2	25.9± 3.6	17.1	20.0	27.6	21.6± 3.1	22.7	70.5	23.0	38.7±15.9	54.3	51.8	41.2	49.1± 4.0
10	17.2	21.8	25.2	21.4± 2.3	15.5	24.7	26.4	22.2± 3.4	23.9	25.7	24.4	24.7± 0.5	37.8	49.2	28.7	38.6± 5.9
12	21.5	22.9	24.7	23.0± 0.9	34.5	23.9	20.3	26.2± 4.3	17.6	44.4	19.0	27.0± 8.7	24.1	32.6	24.5	27.1± 2.8
14	22.2	19.6	25.5	22.4± 1.7	36.7	19.7	22.2	26.2± 5.3	20.9	48.9	29.0	32.9± 8.3	28.4	38.0	30.8	32.4± 2.9
16	29.8	23.5	19.2	24.2± 3.1	20.3	17.5	20.6	19.5± 1.0	19.5	37.2	25.7	27.5± 5.2	27.8	35.5	37.0	33.4± 2.9
18	18.9	24.8	18.9	20.9± 2.0	23.7	16.5	28.4	22.9± 3.5	16.6	29.8	24.5	23.6± 3.8	23.5	20.2	26.2	23.3± 1.7
Days after reinfection																
4	15.8	22.5	19.9	19.4± 2.0	10.0	13.6	17.7	13.8± 2.2	13.5	24.5	17.3	18.4± 3.2	17.0	23.8	21.3	20.7± 2.0
6	17.5	16.2	15.3	16.3± 0.6	5.8	12.1	15.4	11.1± 2.8	12.1	24.8	10.5	15.8± 4.5	12.9	20.4	19.4	17.6± 2.4
8	16.6	12.4	16.9	15.3± 1.5	6.3	13.3	14.7	11.4± 2.6	11.4	30.4	11.8	17.9± 6.3	21.5	14.7	17.6	17.9± 2.0
10	19.6	19.0	15.2	17.9± 1.4	6.8	10.8	18.5	12.0± 3.4	9.4	35.3	11.3	18.7± 8.3	19.0	14.7	15.2	16.3± 1.4
12	23.1	20.1	14.7	19.3± 2.5	5.1	11.0	20.2	12.1± 4.4	8.0	10.4	15.0	11.1± 2.1	19.2	15.7	20.7	18.5± 1.5
14	17.0	24.0	15.8	18.9± 2.6	8.0	13.7	19.3	13.7± 3.3	10.8	8.6	13.4	10.9± 1.4	24.8	23.6	19.2	22.5± 1.7
16	13.4	26.3	14.4	18.0± 4.1	8.8	13.4	16.1	12.8± 2.1	10.8	28.4	15.8	18.3± 5.2	30.0	27.8	20.8	26.2± 2.8
18	10.8	22.8	16.4	16.6± 3.5	6.3	12.1	21.4	13.3± 4.4	16.9	33.3	12.9	21.0± 6.2	22.0	40.5	17.1	26.5± 7.1
20	15.0	19.7	20.0	18.2± 1.6	7.1	13.5	29.0	16.5± 6.5	14.3	41.1	22.0	25.8± 8.0	39.5	47.7	30.0	39.1± 5.1
22	15.0	17.2	18.9	17.0± 1.1	8.6	**	34.0	21.3±12.7	30.3	56.5	19.6	35.5±11.0	44.9	44.7	31.5	40.4± 4.4
24	15.3	19.8	21.6	18.9± 1.9	10.0		37.7	23.9±13.9	27.2	*	28.6	27.9± 0.7	42.2	60.3	32.8	45.1± 8.1
26	18.1	26.4	26.4	23.6± 2.8	9.4		40.0	24.7±15.3	21.0		33.1	27.1± 6.1	41.2	84.9	32.2	52.8±16.3
28	18.5	23.1	27.5	23.0± 2.6	13.0		40.1	26.6±13.6	23.8		31.8	27.8± 4.0	35.4	112.1	35.0	60.8±25.6

* Died on day 23
** Missing value

APPENDIX 3

Studies on the Self-Cure of *H. contortus* Infections: Influence of Breed and Haemoglobin Type.

TABLE 1.

Packed cell volumes (%) of Scottish Blackface sheep.

Days after infection	Blackface HbA 51	86	95	97	Mean ±S.E.	Blackface Hb B 43	45	85	90	Mean ±S.E.
0	31	30	31	29	30.3±0.6	29	31	33	31	31.0±0.8
4	31	29	30	30	30.0±0.4	29	31	32	30	30.5±0.6
7	30	31	34	32	32.0±0.9	32	33	30	30	31.3±0.8
11	30	27	33	30	30.0±1.1	31	32	29	37	29.8±1.1
14	28	27	30	29	28.4±0.7	23	26	25	24	24.5±0.7
18	25	23	24	26	22.0±1.1	20	16	22	21	19.8±1.3
21	20	21	22	25	22.3±0.2	21	16	20	20	19.3±1.1
24	18	20	24	27	23.0±1.1	18	14	17	19	17.0±1.1
28	23	22	21	26	23.0±1.1	17	13	16	18	16.0±1.1
Days after reinfection										
4	23	20	21	26	22.5±1.3	21	18	19	19	19.3±0.6
7	30	23	24	28	26.3±1.7	20	19	18	23	20.0±1.1
11	26	26	22	30	26.0±1.6	24	20	23	17	21.0±1.6
14	27	25	22	30	26.0±1.7	24	24	21	15	21.0±2.1
18	29	24	18	29	25.0±2.5	27	23	19	14	20.8±2.8
21	31	23	18	28	25.0±2.9	26	26	17	15	21.0±2.9
23	32	23	18	28	25.3±3.0	26	28	16	14	21.0±3.5

TABLE 2.

Packed cell volumes (%) of Finn Dorset sheep.

Days after infection	Dorset HbA				Mean ±S.E.	Dorset HbB				Mean ±S.E.
	57	58	59	62		3	40	54	61	
0	32	31	29	28	30.0±0.9	28	28	26	32	28.5±1.3
4	31	31	28	28	29.5±0.9	28	29	26	31	28.5±1.0
7	26	31	30	29	29.0±1.1	22	23	21	24	22.5±0.7
11	25	30	30	29	28.5±1.2	23	24	24	25	24.0±0.4
14	25	27	25	26	25.8±0.5	21	22	19	18	20.0±0.9
18	15	21	19	18	18.3±1.3	14	16	12	17	14.8±1.1
21	13	22	14	17	16.5±2.0	12	14	10	14	12.5±1.0
24	13	21	15	16	16.3±1.7	14	14	10	13	12.8±1.0
28	16	23	14	17	17.6±1.9	12	14	9	13	12.0±1.1
Days after reinfection										
4	14	24	14	15	16.8±2.4	12	13	9	13	11.8±1.0
7	15	28	17	18	19.5±2.9	14	11	10	14	12.3±1.0
11	17	27	17	16	19.3±2.6	13	12	9	12	11.5±0.9
14	14	28	13	14	17.3±3.6	10	12	9	10	10.3±0.6
18	13	30	11	11	16.3±4.6	11	11	7	11	10.0±1.0
21	13	29	11	9	15.5±4.6	8	11	*	10	9.7±0.9
23	13	33	11		20.0±6.6	4	11	*	11	11.0±0.0

* Died

TABLE 3.

Haemoglobin concentrations (g%) of Scottish Blackface sheep.

Days after infection	Blackface HbA 51	86	95	97	Mean ±S.E.	Blackface HbB 43	45	85	90	Mean ±S.E.
0	10.7	10.4	10.9	10.5	10.6±0.1	10.4	11.4	11.7	11.0	11.1±0.3
4	10.4	10.2	11.1	10.6	10.6±0.2	10.1	11.8	11.2	11.0	11.0±0.4
7	10.3	10.1	11.4	10.8	10.6±0.3	11.3	12.3	11.1	11.0	11.4±0.3
11	9.0	9.5	10.5	10.0	9.8±0.3	10.5	10.0	10.5	9.1	10.0±0.3
14	8.9	8.6	10.0	9.1	9.2±0.3	9.2	9.3	9.1	8.8	9.1±0.1
18	7.0	7.8	8.8	9.6	8.3±0.6	8.0	6.7	7.8	7.6	7.5±0.3
21	6.9	7.3	7.4	8.8	7.6±0.4	7.3	5.7	7.1	7.2	6.8±0.4
24	6.9	6.1	6.9	8.7	7.2±0.6	6.0	5.0	5.4	5.5	5.5±0.2
28	7.2	6.4	6.1	8.9	7.1±0.6	6.0	4.6	5.2	6.4	5.6±0.4
Days after reinfection										
4	7.7	6.8	6.8	9.1	7.6±0.5	4.2	5.8	5.8	6.8	5.7±0.5
7	9.2	7.2	6.8	9.0	8.1±0.6	7.0	6.0	5.6	7.6	6.6±0.5
11	7.7	8.9	7.1	10.5	8.5±0.8	8.7	8.6	7.0	5.6	7.5±0.7
14	8.7	8.2	7.0	10.7	8.6±0.8	8.4	8.1	6.4	5.1	7.0±0.8
18	9.7	8.7	6.1	9.9	8.6±0.9	9.6	7.9	6.8	5.0	7.3±1.0
21	10.1	7.8	5.6	9.3	8.2±1.0	9.4	9.2	6.0	5.0	7.4±1.1
23	10.3	7.7	5.4	9.2	8.2±1.1	8.7	9.6	5.2	4.4	7.0±1.3

TABLE 4.

Haemoglobin concentrations (g%) of Finn Dorset sheep.

Days after infection	Dorset HbA 57	58	59	62	Mean ±S.E.	Dorset HbB 3	40	54	61	Mean ±S.E.
0	9.4	11.3	10.9	11.3	10.7±0.4	9.8	9.5	9.8	10.8	10.0±0.3
4	9.2	11.3	11.0	11.0	10.6±0.5	9.2	10.4	9.0	10.6	9.8±0.4
7	9.0	11.4	11.0	10.7±	10.6±0.5	8.7	8.8	7.9	9.1	8.6±0.3
11	8.0	10.0	10.3	10.0	9.6±0.5	8.1	8.4	6.8	8.0	7.8±0.4
14	7.7	9.5	9.3	10.9	9.4±0.7	7.6	8.2	6.2	7.1	7.3±0.4
18	5.[illegible]	8.0	5.8	6.0	6.3±0.6	4.0	6.1	4.3	6.0	5.1±0.6
21	4.6	7.6	4.8	5.8	5.7±0.7	4.0	5.4	3.5	5.2	4.5±0.6
24	5.0	5.9	4.0	4.6	6.6±0.8	3.0	4.3	3.0	3.2	3.4±0.3
28	4.9	7.7	5.0	4.9	5.6±0.7	3.0	4.3	2.8	3.6	3.4±0.3
Days after reinfection										
4	4.5	8.3	4.3	4.9	5.5±0.9	3.[illegible]	4.1	2.9	3.6	3.6±0.3
7	4.9	10.0	5.1	5.3	6.3±1.2	4.3	2.7	3.1	4.1	3.6±0.4
11	5.4	10.3	4.8	4.7	6.3±1.3	3.8	2.9	2.7	4.4	3.5±0.4
14	5.0	9.6	4.0	4.2	5.[illegible]±1.3	3.2	2.9	2.7	3.7	3.1±0.2
18	4.3	10.0	3.1	3.0	5.1±1.7	2.6	2.6	2.0	3.9	2.8±0.4
21	4.2	10.2	3.2	2.5	5.0±1.8	2.2	3.0	*	3.4	2.9±0.4
23	3.8	10.5	3.2	*	5.8±2.3	*	2.3	*	3.0	2.7±0.4

* Died

TABLE 5.

Red cell counts ($x10^6$/cu.mm) of Scottish Blackface sheep.

Days after infection	Blackface HbA 51	86	95	97	Mean ±S.E.	Blackface HbB 43	45	85	90	Mean ±S.E.
0	9.6	9.6	9.5	9.3	9.5±0.1	9.5	9.5	9.6	10.2	9.7±0.2
4	9.0	9.0	10.3	9.3	9.4±0.3	9.7	10.0	9.3	10.1	10.1±0.2
7	9.0	8.8	10.2	9.3	9.3±0.3	9.9	10.4	9.5	10.0	10.0±0.2
11	9.1	8.6	10.0	9.6	9.3±0.3	9.6	10.3	9.2	9.6	9.7±0.2
14	7.9	7.5	8.8	7.7	8.0±0.3	7.7	7.5	7.7	8.6	7.9±0.3
18	7.3	7.4	7.0	7.5	7.3±0.1	7.3	5.0	5.5	7.1	6.2±0.6
21	5.2	5.9	6.3	7.5	6.2±0.5	7.1	4.8	5.0	5.6	5.6±0.5
24	4.9	5.6	7.1	8.4	6.5±0.8	5.3	3.9	4.0	5.2	4.6±0.4
28	5.6	4.9	5.3	7.0	5.7±0.5	4.4	3.5	3.6	5.0	4.1±0.4
Days after reinfection										
4	5.5	5.3	5.2	7.2	5.8±0.5	5.3	4.6	4.2	5.8	5.0±0.4
7	6.7	5.3	5.4	7.1	6.1±0.5	4.9	5.6	4.2	5.9	5.2±0.4
11	5.3	6.4	5.9	8.2	6.4±0.6	6.3	6.5	5.7	4.1	5.7±0.5
14	6.3	6.3	6.0	7.6	6.6±0.4	6.2	6.1	5.6	3.7	5.4±0.6
18	6.8	6.0	5.0	7.3	6.2±0.5	7.1	5.7	4.9	3.5	5.3±0.8
21	7.4	6.2	4.6	7.3	6.4±0.7	6.6	7.0	4.2	3.6	5.4±0.9
23	7.8	5.6	4.4	6.8	6.4±0.7	6.7	7.3	3.6	3.2	5.2±1.0

TABLE 6.

Red cell counts ($x10^6$/cu.mm) of Finn Dorset sheep.

Days after infection	Dorset HbA 57	58	59	62	Mean ±S.E.	Dorset HbB 3	40	54	61	Mean ±S.E.
0	8.3	9.4	9.0	9.5	9.1±0.3	8.8	9.0	8.3	9.8	9.0±0.3
4	8.0	9.3	8.9	9.3	8.9±0.3	8.9	8.9	8.5	9.6	9.0±0.2
7	7.7	9.3	8.7	9.0	8.7±0.3	7.6	7.2	6.7	7.4	7.3±0.2
11	8.1	8.7	8.9	9.1	8.7±0.2	7.8	8.0	8.2	7.2	7.8±0.2
14	6.5	8.0	7.2	9.2	7.2±0.6	6.7	6.4	6.0	5.8	6.3±0.2
18	3.9	6.5	5.0	5.2	5.2±0.5	3.7	5.1	3.5	4.2	4.1±0.4
21	3.0	6.3	3.6	4.5	4.3±0.7	3.0	4.3	2.5	3.6	3.4±0.4
24	3.1	6.7	3.8	3.9	4.4±0.8	3.3	3.2	2.0	2.6	3.9±0.4
28	2.9	6.0	3.1	3.9	4.0±0.7	2.5	3.6	2.1	2.7	2.8±0.3
Days after reinfection										
4	2.3	6.0	2.5	3.2	3.5±0.9	2.3	2.8	1.8	3.3	2.6±0.3
7	2.9	8.3	3.0	3.7	4.5±1.3	2.8	1.9	2.1	3.6	2.6±0.4
11	3.5	7.0	3.0	3.6	4.3±0.9	2.3	2.1	2.1	2.7	2.3±0.2
14	3.0	7.3	2.4	2.8	3.9±1.1	2.0	2.4	1.6	2.4	2.1±0.2
18	2.6	7.4	1.6	2.2	3.5±1.3	1.6	2.4	1.3	2.6	2.0±0.3
21	2.4	7.4	1.7	1.9	3.4±1.4	1.5	2.6	*	2.5	2.2±0.4
23	2.3	8.2	1.7	*	4.1±2.1	*	2.5	*	2.1	2.3±0.2

* Died

TABLE 7.

Mean corpuscular volumes (μ^3) of Scottish Blackface sheep.

Days after infection	Blackface HbA 51	86	95	97	Mean ±S.E.	Blackface HbB 43	45	85	90	Mean ±S.E.
0	32.9	30.7	32.5	31.1	31.8±0.4	30.4	33.3	34.3	30.9	32.2±0.9
4	34.4	32.2	29.3	32.3	32.1±1.0	29.9	30.9	34.5	29.7	31.3±1.1
7	33.3	34.7	33.2	34.4	33.9±0.4	32.3	31.7	31.4	29.9	31.3±0.5
11	32.3	31.3	32.4	31.1	31.8±0.3	32.2	31.1	31.7	28.1	30.8±0.9
14	35.4	36.0	34.1	37.2	35.7±0.6	29.9	34.6	32.5	28.6	31.4±1.3
18	34.2	31.1	34.3	34.7	33.6±0.8	27.4	32.0	39.9	29.6	32.2±2.7
21	38.8	35.8	35.9	33.4	36.0±1.1	29.6	33.8	39.7	35.8	34.7±2.1
24	36.9	35.6	33.7	32.1	34.6±1.1	34.2	35.5	43.0	36.3	37.3±2.0
28	41.4	44.9	39.6	36.9	40.7±1.7	38.5	36.9	44.1	35.7	38.8±1.9
Days after reinfection										
4	41.7	37.6	40.7	36.6	39.2±1.2	39.9	39.0	44.9	33.0	39.2±2.4
7	44.8	43.4	44.6	39.3	43.0±1.3	40.9	34.1	43.1	39.3	39.4±1.9
11	49.4	40.5	37.3	36.8	41.0±2.9	37.9	30.6	40.2	41.5	37.6±2.4
14	43.0	39.4	36.8	39.5	39.7±1.3	39.0	39.1	37.2	41.0	39.1±0.8
18	42.9	40.3	37.3	39.9	40.1±1.1	37.9	40.1	32.7	40.2	37.7±1.8
21	42.1	37.3	39.6	37.6	39.2±1.1	39.3	37.4	40.0	41.7	39.6±0.9
23	40.8	41.0	41.3	41.2	41.1±0.1	38.6	38.3	44.2	43.2	41.1±1.5

TABLE 8.

Mean corpuscular volumes (μ^3) of Finn Dorset sheep.

Days after infection	Dorset HbA 57	58	59	62	Mean ±S.E.	Dorset HbB 3	40	54	61	Mean ±S.E.
0	38.5	32.6	31.7	29.5	33.1±0.9	31.7	31.2	31.3	32.7	31.7±0.3
4	38.8	33.3	31.5	30.3	33.5±1.9	31.5	32.5	30.6	36.0	32.7±1.2
7	33.6	33.3	34.4	32.4	33.4±0.4	28.9	31.9	31.2	32.3	31.1±0.8
11	31.0	34.4	33.6	31.7	32.7±0.8	29.6	29.9	29.1	33.9	30.6±1.1
14	38.2	33.8	34.9	28.3	33.8±2.1	32.2	34.3	31.6	30.8	32.2±0.8
18	38.5	32.3	37.8	34.6	35.8±1.4	36.5	31.4	34.3	39.3	35.4±1.7
21	43.9	35.1	38.7	38.0	38.9±1.8	40.3	32.4	39.4	38.9	37.8±1.8
24	41.7	31.3	39.5	40.6	38.3±2.4	42.9	44.3	49.5	50.8	46.9±1.9
28	45.9	38.6	46.5	43.8	43.7±1.8	47.6	39.3	43.7	45.1	43.9±1.7
Days after reinfection										
4	50.6	39.9	57.8	46.7	48.8±3.7	51.2	46.3	49.5	40.4	46.9±2.4
7	51.7	33.7	50.0	49.2	46.2±4.2	49.3	56.7	46.7	38.7	47.9±3.7
11	48.0	38.8	55.9	44.0	46.7±3.6	54.8	57.6	43.7	45.1	50.3±3.5
14	46.4	37.7	53.7	47.5	46.3±3.3	51.0	49.2	51.8	42.0	48.5±2.2
18	50.0	40.5	70.1	49.5	52.5±6.3	57.5	46.8	55.1	42.6	50.5±3.5
21	53.7	39.3	64.0	46.6	50.9±5.3	58.3	43.0	*	43.4	48.2±5.0
23	57.0	40.0	63.2	*	53.4±6.9	*	43.7	*	47.2	45.5±1.8

* Died

TABLE 9.

Mean corpuscular haemoglobin concentrations (%) of Scottish Blackface sheep.

Days after infection	Blackface HbA 51	86	95	97	Mean ±S.E.	Blackface HbB 43	45	85	90	Mean ±S.E.
0	33.8	35.3	35.1	36.3	35.1±0.5	35.9	36.3	35.0	35.0	35.6±0.3
4	33.4	35.2	37.1	35.4	35.3±0.8	34.8	38.1	35.0	36.9	36.2±0.8
7	34.2	33.1	33.5	33.7	33.6±0.2	35.2	37.2	36.9	36.6	36.5±0.4
11	30.5	35.2	32.3	33.3	32.8±1.0	33.9	31.3	36.3	33.8	33.8±1.0
14	31.9	32.0	33.5	32.4	32.5±0.4	39.8	35.7	36.4	36.0	37.0±1.0
18	28.0	33.9	36.7	36.7	33.8±2.1	40.0	41.9	35.6	36.1	38.4±1.5
21	34.4	37.7	33.4	33.3	34.0±0.4	34.7	35.9	35.7	36.2	35.6±0.3
24	38.1	35.5	33.0	32.2	34.7±1.3	34.1	35.6	32.2	29.1	32.8±1.4
28	31.2	28.9	28.9	34.2	30.8±1.3	36.3	35.4	32.7	35.8	35.1±0.8
Days after reinfection										
4	33.6	34.0	32.6	34.4	33.7±0.4	20.0	32.3	30.7	36.0	29.8±3.4
7	30.8	31.2	28.5	32.0	30.6±0.8	35.1	31.4	31.2	33.1	32.7±0.9
11	29.7	34.2	32.1	34.9	32.7±1.2	36.9	34.2	32.9	33.0	34.3±0.9
14	32.3	32.6	31.6	35.5	33.0±0.9	35.1	33.7	30.4	33.7	33.2±1.0
18	33.5	36.2	32.8	34.2	34.2±0.7	35.6	34.4	36.0		35.3±0.5
21	32.5	34.0	31.1	33.8	32.9±0.7	36.2	35.4	35.1	33.2	35.0±0.6
23	32.2	32.5	29.8	32.9	31.9±0.7	33.5	34.2	32.6	31.6	33.0±0.6

TABLE 10.

Mean corpuscular haemoglobin concentrations (%) of Finn Dorset sheep.

Days after infection	Dorset HbA 57	58	59	62	Mean ±S.E.	Dorset HbB 3	40	54	61	Mean ±S.E.
0	39.3	36.9	38.1	40.2	38.6±0.7	35.0	34.3	38.1	33.7	35.3±1.0
4	29.6	36.5	39.3	39.4	36.2±2.3	33.2	36.3	35.1	34.0	34.7±0.7
7	34.7	36.9	36.6	37.0	36.3±0.5	39.5	38.2	37.8	37.7	38.3±0.4
11	32.0	33.3	34.3	34.5	33.5±0.6	35.0	35.0	32.5	32.7	33.8±0.7
14	30.8	35.3	37.2	41.7	36.3±2.3	37.0	37.1	32.7	39.4	36.6±1.4
18	36.7	38.1	30.5	33.3	34.7±1.7	29.6	37.8	35.4	36.4	34.8±1.8
21	38.3	34.5	34.4	38.1	33.8±2.1	33.1	31.5	34.6	22.5	30.4±2.7
24	38.5	27.9	32.1	29.8	32.1±2.3	28.3	30.0	29.6	24.5	28.1±1.3
28	30.8	33.4	34.5	29.0	31.9±1.3	25.1	30.5	32.7	27.3	28.9±1.7
Days after reinfection										
4	31.8	34.5	29.9	32.0	32.2±1.0	32.2	31.2	32.7	27.6	30.9±1.2
7	32.6	37.1	29.5	29.4	32.2±1.8	31.0	24.1	30.1	29.6	28.7±1.6
11	31.6	38.0	28.4	29.4	31.9±2.2	30.0	24.3	30.2	36.5	30.3±2.5
14	35.4	34.9	30.5	31.0	33.0±1.3	32.0	23.8	31.2	36.7	30.9±2.7
18	33.1	33.2	26.5	27.5	30.6±1.5	23.7	24.0	27.9	36.0	27.9±2.9
21	32.2	36.3	28.7	22.8	30.0±2.9	19.6	26.9	*	31.2	25.9±3.4
23	29.1	37.0	28.9	*	31.7±2.7	*	20.9	*	29.6	25.3±4.3

* Died

TABLE 11.

Serum iron (µg%).

	Sheep No.	Days after infection 0	7	14	21	28	Days after refinfection 7	14	21
	51	136	126	116	118	105	100	119	120
Blackface HbA	86	165	163	160	169	165	188	116	114
	95	120	107	124	129	118	93		89
	97	150	135	120	118	129	111	121	138
Mean		143	133	130	134	129	123	119	115
S.E.		10	12	10	12	13	22	2	10
	43	169	159	135	106	91	120	109	132
Blackface HbB	45	130	120	130	98	100	116	128	134
	85	109	133	137	142	139	130	109	73
	90	130	122	131	114	110	120	108	97
Mean		135	134	133	115	110	122	114	109
S.E.		13	9	2	10	10	3	5	15
	57	162	156	148	160	132	120	121	118
Dorset HbA	58	176	167	143	120	102	89	118	124
	59	178	176	143	120	110	100	89	79
	62	173	165	139	106	88	109	84	26
Mean		172	166	143	127	108	105	103	87
S.E.		4	4	2	12	9	7	10	23
	3	158	157	128	96	70	98	65	23
Dorset HbB	40	191	184	133	51	91	88	81	59
	54	150	130	119	98	88	53	49	*
	61	122	141	130	109	105	100	100	58
Mean		155	153	127	89	89	85	74	47
S.E.		14	12	3	13	7	11	11	12

* Died

TABLE 12.

Total serum proteins (g%).

	Sheep No.	Days after infection 0	7	14	21	28	Days after reinfection 7	14	21
	51	6.8	7.2	6.4	5.3	5.8	6.2	5.7	5.7
Blackface HbA	86	6.8	6.8	6.3	6.5	5.1	5.3	5.0	5.3
	95	6.9	6.3	6.1	5.7	5.3	4.3	4.1	4.0
	97	7.0	6.4	6.3	5.5	6.4	6.4	6.0	6.1
Mean		6.9	6.7	6.3	5.8	5.7	5.6	5.2	5.3
S.E.		0.1	0.2	0.1	0.3	0.3	0.5	0.4	0.5
	43	7.2	6.8	6.1	5.3	5.4	5.4	6.6	5.7
Blackface HbB	45	7.2	7.1	6.1	6.1	4.8	5.0	5.5	5.3
	85	7.2	6.5	6.1	6.2	5.8	6.6	5.7	5.0
	90	7.2	6.5	6.0	6.0	4.9	4.8	5.0	5.2
Mean		7.2	6.7	6.1	5.9	5.2	5.5	5.7	5.3
S.E.		0.0	0.1	0.0	0.2	0.2	0.4	0.3	0.1
	57	6.5	6.4	5.8	5.2	4.0	4.5	4.0	3.7
Dorset HbA	58	7.6	7.5	6.9	6.7	6.4	7.2	6.0	6.5
	59	7.2	6.4	6.0	4.9	4.7	4.4	3.9	3.5
	62	7.1	6.5	6.4	5.3	4.3	4.5	3.5	3.5
Mean		7.1	6.7	6.3	5.5	4.9	5.2	4.4	4.3
S.E.		0.2	0.3	0.2	0.4	0.5	0.7	0.6	0.7
	3	6.8	6.6	5.8	4.8	4.0	3.9	3.6	3.4
Dorset HbB	40	6.6	6.5	6.4	6.3	5.9	5.9	5.3	4.8
	54	7.4	7.0	6.3	6.1	4.4	3.5	2.9	*
	61	6.9	6.4	5.4	4.9	5.0	4.8	4.6	4.5
Mean		6.9	6.6	6.0	5.5	4.8	4.5	4.1	4.2
S.E.		0.2	0.1	0.2	0.4	0.4	0.5	0.5	0.4

* Died

TABLE 13.

Serum albumin (g%).

	Sheep No.	Days after infection 0	7	14	21	28	Days after reinfection 7	14	21
	51	3.7	4.0	3.0	2.8	2.5	2.3	3.0	2.9
Blackface HbA	86	3.8	3.3	2.9	2.8	2.5	2.5	2.3	2.3
	95	4.0	3.8	3.6	2.6	2.2	2.1	3.0	2.0
	97	3.9	3.8	3.3	3.0	2.8	2.5	2.8	2.6
Mean		3.9	3.7	3.2	2.8	2.5	2.4	2.8	2.5
S.E.		0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.2
	43	3.8	3.4	2.9	2.2	2.6	2.9	2.8	2.8
Blackface HbB	45	3.9	3.6	2.9	2.5	2.3	2.3	2.7	2.7
	85	3.8	3.5	2.6	2.3	2.1	2.6	2.9	2.3
	90	3.4	3.3	2.9	3.2	2.3	2.0	2.3	2.0
Mean		3.7	3.5	2.8	2.6	2.3	2.5	2.7	2.5
S.E.		0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.2
	57	3.6	3.1	2.7	2.1	1.6	1.3	1.5	1.2
Dorset HbA	58	3.4	3.4	2.7	2.5	2.5	2.8	3.0	3.4
	59	3.5	3.2	2.5	2.0	1.8	1.7	1.2	1.4
	62	3.5	2.9	2.6	2.0	1.7	1.7	1.4	1.2
Mean		3.5	3.2	2.6	2.2	1.9	1.9	1.8	1.8
S.E.		0.1	0.1	0.5	0.1	0.3	0.3	0.4	0.5
	3	4.0	3.6	2.5	1.9	1.3	1.1	0.9	0.4
Dorset HbB	40	3.3	2.8	2.4	1.8	1.4	1.5	1.9	1.0
	54	3.2	2.8	2.4	1.8	1.4	1.2	0.5	*
	61	3.2	2.6	2.0	2.0	1.6	1.4	1.8	1.2
Mean		3.4	3.0	2.3	1.9	1.4	1.3	1.3	0.9
S.E.		0.2	0.2	0.1	0.5	0.1	0.1	0.3	0.2

* Died

TABLE 14.

Serum globulins (g%).

	Sheep No.	Days after infection 0	7	14	21	28	Days after reinfection 7	14	21
	51	3.1	3.2	3.4	2.5	3.3	3.9	2.7	2.8
Blackface HbA	86	3.0	3.5	3.4	3.7	2.6	2.8	2.7	3.0
	95	2.9	2.5	2.5	3.1	3.1	2.2	1.1	1.0
	97	2.5	2.6	3.0	2.5	3.6	3.9	3.2	3.5
Mean		2.9	3.0	3.1	3.0	3.2	3.2	2.4	2.6
S.E.		0.1	0.2	0.2	0.3	0.2	0.4	0.5	0.5
	43	3.4	3.4	3.2	3.1	2.8	2.5	3.8	3.2
Blackface HbB	45	3.3	3.5	3.2	3.6	2.5	2.7	2.8	2.6
	85	3.4	3.0	3.5	3.8	3.7	4.0	2.8	2.7
	90	3.8	3.2	3.1	2.8	2.6	2.8	2.7	3.2
Mean		3.5	3.3	3.3	3.3	2.9	3.0	3.0	2.9
S.E.		0.1	0.1	0.1	0.2	0.3	0.3	0.3	0.2
	57	2.9	3.3	3.1	3.1	2.4	3.2	2.5	2.5
Dorset HbA	58	4.2	4.1	4.2	4.2	3.9	4.4	3.0	3.1
	59	3.7	3.2	3.5	2.9	2.9	2.7	2.7	2.1
	62	3.6	3.6	3.8	3.3	2.6	2.8	2.1	2.3
Mean		3.6	3.6	3.7	3.4	3.0	3.3	2.6	2.5
S.E.		0.3	0.2	0.2	0.3	0.3	0.4	0.2	0.2
	3	2.8	3.0	3.3	2.9	2.7	2.8	2.5	3.0
Dorset HbB	40	3.3	4.1	4.0	4.5	4.5	4.4	3.4	3.8
	54	4.2	4.2	3.9	4.3	3.0	2.3	2.4	*
	61	3.7	3.8	3.4	2.9	3.4	3.4	2.8	3.3
Mean		3.5	3.8	3.7	3.8	3.4	3.2	2.8	3.4
S.E.		0.3	0.3	0.2	0.4	0.4	0.5	0.2	0.2

* Died

TABLE 15.

Percentage of HbC in the blood following infection and reinfection.

	Sheep No.	Weeks after infection 0	1	2	3	4	Weeks after reinfection 1	2	3
Blackface HbA	51	2	2	2	5	8	11	4	4
	86	3	4	6	7	12	23	25	14
	95	2	2	2	10	15	15	9	13
	97	3	3	2	4	9	28	27	27
Mean		2.5	2.8	3.0	6.5	11.0	19.3	16.3	14.5
S.E.		0.3	0.5	1.0	1.3	1.6	3.9	5.7	4.7
Dorset HbA	57	3	2	7	15	29	38	29	55
	58	1	4	3	12	19	19	15	12
	59	2	3	3	18	33	69	78	95
	62	2	4	7	14	23	36	64	-
Mean		2.0	3.3	5.0	14.8	26.0	40.5	46.5	54.0
S.E.		0.5	0.5	1.2	1.3	3.1	10.4	14.7	24.0

TABLE 16.

Faecal egg output ($x10^6$) of Scottish Blackface sheep.

Days after infection	Blackface HbA 51	86	95	97	Mean ±S.E.	Blackface HbB 43	45	85	90	Mean ±S.E.
16	N	N	N	N	N	N	N	N	N	N
17	N	N	N	N	N	N	0.1	N	N	0.03±0.03
18	0.1	N	N	N	0.03±0.03	N	0.6	1.3	N	0.5±6.3
19	1.1	N	N	N	0.3±0.3	0.1	5.5	2.3	0.1	2.0±1.3
20	6.7	0.6	1.4	0.1	2.2±1.5	0.2	8.3	7.1	1.0	4.2±2.1
21	12.0	1.2	3.3	0.1	4.2±2.7	0.8	15.9	12.1	2.2	7.8±3.7
22	13.6	3.4	5.3	1.1	5.9±2.7	3.2	14.2	13.9	4.0	8.8±3.0
23	14.6	5.5	12.8	1.5	8.6±3.1	10.6	14.2	15.6	8.1	12.1±1.7
24	9.4	5.4	10.6	2.6	7.0±1.8	7.6	9.8	17.3	12.3	11.8±2.1
26	13.6	12.6	23.9	8.8	14.7±3.2	16.2	15.8	12.8	18.6	15.9±1.2
27	22.9	25.4	26.4	6.3	20.3±4.7	18.2	17.5	14.3	17.2	16.8±0.9
28	25.7	18.4	24.4	7.8	19.1±4.1	20.6	16.2	12.7	19.4	17.2±1.8
Days after reinfection										
1	23.1	27.6	18.7	9.9	19.8±3.8	26.8	13.8	15.6	15.9	18.0±3.0
2	23.3	26.8	17.4	7.2	18.7±4.3	23.7	14.1	14.3	14.1	16.6±2.4
3	32.2	38.1	30.9	8.3	27.4±6.6	20.7	14.9	12.1	16.9	16.2±1.8
4	28.6	27.3	31.5	10.9	24.6±4.6	13.8	14.8	14.6	10.7	13.5±1.0
5	21.4	16.4	16.3	9.0	15.8±2.6	13.3	14.7	12.3	11.3	12.9±0.7
6	26.0	9.2	11.3	6.6	13.3±4.3	11.2	11.1	8.7	11.5	10.6±0.7
8	14.8	14.6	2.0	2.1	8.4±3.7	0.6	8.6	8.1	2.8	5.0±2.0
9	8.3	2.1	2.9	1.4	3.7±1.6	N	2.6	5.2	2.1	2.5±1.1
10	3.0	1.2	9.0	0.6	3.5±1.9	N	0.8	5.4	1.6	2.0±1.2
11	2.1	2.9	6.0	0.6	2.9±1.1	N	0.6	5.7	6.7	3.3±1.7
12	0.8	3.4	2.0	0.5	1.7±0.7	N	0.6	5.3	5.8	2.9±1.5
13	0.6	3.8	2.2	0.7	1.8±0.8	N	0.6	3.8	7.4	3.0±1.7
14	N	3.7	2.1	1.7	1.9±0.8	N	1.9	2.6	13.4	4.5±3.0
15	N	4.4	1.7	1.8	2.0±0.9	N	1.3	3.1	12.6	5.8±4.0
16	N	6.7	5.0	2.3	3.5±1.5	N	2.9	7.3	10.7	5.2±2.4
17	N	6.4	7.6	0.7	3.7±1.9	N	1.2	4.2	6.5	3.0±1.5
18	N	11.6	11.8	1.0	6.1±3.2	N	1.2	5.9	9.5	4.2±2.2
19	N	13.0	3.7	2.0	4.7±2.9	N	1.4	8.6	9.6	4.9±2.5
20	N	1.0	5.4	3.0	2.4±1.2	N	1.5	4.4	9.2	3.8±2.0
21	N	3.2	3.0	1.3	1.9±0.8	N	N	4.9	3.5	2.1±1.3
22	N	2.2	2.5	1.9	1.7±0.6	N	N	6.4	3.4	2.5±1.5
23	N	4.8	2.4	1.9	2.3±1.0	N	N	3.8	2.2	1.5 0.9

TABLE 17.

Faecal egg output ($x10^6$) of Finn Dorset sheep.

Days after infection	Dorset HbA 57	58	59	62	Mean ±S.E.	Dorset HbB 3	40	54	61	Mean ±S.E.
16	N	N	N	N	N	0.1	N	N	N	0.03±0.03
17	N	N	N	0.1	0.03±0.03	0.1	N	N	N	0.03±0.03
18	0.1	N	N	0.6	0.2±0.1	0.5	N	N	N	0.1±0.1
19	1.5	0.1	0.5	3.6	1.4±0.8	17.5	0.1	0.1	0.7	4.6±4.3
20	9.3	2.4	5.8	11.8	7.3±2.1	29.7	5.3	6.5	2.9	11.1±6.2
21	18.1	9.4	7.9	25.8	15.3±4.2	49.8	6.0	12.7	10.8	19.8±10.1
22	23.1	18.2	19.3	27.4	22.0±2.1	34.0	9.2	20.5	10.7	18.6±5.7
23	40.0	16.5	24.6	30.7	28.0±5.0	61.2	14.8	22.2	22.2	30.1±0.5
24	16.2	18.3	23.3	29.5	21.8±3.0	62.3	21.1	25.7	21.9	32.8±9.9
26	33.8	18.1	19.4	18.6	22.5±3.8	77.3	29.0	38.7	20.1	41.3±12.6
27	38.3	28.0	64.4	48.8	44.9±7.8	45.4	28.0	33.5	24.9	33.0±4.5
28	44.9	28.6	60.9	55.5	47.5±7.1	59.7	27.2	41.5	33.9	40.6±7.0
Days after reinfection										
1	45.7	24.7	59.1	65.1	48.7±9.0	61.5	35.3	57.7	38.5	48.3±6.6
2	47.7	23.6	41.6	58.1	42.8±7.2	57.1	22.6	35.2	48.0	40.7±7.5
3	44.3	24.4	43.5	65.3	44.4±8.4	41.3	38.7	56.0	33.9	42.5±4.8
4	44.1	26.5	39.4	32.9	35.7±3.8	43.5	25.9	49.8	27.3	36.6±5.9
5	43.9	21.5	49.5	62.0	44.2±8.5	60.4	19.5	45.8	22.5	37.1±9.8
6	46.2	12.9	58.0	67.0	46.0±11.8	51.5	27.6	23.0	25.0	31.8±6.6
8	55.5	11.9	53.1	59.0	44.9±11.1	51.1	30.1	35.4	18.6	33.8±6.8
9	25.8	11.2	53.2	62.8	38.3±12.0	46.8	20.5	57.8	19.1	36.1±9.7
10	17.1	11.0	50.0	70.6	37.2±14.1	42.7	20.6	38.5	26.5	34.6±6.6
11	14.8	7.0	53.7	68.6	36.0±15.0	46.8	21.1	45.5	19.8	33.3±7.4
12	25.7	3.0	51.2	57.5	34.3±12.5	49.7	30.7	51.4	24.7	39.1±6.7
13	18.9	1.3	52.8	63.3	34.0±14.5	46.1	33.7	40.3	30.2	37.6±3.5
14	28.4	1.0	55.1	53.3	34.5±12.7	33.4	37.9	41.0	28.3	35.2±2.8
15	46.3	1.2	59.3	53.1	40.0±13.0	38.4	40.9	30.5	27.6	34.4±3.2
16	27.3	0.6	56.0	61.2	36.3±14.0	30.7	41.8	30.2	23.3	31.5±3.8
17	22.0	0.7	65.0	62.4	37.5±15.7	37.8	48.9	37.8	14.2	34.7±7.3
18	25.4	N	46.5	39.6	27.9±10.3	32.7	45.1	31.3	36.4	36.4±3.1
19	35.5	1.8	44.8	48.3	32.6±10.6	35.3	32.5	28.4	39.3	33.9±2.3
20	47.0	0.8	42.0	30.2	30.0±10.4	30.8	27.8	*	20.2	26.2±3.2
21	33.6	0.5	54.5	*	29.5±15.7	32.3	27.8	*	19.9	26.7±3.6
22	37.3	0.2	42.9	*	26.8±13.4	*	25.4	*	11.5	18.5±7.0
23	26.7	0.1	46.7	*	24.5±13.5	*	16.8	*	14.0	15.4±1.4

* Died

TABLE 18.

Faecal blood clearances (ml/day) of Scottish Blackface sheep.

Days after infection	Blackface HbA 51	86	95	97	Mean ±S.E.	Blackface HbB 43	45	85	90	Mean ±S.E.
22	113	123	102	25	90.8±22.3	66	198	176	88	132.0±32.4
24	120	154	137	24	108.8±29.1	97	171	176	83	131.8±24.3
26	109	146	139	34	107.0±25.6	103	166	154	94	129.3±18.0
28	134	136	135	26	107.8±27.3	99	73	193	148	128.3±26.6
Days after reinfection										
2	123	113	115	25	94.0±23.0	76	94	175	90	108.8±22.4
4	99	102	115	40	89.0±16.7	65	87	167	79	99.5±23.0
6	79	47	79	26	57.8±13.0	37	63	122	56	69.5±18.3
8	80	20	55	11	41.5±1.60	6	52	171	34	65.8±36.3
10	67	23	76	20	46.5±14.6	4	12	53	58	31.8±13.9
12	3	29	108	24	41.0±23.0	2	10	39	52	25.8±11.8
14	2	45	141	27	53.8±30.4	2	18	78	69	41.8±18.7
16	2	50	165	31	62.0±35.7	3	25	58	77	40.8±16.5
18	4	33	195	38	67.5±43.2	4	32	88	87	52.8±20.9
20	7	78	166	24	68.8±35.8	5	23	129	120	69.3±20.9
22	7	75	156	32	67.5±32.7	5	9	128	123	66.3±34.2

TABLE 19.

Faecal blood clearances (ml/day) of Finn Dorset sheep.

Days after infection	Dorset HbA 57	58	59	62	Mean ±S.E.	Dorset HbB 3	40	54	61	Mean ±S.E.
22	395	139	244	295	268.3±53.0	421	178	410	261	317.5±59.0
24	371	161	296	354	295.5±48.0	496	220	431	272	354.8±65.0
26	427	151	195	441	303.5±76.0	418	233	480	241	343.0±63.0
28	361	154	412	396	330.8±60.0	410	226	461	239	334.0±60.0
Days after reinfection										
2	304	46	324	525	300.0±98.0	318	236	446	187	297.0±57.0
4	262	31	342	435	267.5±86.0	317	213	431	151	278.0±61.0
6	200	27	324	403	238.5±82.0	337	224	332	129	255.5±50.0
8	248	21	369	433	267.8±91.0	398	255	683	155	372.8±115.0
10	181	8	462	623	318.5±138.0	472	278	616	179	386.3±98.0
12	253	6	618	687	391.0±160.0	515	267	677	185	411.0±113.0
14	262	5	634	602	375.8±150.0	590	367	640	217	453.5±99.0
16	370	6	618	639	408.0±147.0	639	280	726	235	470.0±124.0
18	558	6	647	562	443.0±147.0	587	343	682	350	490.5±85.0
20	514	6	474	586	395.0±132.0	684	296	*	364	448.0±120.0
22				*		*		*	381	

* Died

TABLE 20.

Faecal red cell clearances (ml/day) of Scottish Blackface sheep.

Days after infection	Blackface HbA 51	86	95	97	Mean ±S.E.	Blackface HbB 43	45	85	90	Mean ±S.E.
22	24.0	31.0	21.0	6.0	30.5±5.3	12.0	32.0	35.0	16.0	23.8±5.7
24	22.0	31.0	33.0	7.0	23.3±5.9	18.0	24.0	36.0	16.0	23.5±4.5
26	25.0	32.0	31.0	9.0	24.3±5.3	19.0	25.0	30.0	20.0	23.5±2.5
28	30.0	27.0	26.0	6.0	22.3±5.5	17.0	16.0	26.0	21.0	20.0±2.3
Days after reinfection										
2	31.0	26.0	24.0	7.0	22.0±5.2	13.0	13.0	30.0	16.0	18.0±4.1
4	23.0	20.0	24.0	11.0	19.5±3.0	14.0	16.0	28.0	15.0	18.3±3.3
6	21.0	9.0	16.0	7.0	13.3±3.2	7.0	11.0	22.0	11.0	12.8±3.2
8	19.0	4.0	13.0	3.0	9.8±3.8	1.0	9.0	16.0	7.0	8.3±3.1
10	17.0	5.0	18.0	6.0	11.5±3.5	0.5	3.0	12.0	12.0	6.9±3.0
12	0.5	7.0	23.0	6.0	9.1±4.8	0.5	2.0	9.0	11.0	5.6±2.6
14	0.5	11.0	31.0	8.0	12.6±6.5	0.5	4.0	16.0	14.0	8.6±3.8
16	0.5	13.0	36.0	8.0	14.4±7.7	0.5	6.0	13.0	18.0	9.4±3.9
18	1.0	8.0	35.0	11.0	13.8±7.4	1.0	7.0	15.0	19.0	10.5±4.0
20	2.0	19.0	30.0	7.0	14.5±6.3	1.0	5.0	22.0	18.0	11.5±5.0
22	2.0	20.0	31.0	9.0	15.5±6.4	1.0	2.0	22.0	19.0	11.0±5.5

TABLE 21.

Faecal red cell clearances (ml/day) of Finn Dorset sheep.

Days after infection	Dorset HbA 57	58	59	62	Mean ±S.E.	Dorset HbB 3	40	54	61	Mean ±S.E.
22	55	53	37	50	48.8± 4.1	55	25	56	34	42.5±7.7
24	48	35	45	57	46.3± 4.5	69	37	52	35	48.3±7.9
26	55	31	35	75	49.0±10.1	59	28	48	39	43.5±6.6
28	54	32	46	59	47.8± 5.9	49	29	50	36	41.0±5.1
Days after reinfection										
2	46	31	49	84	52.5±11.2	45	33	49	30	39.3±4.6
4	37	27	51	65	45.0± 8.3	38	28	39	24	32.3±3.7
6	28	7	49	61	36.3±11.9	40	36	33	26	33.8±3.0
8	35	6	55	65	40.3±13.0	48	33	61	28	42.5±7.5
10	29	2	74	100	51.3±22.0	61	34	55	31	45.3±7.5
12	38	2	86	110	59.0±24.2	57	37	61	30	46.3±7.5
14	37	1	82	78	49.5±19.1	59	55	51	35	50.0±5.3
16	52	2	80	77	52.8±18.0	49	42	58	23	43.0±7.4
18	59	2	71	62	48.5±15.7	53	38	48	39	44.5±3.6
20	48	1	52	53	38.5±12.5	42	44	*	40	42.0±1.2
22						*		*	38	

* Died

TABLE 22.

Fractional catabolic rate of albumin F(CA) in Scottish Blackface sheep.

Days after infection	Blackface HbA 51	86	85	90	Mean ±S.E.	Blackface HbB 43	45	85	90	Mean ±S.E.
22	.124	.135	.232	.080	.143±.030	.088	.114	.176	.145	.131±.019
24	.181	.139	.181	.083	.146±.023	.140	.141	.148	.131	.140±.003
26	.116	.089	.184	.067	.114±.025	.108	.188	.156	.150	.151±.016
28	.138	.126	.242	.076	.146±.035	.151	.192	.186	.161	.173±.010
Days after reinfection										
2	.189	.131	.235	.052	.152±.039	.187	.199	.148	.137	.168±.015
4	.186	.132	.237	.075	.158±.035	.151	.233	.102	.141	.157±.028
6	.127	.122	.160	.091	.125±.014	.095	.101	.122	.134	.113±.009
8	.131	.100	.171	.066	.117±.022	.040	.141	.108	.091	.095±.021
10	.092	.068	.138	.050	.087±.019	.060	.081	.119	.091	.088±.012
12	.080	.120	.172	.058	.108±.025	.044	.070	.111	.088	.078±.014
14	.042	.187	.215	.050	.124±.045	.039	.074	.136	.118	.092±.021
16	.062	.198	.226	.071	.139±.042	.070	.064	.158	.097	.097±.022
18	.057	.188	.245	.060	.138±.047	.051	.076	.126	.106	.090±.017
20	.036	.189	.210	.069	.126±.043	.051	.061	.134	.192	.110±.033
22	.052	.166	.262	.070	.138±.049	.045	.063	.144	.186	.110±.033

TABLE 23.

Fractional catabolic rate of albumin F(CA) in Finn Dorset sheep.

Days after infection	Dorset HbA 57	58	59	62	Mean ±S.E.	Dorset HbB 3	40	54	61	Mean ±S.E.
22	.207	.083	.115	.147	.138±.026	.224	.185	.217	.197	.206±.009
24	.222	.100	.223	.230	.194±.031	.340	.172	.184	.244	.235±.038
26	.309	.088	.172	.174	.186±.046	.312	.219	.316	.192	.260±.032
28	.249	.100	.322	.256	.232±.047	.438	.359	.480	.276	.388±.045
Days after reinfection										
2	.348	.071	.351	.330	.275±.068	.316	.198	.552	.254	.330±.078
4	.518	.120	.398	.402	.360±.085	.390	.312	.731	.263	.424±.012
6	.335	.070	.414	.374	.298±.078	.432	.278	.171	.236	.279±.055
8	.347	.062	.412	.366	.297±.079	.496	.275	.520	.145	.359±.090
10	.283	.079	.500	.375	.309±.089	.517	.271	.618	.149	.389±.110
12	.165	.057	.430	.720	.343±.148	.593	.249	.693	.245	.445±.120
14	.300	.072	.400	.431	.300±.081	.629	.307	.601	.269	.452±.090
16	.219	.073	.466	.378	.284±.087	.511	.211	.601	.217	.385±.100
18	.302	.082	.451	.412	.312±.082	.508	.261	.537	.287	.398±.072
20	.304	.080	.393	.317	.274±.067	.434	.258	.514	.219	.356±.070
22	.338	.036	.317	*	.230±.097	*	.263	*	.242	.253±.010

* Died

TABLE 24.

Faecal plasma clearances (ml/day) of Scottish Blackface sheep.

Days after infection	Dorset HbA 57	58	59	62	Mean ±S.E.	Dorset HbB 3	40	54	61	Mean ±S.E.
22	25	61	43	40	42.3± 7.4	110	81	70	54	78.8±11.8
24	41	64	84	68	64.3± 8.9	168	88	111	54	105.3±24.0
26	57	46	55	92	62.5±10.1	220	59	119	101	124.8±34.1
28	41	36	88	71	59.0±12.4	246	57	141	164	152.0±38.9
Days after reinfection										
2	54	43	114	145	89.0±24.3	189	68	124	70	112.8±28.5
4	48	48	126	84	76.5±18.6	303	59	140	59	140.3±57.5
6	41	36	134	98	77.3±23.6	244	75	147	42	127.0±44.7
8	40	33	124	89	71.5±21.5	398	77	223	53	187.8±79.5
10	55	40	175	155	106.3±34.3	423	95	362	70	237.5±90.5
12	41	30	258	164	123.3±54.2	402	94	280	73	212.3±78.5
14	58	29	235	138	115±46.2	468	100	500	59	281.8±117.0
16	81	35	284	248	162.0±61.2	542	99	52	39	183.0±120.0
18	88	27	245	219	144.8±52.2	506	98	63	69	184.0±107.6
20	86	41	329	179	158.9±63.6	260	95	*	76	143.7±58.4
22	128	26	322	*	158.7±86.8	*	98	*	76	87.0±11.0

* Died

TABLE 25.

Faecal plasma clearances (ml./day) of Scottish Blackface sheep.

Days after infection	Blackface HbA 51	86	95	97	Mean ±S.E.	Blackface HbB 43	45	85	90	Mean ±S.E.
22	37	31	33	20	30.3± 3.6	20	32	37	48	34.3± 5.8
24	51	51	43	12	39.2± 9.3	25	35	29	58	36.8± 7.4
26	44	31	38	16	32.3± 6.0	24	43	36	49	38.0± 5.4
28	49	40	36	35	40.0± 3.2	22	53	38	67	45.0± 9.7
Days after reinfection										
2	53	36	32	27	37.0± 5.6	10	36	36	57	34.8± 9.6
4	33	42	31	20	31.5± 4.5	10	29	30	41	27.5± 6.4
6	26	19	20	9	18.5± 3.5	7	16	34	40	24.3± 7.7
8	28	15	15	6	16.0± 4.5	6	15	50	35	26.5± 9.9
10	17	26	19	7	17.3± 3.9	6	13	37	31	21.8± 7.3
12	25	16	21	6	17.0± 4.1	5	22	46	29	25.5± 8.5
14	16	18	41	10	21.3± 6.8	5	19	60	31	28.8±11.7
16	12	35	50	13	27.5± 9.2	8	19	37	30	23.5± 6.4
18	21	16	68	7	28.0±13.6	11	13	22	24	17.5± 3.2
20	16	32	80	13	35.3±15.5	11	18	41	26	24.0± 6.4
22	18	32	61	12	30.8±10.9	10	18	38	23	22.3± 5.9

TABLE 26.

Haematological indices of challenge controls.

Packed cell volumes (%)

		Sheep No.	Days before infection 0	7	14	21	28	Days after infection 7	14	21	23
Blackface	HbA	1	30	30	30	30	29	24	22	18	19
	HbAB	65									
Mean			30.5	30.5	31.0	31.5	28.5	25.0	22.0	17.0	17.0
S.E.			0.5	0.5	1.0	1.5	0.5	1.0	-	1.0	2.0
Dorset	HbA	52	30	30	30	30	27	25	22	19	13
	HbB	56	29	29	28	30	28	25	20	13	10
Mean			29.5	29.5	29.0	30.0	27.5	25.0	21.0	16.0	11.5
S.E.			0.5	0.5	1.0	-	0.5	-	1.0	3.0	1.5

Haemoglobin concentrations (g%)

Blackface	HbA	1	9.7	9.7	9.7	8.8	8.9	8.2	8.0	6.2	6.2
	HbAB	65	12.0	12.0	12.0	10.5	10.5	9.5	8.5	4.1	4.1
Mean			10.9	10.9	10.9	9.7	9.7	8.9	8.3	5.2	5.2
S.E.			1.2	1.2	1.2	0.9	0.8	0.7	0.3	1.1	1.1
Dorset	HbA	52	9.9	9.9	9.9	8.8	8.8	8.4	6.7	3.9	3.1
	HbB	56	10.1	10.0	10.0	9.7	9.3	8.6	7.6	5.1	4.7
Mean			10.0	10.0	10.0	9.3	9.1	8.5	7.2	4.5	3.9
S.E.			0.1	0.1	0.1	0.5	0.3	0.1	0.5	0.6	0.8

Red cell counts ($x10^6$/cu.mm)

Blackface	HbA	1	9.1	9.0	8.6	7.9	7.8	7.2	6.9	4.7	4.3
	HbAB	65	9.8	10.2	10.2	10.0	8.6	8.5	7.8	3.7	3.3
Mean			9.5	9.6	9.4	9.0	8.2	7.9	7.4	4.2	3.8
S.E.			0.4	0.6	0.8	1.0	0.4	0.7	0.5	0.5	0.5
Dorset	HbA	52	9.0	9.5	8.7	8.5	7.8	7.2	5.8	2.7	2.3
	HbB	56	8.9	8.9	9.0	10.5	7.8	7.2	6.1	4.0	3.7
Mean			9.4	9.2	8.9	9.5	7.8	7.2	6.0	3.4	3.0
S.E.			0.5	0.3	0.2	1.0	-	-	0.2	0.7	0.7

TABLE 27.

Red cell indices of challenge controls.

Mean corpuscular volumes (μ^3)

		Sheep No.	Days before infection 0	7	14	21	28	Days after infection 7	14	21	23
Blackface	HbA	1	32.9	33.3	34.9	37.9	36.8	33.4	31.9	38.5	44.6
	HbAB	65	32.0	30.3	31.4	29.4	33.3	30.6	28.4	42.0	45.2
Mean			32.5	31.8	33.2	33.7	35.1	32.0	30.2	40.3	44.9
S.E.			0.5	1.5	1.8	4.3	1.8	1.4	1.8	1.8	1.3
Dorset	HbA	52	30.6	31.6	34.5	35.1	31.5	34.9	34.5	45.1	44.2
	HbAB	56	32.4	32.5	31.1	28.5	35.9	34.6	36.3	34.9	34.8
Mean			31.5	32.1	32.8	31.8	33.7	34.8	35.4	40.0	39.5
S.E.			0.9	0.5	1.7	3.3	2.2	0.2	0.9	5.1	4.7

Mean corpuscular haemoglobin concentrations (%)

Blackface	HbA	1	32.0	32.3	32.4	29.2	30.8	34.2	36.5	34.3	32.3
	HbAB	65	38.0	38.7	37.3	31.9	44.3	36.5	38.6	25.5	27.3
Mean			35.0	35.5	34.9	30.6	37.6	35.4	37.6	29.9	29.8
S.E.			3.0	3.2	2.5	1.4	6.8	1.2	1.1	4.4	2.5
Dorset	HbA	52	40.0	40.0	33.1	28.9	36.8	33.5	33.7	32.5	30.8
	HbAB	56	35.0	34.8	35.9	32.5	34.6	34.3	34.6	36.4	35.9
Mean			37.5	37.4	34.5	30.7	35.7	33.9	34.2	34.5	33.4
S.E.			2.5	2.6	1.4	1.7	1.1	0.4	0.5	2.0	2.6

TABLE 28.

Serum protein and iron concentrations of challenge controls.

		Sheep No.	Days before infection 0	7	14	21	28	Days after reinfection 7	14	21
Total proteins (g%)										
Blackface	HbA	1	6.2	6.4	5.8	6.1	6.2	6.0	6.1	5.6
	HbAB	65	5.8	5.4	5.9	6.6	6.5	6.3	5.7	3.9
Mean			6.0	5.9	5.9	6.4	6.4	6.2	5.9	4.8
S.E.			0.2	0.5	0.1	0.3	0.2	0.2	0.2	0.9
Dorset	HbA	52	6.2	6.6	6.2	5.9	6.1	6.0	6.0	4.8
	HbAB	56	6.7	6.6	6.0	5.7	5.9	5.9	5.7	5.5
Mean			6.5	6.6	6.1	5.8	6.0	6.0	5.9	5.2
S.E.			0.3	-	0.1	0.1	0.1	0.1	0.2	0.4
Albumin (g%)										
Blackface	HbA	1	3.4	3.6	3.3	3.3	3.5	3.5	3.8	3.5
	HbAB	65	3.7	3.4	3.6	3.5	3.3	3.2	2.7	1.7
Mean			3.6	3.5	3.5	3.4	3.4	3.4	3.3	2.6
S.E.			0.2	0.2	0.2	0.1	0.1	0.2	0.6	0.9
Dorset	HbA	52	3.6	3.3	3.2	3.4	3.0	3.0	3.0	2.4
	HbAB	56	3.8	3.5	3.6	3.3	3.2	2.9	3.1	2.8
Mean			3.7	3.4	3.4	3.4	3.1	3.0	3.1	2.6
S.E.			0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.2
Globulins (g%)										
Blackface	HbA	1	2.8	2.8	2.5	2.8	2.7	2.5	2.3	2.1
	HbAB	65	2.1	2.0	2.3	3.1	3.2	3.1	3.0	2.2
Mean			2.5	2.4	2.4	3.0	3.0	2.8	2.7	2.3
S.E.			0.4	0.4	0.1	0.2	0.3	0.3	0.4	0.1
Dorset	HbA	52	3.4	3.3	3.0	2.5	3.4	3.0	3.0	2.4
	HbAB	56	2.9	3.1	2.4	2.4	2.7	3.0	2.6	2.7
Mean			3.2	3.2	2.7	2.5	3.1	3.0	2.8	2.6
S.E.			0.3	0.1	0.3	0.1	0.4	-	0.2	0.2
Iron (µg%)										
Blackface	HbA	1	154	168	141	187	149	131	123	120
	HbAB	65	163	147	131	138	128	132	121	87
Mean			159	158	136	163	139	132	122	104
S.E.			4	11	5	25	11	0.5	1	17
Dorset	HbA	52	137	122	118	116	163	116	149	108
	HbAB	56	186	207	170	202	163	165	151	110
Mean			162	165	144	159	163	141	150	109
S.E.			25	43	26	43	-	25	1	1

TABLE 29.

Faecal Egg Output ($x10^6$) of Challenge Controls.

Days after infection	Scottish Blackface HbA 1	Scottish Blackface HbAB 65	Scottish Blackface Mean ±S.E.	Finn Dorset HbA 52	Finn Dorset HbAB 56	Finn Dorset Mean ±S.E.
16	N	N	N	N	N	N
17	N	0.2	0.1±0.1	0.1	N	0.1±0.1
18	N	0.6	0.3±0.3	3.0	4.2	3.6±0.6
19	2.6	3.2	2.9±0.3	7.5	15.6	11.6±4.1
20	7.8	7.9	7.9±0.1	5.9	29.6	17.8±11.9
21	14.4	15.8	15.1±0.7	15.7	30.2	23.0±7.2
22	10.0	17.0	13.5±3.5	24.6	47.8	36.2±11.6
23	17.5	19.8	18.7±1.2	29.2	54.5	41.9±12.7

TABLE 30.

Faecal Red Cell "Clearances" (ml/day) of Challenge Controls.

Days before infection	Scottish Blackface HbA 1	HbAB 65	Mean ±S.E.	Finn Dorset HbA 52	HbAB 56	Mean ±S.E.
22	0.7	0.8	0.8±0.1	0.9	0.8	0.9±0.1
20	0.6	0.9	0.8±0.2	1.1	0.7	0.9±0.2
18	0.3	1.2	0.8±0.5	1.5	0.8	1.2±0.4
16	0.4	1.7	1.1±0.7	0.7	0.6	0.7±0.1
14	0.4	1.3	0.9±0.5	1.4	0.8	1.1±0.3
12	0.8	1.2	1.0±0.2	1.1	0.5	0.8±0.3
10	0.6	0.9	0.8±0.2	0.8	0.9	0.9±0.1
8	0.5	0.6	0.6±0.1	0.8	0.8	0.8±0.0
6	0.4	0.7	0.6±0.2	0.6	0.9	0.8±0.2
4	0.5	0.9	0.7±0.2	0.9	1.0	1.0±0.1
2	0.5	1.0	0.8±0.3	0.8	0.9	0.9±0.1
0	0.4	0.6	0.5±0.1	0.9	0.7	0.9±0.1
Days after infection						
2	0.5	0.9	0.7±0.2	0.8	1.0	0.9±0.1
4	0.5	1.2	0.9±0.4	0.9	0.9	0.9±0.0
6	0.5	0.7	0.6±0.1	0.6	1.0	0.8±0.2
8	0.6	1.3	1.0±0.4	1.2	1.9	1.6±0.4
10	1.2	0.8	1.0±0.2	2.0	5.1	3.6±1.6
12	12.2	17.5	14.9±2.7	19.2	23.7	21.5±2.3
14	13.3	20.0	16.2±3.9	29.3	40.5	34.9±5.6
16	27.0	27.8	27.4±0.4	43.7	55.0	49.4±5.6
18	35.4	37.2	36.3±0.9	43.5	56.2	49.9±6.4
20	33.0	47.3	40.2±7.2	44.5	63.4	54.0±9.5
22	37.2	56.1	46.7±9.5	48.5	62.7	55.6±7.1

APPENDIX 4

The Influence of Haemoglobin Type on the Response of Scottish Blackface Sheep to Infection with 100,000 *O. circumcincta* Larvae.

TABLE 1.

Packed cell volumes (%) after infection with 100,000 *O. circumcincta* larvae.

Sheep No.	Hb Type	Days after infection 0	7	14	16
33	A	36.0	35.0	35.0	32.5
34	A	36.0	38.0	35.0	34.5
35	A	35.0	32.0	32.0	30.0
37	A	33.0	36.0	37.0	34.5
40	A	35.0	34.0	33.0	32.5
Mean		35.0	35.0	34.4	32.8
S.E.		0.5	1.0	0.9	0.8
31	B	27.0	27.0	29.0	27.0
36	B	28.0	28.0	28.0	27.5
38	B	30.0	32.0	34.0	30.0
39	B	32.0	30.0	29.0	29.5
Mean		29.3	29.3	30.0	28.5
S.E.		1.1	1.1	1.4	0.7
P		<0.01	<0.01	<0.05	<0.01

TABLE 2.

Total serum protein (g %) after infection with 100,000 *O. circumcincta* larvae.

Sheep No.	Hb Type	Days after infection 0	7	10	14
33	A	6.6	7.0	6.7	6.6
34	A	6.9	7.2	6.7	6.1
35	A	7.0	7.4	6.9	6.7
37	A	6.9	8.0	7.1	7.3
40	A	7.2	7.2	7.0	7.1
Mean		6.9	7.4	6.9	6.8
S.E.		0.1	0.2	0.1	0.2
31	B	8.0	6.5	6.9	6.4
36	B	6.0	6.2	6.1	5.8
38	B	7.5	8.3	9.1	8.8
39	B	6.6	6.6	6.7	7.0
Mean		7.0	6.9	7.2	7.0
S.E.		0.5	0.5	0.7	0.6
P		N.S.	N.S.	N.S.	N.S.

TABLE 3.

Serum albumin (g %) after infection with 100,000 *O. circumcincta* larvae.

Sheep No.	Hb Type	Days after infection 0	7	10	14
33	A	3.5	3.6	3.8	2.8
34	A	3.7	3.5	2.6	3.2
35	A	3.3	3.1	3.3	2.5
37	A	3.3	2.9	3.2	2.8
40	A	2.9	2.6	2.5	2.6
Mean		3.4	3.1	3.1	2.8
S.E.		0.1	0.2	0.2	0.1
31	B	3.2	3.3	2.8	2.3
36	B	2.7	2.5	2.5	2.0
38	B	3.3	2.9	2.4	2.2
39	B	3.6	3.8	3.2	3.0
Mean		3.2	3.1	2.7	2.4
S.E.		0.2	0.3	0.2	0.2
P		N.S.	N.S.	N.S.	N.S.

TABLE 4.

Serum globulins (g%) after infection with 100,000 *O. circumcincta* larvae.

Sheep No.	Hb Type	Days after infection 0	7	10	14
33	A	3.1	3.4	2.9	3.8
34	A	3.2	3.7	4.1	2.9
35	A	3.7	4.3	3.6	4.2
37	A	3.6	5.1	3.9	4.5
40	A	4.3	4.6	4.5	4.5
Mean		3.6	4.2	3.8	4.0
S.E.		0.2	0.3	0.3	0.3
31	B	4.8	3.2	4.1	4.1
36	B	3.3	3.7	3.6	3.8
38	B	4.2	5.4	6.7	6.6
39	B	3.0	2.8	3.5	4.0
Mean		3.8	3.8	4.5	4.6
S.E.		0.4	0.4	0.8	0.7
P		N.S.	N.S.	N.S.	N.S.

TABLE 5.

Albumin:Globulin ratios after infection with 100,000 O. circumcincta larvae.

Sheep No.	Hb Type	Days after infection 0	7	10	14
33	A	1.1	1.1	1.3	0.7
34	A	1.2	0.9	0.6	1.1
35	A	0.9	0.7	0.9	0.6
37	A	0.9	0.6	0.8	0.6
40	A	0.7	0.6	0.6	0.6
Mean		1.0	0.8	0.8	0.7
S.E.		0.1	0.1	0.1	0.1
31	B	0.7	1.0	0.7	0.6
36	B	0.8	0.7	0.7	0.5
38	B	0.8	0.5	0.4	0.4
39	B	1.2	1.4	0.9	0.8
Mean		0.9	0.9	0.7	0.6
S.E.		0.1	0.2	0.1	0.1
P		N.S.	N.S.	N.S.	N.S.

TABLE 6.

Plasma pepsinogen levels (mU Tyrosine) after infection with 100,000 O. circumcincta larvae.

Sheep No.	Hb Type	Days after infection 0	4	7	10	14	16
33	A	2000	3100	4000	6500	8000	8900
34	A	1300	1200	2100	2500	3700	4500
35	A	1300	2700	2400	3400	4500	5000
37	A	1700	2400	2500	2000	5000	7200
40	A	1500	1400	1600	3300	6100	7200
Mean		1560	2160	2520	3540	5460	6560
S.E.		130	370	400	780	750	800
31	B	1900	2300	2500	5100	6000	7700
36	B	900	1100	900	1200	2550	6350
38	B	380	380	400	1200	2400	2500
39	B	1800	2300	2400	3000	3200	5000
Mean		1245	1520	1550	2620	3540	5400
S.E.		360	470	530	930	840	1100
P		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

TABLE 7.

Apparent half-life ($t_{\frac{1}{2}}$) of ^{125}I-labelled albumin after infection with 100,000 O. circumcincta larvae.

Sheep No.	Hb Type	$t_{\frac{1}{2}}$ (hr)
33	A	439
34	A	545
35	A	531
37	A	441
40	A	619
Mean		515
S.E.		34
31	B	445
36	B	403
38	B	309
39	B	482
Mean		409
S.E.		37
P		N.S.

TABLE 8.

Fractional catabolic rate of albumin (F(CA)) after infection with 100,000 *O. circumcincta* larvae.

Days after infection	Hb.A Group 33	34	35	37	40	Mean (±S.E.)	Hb.B Group 31	36	38	39	Mean (±S.E.)	P
0	0.058	0.067	0.054	0.056	0.059	0.059 ± 0.002	0.041	0.029	0.067	0.047	0.046 ± 0.008	N.S.
1	0.073	0.054	0.058	0.031	0.081	0.059 ± 0.009	0.059	0.054	0.067	0.071	0.063 ± 0.004	N.S.
2	0.075	0.048	0.079	0.047	0.077	0.065 ± 0.007	0.038	0.054	0.068	0.083	0.061 ± 0.010	N.S.
3	0.072	0.041	0.083	0.076	0.092	0.073 ± 0.009	0.102	0.057	0.081	0.069	0.077 ± 0.010	N.S.
4	0.078	0.063	0.069	0.057	0.073	0.068 ± 0.004	0.069	0.061	0.086	0.073	0.072 ± 0.005	N.S.
5	0.085	0.051	0.081	0.088	0.057	0.072 ± 0.008	0.045	0.070	0.089	0.056	0.065 ± 0.009	N.S.
6	0.070	0.044	0.070	0.048	0.068	0.060 ± 0.006	0.055	0.058	0.046	0.064	0.056 ± 0.004	N.S.
7	0.071	0.042	0.071	0.050	0.067	0.060 ± 0.006	0.056	0.063	0.068	0.076	0.066 ± 0.004	N.S.
8	0.083	0.063	0.062	0.090	0.062	0.072 ± 0.006	0.048	0.070	0.081	0.080	0.070 ± 0.008	N.S.
9	0.054	0.055	0.059	0.054	0.058	0.056 ± 0.001	0.043	0.045	0.054	0.061	0.051 ± 0.004	N.S.
10	0.054	0.053	0.071	0.059	0.072	0.062 ± 0.004	0.034	0.053	0.072	0.065	0.056 ± 0.009	N.S.
11	0.082	0.040	0.033	0.060	0.060	0.055 ± 0.009	0.049	0.079	0.078	0.054	0.065 ± 0.008	N.S.
12	0.048	0.064	0.070	0.059	0.045	0.057 ± 0.005	0.071	0.057	0.075	0.061	0.066 ± 0.004	N.S.
13	0.062	0.043	0.035	0.029	0.088	0.051 ± 0.011	0.050	0.050	0.080	0.044	0.056 ± 0.008	N.S.
14	0.062	0.063	0.055	0.059	0.043	0.056 ± 0.004	0.052	0.064	0.074	0.061	0.063 ± 0.005	N.S.
15	0.059	0.035	0.075	0.067	0.063	0.060 ± 0.007	0.052	0.079	0.064	0.060	0.064 ± 0.006	N.S.
16	0.043	0.046	0.066	0.036	0.067	0.052 ± 0.006	0.033	0.087	0.079	0.059	0.069 ± 0.014	N.S.

TABLE 9.

Faecal plasma clearances (ml/day) after infection with 100,000 *O. circumcincta* larvae.

Days after infection	Hb.A Group					Mean (±S.E.)	Hb.B Group				Mean (±S.E.)	P
	33	34	35	37	40		31	36	38	39		
0	53.5	39.4	47.6	46.5	54.2	48.2 ± 2.7	29.7	45.7	44.9	50.0	42.6 ± 4.4	N.S.
1	72.2	27.5	65.8	56.0	50.9	54.5 ± 7.7	37.5	70.5	45.5	53.5	51.8 ± 7.1	N.S.
2	76.0	30.6	70.7	45.2	54.1	55.3 ± 8.3	35.4	62.2	53.5	64.8	54.0 ± 6.6	N.S.
3	67.2	30.3	65.5	55.0	63.7	56.3 ± 6.8	33.0	78.6	61.7	56.4	57.4 ± 9.4	N.S.
4	56.4	37.8	70.4	62.2	57.6	56.9 ± 5.4	39.3	62.8	57.6	52.4	53.0 ± 5.0	N.S.
5	65.5	34.3	64.5	52.9	34.7	50.4 ± 6.9	39.5	62.4	55.2	54.1	52.8 ± 4.8	N.S.
6	65.0	48.6	54.1	46.3	34.8	49.8 ± 4.9	45.9	69.9	53.3	59.1	57.1 ± 5.1	N.S.
7	62.6	43.6	53.5	59.6	47.9	53.4 ± 3.5	46.9	70.5	57.0	52.1	56.6 ± 5.1	N.S.
8	61.6	44.5	54.2	39.5	46.7	49.3 ± 3.9	36.8	73.2	58.5	64.6	58.3 ± 7.8	N.S.
9	55.0	37.4	46.4	40.3	49.0	45.6 ± 3.1	43.6	62.0	59.8	54.9	55.1 ± 4.1	N.S.
10	56.4	38.4	48.7	34.0	56.2	46.7 ± 4.6	37.1	70.4	57.3	61.6	56.6 ± 7.0	N.S.
11	58.1	33.0	52.8	37.9	44.2	45.2 ± 4.6	36.4	64.1	62.3	54.8	54.4 ± 6.3	N.S.
12	65.6	44.5	45.8	45.5	67.3	53.7 ± 5.2	34.4	70.2	66.7	60.2	57.9 ± 8.1	N.S.
13	58.1	49.7	45.3	46.0	64.5	52.7 ± 3.7	42.6	110.3	71.3	64.9	72.3 ±14.0	N.S.
14	51.8	43.6	43.6	43.9	55.1	47.6 ± 2.5	38.3	92.6	71.9	51.9	63.7 ±11.9	N.S.
15	62.2	45.0	55.5	40.1	59.0	52.4 ± 4.2	36.5	92.1	76.3	46.5	62.9 ±12.9	N.S.
16	59.3	41.2	42.7	32.4	45.2	44.2 ± 4.4	34.9	107.6	71.6	44.1	64.6 ±16.3	N.S.

APPENDIX 5

Influence of Haemoglobin Type and Breed on the Immune Response of Sheep to Non-Parasitic Antigens.

TABLE 1.

The half-lives ($t_{\frac{1}{2}}$) of ^{125}I-labelled horse gamma globulin in Blackface and Finn Dorset sheep of different Hb type.

	Sheep No.	Half-life ($t_{\frac{1}{2}}$) Primary Injection (hr)	Secondary injection (hr)
Blackface HbA	51	354	44
	86	226	73
	95	309	311
	97	351	254
Mean		285	171
S.E.		29	66
Blackface HbB	43	167	174
	45	267	48
	85	92	330
	90	264	362
Mean		198	229
S.E.		42	73
Dorset HbA	52	490	82
	57	229	56
	58	362	320
	59	83	152
Mean		291	153
S.E.		87	59
Dorset HbB	40	84	55
	54	432	233
	56	94	338
	61	111	46
Mean		180	168
S.E.		84	71

TABLE 2.

Haemagglutinin titres in sera of Blackface sheep of different haemoglobin types immunised with rabbit r.b.c's.

	Sheep No.	Days after last injection 7	14	21	28
	33	160	80	20	20
	34	40	10	20	20
	35	1280	640	640	320
	37	1280	320	160	160
Blackface HbA	40	1280	640	640	320
	51	1280	640	*	320
	86	640	640	*	160
	95	1280	320	*	160
	97	1280	640	*	320
Mean		947	437	296	200
S.E.		175	87	143	42
	31	640	160	40	20
	32	640	160	160	160
	36	1280	640	640	320
	39	640	640	320	40
Blackface HbB	43	640	640	*	80
	45	640	320	*	160
	85	1280	320	*	160
	90	640	160	*	160
Mean		800	380	290	138
S.E.		105	80	130	33
p		N.S.	N.S.	N.S.	N.S.

* Missing values

TABLE 3.

Antigen binding capacity (33% end-point) of sera of Blackface sheep of different haemoglobin types after a course of injections of human serum albumin.

	Sheep No.	Days after last Injection 7	14	21	28
	33	0.4	2.0	1.2	1.1
	34	1.0	2.0	1.6	0.8
	35	2.5	2.4	2.2	2.0
	37	0.6	0.7	0.6	0.5
Blackface HbA	40	1.3	1.7	1.5	1.2
	51	4.5	9.1	*	5.2
	86	2.6	9.5	*	3.8
	95	1.4	1.3	*	0.7
	97	1.6	6.1	*	3.3
Mean		1.8	3.9	1.4	2.1
S.E.		0.4	1.2	0.3	0.6
	31	0.1	0.1	0.2	0.1
	32	0.1	0.2	0.4	0.8
	36	0.1	0.1	0.1	0.1
	39	1.1	1.4	1.0	0.8
Blackface HbB	43	4.3	3.8	*	2.9
	45	1.0	1.0	*	0.4
	85	0.4	0.9	*	0.7
	90	0.8	0.6	*	0.3
Mean		1.0	1.0	0.4	0.8
S.E.		0.5	0.4	0.2	0.3
p		N.S.	<0.05	<0.05	N.S.