RADIOISOTOPIC TECHNIQUES IN THE STUDY OF ANAEMIA

by

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CONTENTS.

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SUMMARY.

The thesis consists of six reviews and a dissertation on the research project.

In Chapter I various aspects of the epidemiology and pathogenesis of haemonchosis are reviewed. The disease is highly seasonal being more prevalent during the warm humid months, although chronic infections can occur almost all the year round. The parasites suck blood causing haemorrhage into the host's abomasum and the pathogenesis of the disease depends on this blood loss. Factors such as age, breed, haemoglobin type and nutritional status of the host influence the pathogenesis of the disease.

The pathogenesis of bovine trypanosomiasis which depends on factors such as anaemia, pathological lesions and immunodepression is described in Chapter II. Although the anaemia is classified as haemolytic, the precise mechanism appears multi-factorial.

In Chapter III the use of radiochromium (^{51}Cr) and radioiron (^{59}Fe) in studying the mechanism of anaemias of parasitic infections is examined. Red cells labelled with ^{51}Cr provide information regarding the contribution made by haemodilution and increased erythrocyte breakdown while ^{59}Fe may be used to monitor the animals'capacity to synthesise red cells.

In the study of albumin metabolism in parasitised animals, radioiodine (^{125}I or ^{131}I) is often employed (Chapter IV). ^{125}I or ^{131}I-labelled polyvinylpyrrolidone (PVP) or ^{51}Cr (as $^{51}CrCl_3$) are the isotopes of choice in providing information regarding the aetiology of gastrointestinal albumin losses in parasitic infections.

In/

In Chapter V most parasitic infections are shown to upset the nutritional status of the host through depression of food intake, disturbances of post-absorptive N and energy metabolism and poor feed conversion efficiency. Adequately fed hosts develop immunity earlier which also persists longer than their poorly fed counterparts.

In Chapter VI the limited value of conventional methods of control of haemonchosis and trypanosomiasis is highlighted. Similarly, the limited success of vaccination trials is also discussed.

In Chapter VII the results of a project carried out to determine the effects of different levels of protein intake on the pathophysiology of acute haemonchosis and on the self-cure phenomenon are presented. Animals on the high protein diet performed better than their poorly fed counterparts as judged by a variety of parasitological, haematological and radioisotopic criteria. The better performance of this group was concluded to reflect a better immune response against the parasite. The nature of this immune response was not determined but was thought to act either against parasite establishment or through suppression of egg laying capability of the female worms. A slight fall in total egg production was observed following reinfection. However pathophysiological changes following reinfection were as severe as recorded following primary infection with the low protein group being more severely affected.

CHAPTER I

EPIDEMIOLOGY AND PATHOGENESIS OF HAEMONCHOSIS

EPIDEMIOLOGY AND PATHOGENESIS OF HAEMONCHOSIS.

Introduction:

Haemonchosis in its classical form has been described as an acute syndrome characterised by severe anaemia, hypoalbuminaemia, sudden death and weight loss associated with the rapid acquisition of a large worm burden during periods of warm wet weather (Veglia, 1915; Fourie, 1931; Andrews, 1942; Gordon, 1950; Soulsby, 1965). Anaemia is probably the most important manifestion of haemonchosis.

Initially the anaemia is normocytic and normochromic but in heavy or long standing infections it becomes macrocytic and hypochromic with low serum iron levels and depressed iron stores (Dargie, 1973; Dargie and Allonby, 1975). Allonby (1973) and Allonby and Urquhart (1975) drew attention to a previously unrecognised chronic syndrome encountered in natural cases of the disease and characterised by persistent anaemia without obvious clinical signs, widespread incidence, very high morbidity and a progressive insidious weight loss not unlike malnutrition. They considered this form of the disease more important than the classical form economically.

Most of the literature deals with the disease in sheep, goats and cattle in decreasing order of susceptibility and ascribes its cause to either Haemonchus contortus or Haemonchus placei; both of which are trichostrongylid worms inhabiting the abomasum of their hosts. H. contortus is mainly a parasite of sheep and goats while H. placei infects cattle. There is no claim of absolute host specificity as H. contortus has been recovered from cattle and H. placei from sheep (Skryabin, Shikhobalova, Shults, 1954).

The disease has been recognised for a very long time in various parts of the world (Fitzsimmons, 1969; Edgar, 1933; Hall, 1936; Gordon, 1950; Lee, 1955; Adams and McKay, 1966; Fabiyi, 1970; Schillhorn van Veen, 1973; Allonby, 1973). Gordon (1950) attributed the importance of the disease to:-

(a)/

(a) the high biotic potential of the worm; The females lay 5,000 to 10,000 eggs per 24 hours which is higher than any other species;

(b) development of eggs to infective stage under optimal environmental conditions is rapid lasting only 5 days;

(c) the sudden onset of acute clinical disease and death in previously healthy flock, typically after a period of heavy rainfall;

(d) the relative lack of age resistance in the occurrence of the disease.

The disease is characteristically that of tropical and sub-tropical regions (Allonby and Urquhart, 1975) and in most of these areas is probably the most important disease of sheep economically (Schillhorn van Veen, 1973, 1978; Allonby and Urquhart, 1975). According to Allonby (1973) the disease may also present problems in the warmer temperate regions of Southern Europe and elsewhere.

The epidemiology and pathogenesis of the naturally occurring and experimentally produced disease in ruminants are reviewed forthwith.

A. Epidemiology:

Epidemiological studies in various parts of the world showed that larval challenge and faecal egg counts are highly seasonal.

Gordon (1948) in Australia regarded haemonchosis as a disease of the warmer months of the year, i.e. spring and summer. Later works by Gordon (1950) and Swan (1970) also in Australia confirmed the seasonal fluctuations of H. contortus eggs in the faeces of infected sheep and the periods when these eggs increased markedly. Such periods have rainfall above 2 ins. and temperature above 18°C. In England studies by Boag and Thomas (1971, 1973); Thomas and Boag (1972, 1973); Waller and Thomas (1975) and Thomas and Waller (1979) revealed that clinical haemonchosis was mainly a problem of the spring and summer months.

Observations/

Observations made in Nigeria, fairly typical of most tropical areas, by Lee, Armour and Ross (1960); Hart (1964); Fabiyi (1970); Okon and Enyenihi (1975) confirmed the seasonal nature of haemonchosis in ruminants and regarded the length of rainfall as the key factor in the pattern of infections. Outbreaks therefore occur during the rainy season lasting from 5 to 9 months of the year depending on the ecological zone. Lee, Armour and Ross (1960) postulated a monthly rainfall of 6 inches as being necessary for the development of the disease in the Savannah zone of Nigeria. This value is likely to be lower in the Southern forest zones due to differences in soil evaporation rate, leading to a longer period of heavy worm burden (Okon and Enyenihi, 1975). Allonby and Urquhart (1973, 1975) also confirmed this seasonal fluctuation in faecal egg counts, worm burdens and pasture larval contamination in the semi-arid regions of Kenya in East Africa. These variables were at their peak during periods of heavy rainfall with temperatures above 18°C. in mid-March to mid-June and between September to November. When they failed to find significant worm burdens in months with optimal environmental conditions, they concluded that factors other than rainfall and temperature were important in the development and availability of H. contortus larvae on herbage. Such factors were not determined. A deviation from this seasonal occurrence of haemonchosis was reported by Schillhorn van Veen (1978) in Northern Nigeria, when he observed clinical haemonchosis during the dry season in 6 weeks old lambs. He attributed this to the unusually high rainfall recorded at the time of birth of the lambs which produced a high level of pasture contamination during subsequent grazing. Similar observations were made by Gordon (1950); Connan (1975); Allonby and Urquhart (1975) when clinical haemonchosis was observed during period of high rainfall in 2 months old lambs.

The/

The seasonal occurrence of haemonchosis is closely linked with certain aspects of husbandry and management. Birth of offspring in temperate areas coincides with the season when the disease is at its peak (Gordon, 1950). The availability of susceptible age group, i.e. the lambs and the breeding ewe, coupled with the failure of the infective free living stage of the parasite to find optimum conditions during the cooler months of the year (Gordon, 1948), are further factors responsible for the seasonal occurrence of this disease. While this observation may be largely true in the drier parts of the tropics where there is a sharp distinction between the dry and rainy season and births restricted to particular periods, the more humid regions experience slightly different epizootiological conditions. In these regions the dry season is either short or absent and births are not restricted to any particular time of the year. What appears to be more important is the stocking density of pastures which determines the level of pasture contamination since the pastures are suitable for larval development and survival almost all the year round. This observation is borne out by the work of Okon and Enyenihi (1975) who found worm burdens in goats during the peak of the dry season in the forest zone (January) which are comparable to the burdens of those in the savannah zone during the peak of the rainy season (August).

The 'rain-rise' or 'spring-rise' in faecal egg count exhibited in haemonchosis has been described in several parts of the world (Proctor and Gibbs, 1968; Gibbs, 1973, 1977; Van Geldorp and Schillhorn van Veen, 1976; and Schillhorn van Veen and Ogunsusi, 1978). This rise was attributed by Gibbs (1973) to the resumed development of the hypobiotic 4th stage larvae of the parasite as a result of seasonal influence. This resumed development causes an increase in the adult helminth population which is detectable through increased parasite egg output. The rise coincides with the onset of rains or spring/

spring when pastures are optimal for larval development and there is availability of susceptible hosts, hence the name'spring-rise'or'rain-rise'. This rise in faecal egg counts is commonly associated with the peripaturient period in ewes (O'Sullivan and Donald, 1970; Michel, 1970; Connan, 1976; Schillhorn and Ogunsusi, 1978; Gibbs, 1977; Donald and Waller, 1973). Allonby and Urquhart (1975) could observe no such peripaturient effect. Michel (1974) and Connan (1976) concluded that there was a reduction in the turnover of helminths in lactating animals leading to a relative increase in worm burdens. This observed phenomenon coupled with the high fecundity of the worms was presumed to cause the observed rise in lactating ewes (Schillhorn van Veen and Ogunsusi, 1978). Once the hypobiotic larvae responsible for the spring-rise is established, parturition or lactation has no effect on its development since prolactin or diethylstilboestrol did not advance the onset of spring-rise in haemonchosis infected ewes (Blitz and Gibbs, 1972; Schillhorn van Veen and Ogunsusi, 1978).

'Self-cure' was first used by Stoll (1929) to describe a sudden fall in the faecal egg count occurring in lambs subjected to continual reinfection with Haemonchus contortus. Gordon (1948) showed that self-cure was a regular and consistent flock phenomenon in endemic areas and that it not only causes a dramatic fall in faecal egg count but led to expulsion of the adult worm burden of the affected sheep. Two widely different hypotheses were put forward to explain this phenomenon. Gordon (1948) on always observing this phenomenon following a period of rainfall, incriminated an'anthelmintic' factor in freshly growing pasture as the probable cause. Stewart (1950a, b, 1953) experimentally challenged sheep with infective larvae and observed self-cure which led him to believe that an "immediate-type hypersensitivity reaction" in the abomasal mucosa was responsible. The works of Gordon (1967); Lopez/

Lopez and Urquhart (1967); Allonby and Urquhart (1973) failed to find any immunological basis in explaining the phenomenon, as self-cure occurred in the absence of reinfection; was seen at exactly the same point in time in mature ewes and young lambs irrespective of their worm burdens and, more importantly, was not followed by obvious resistance. The only common factor to all these field cases was the onset of rains and this led Allonby and Urquhart (1973) to surmise that:-

(a) The shoot of freshly growing grass contains a substance toxic to Haemonchus;

(b) the sudden dietary change caused the abomasum to undergo certain physiological alterations, e.g. pH making existence intolerable for adult Haemonchus;

(c) an anthelmintic factor in new pasture could produce a hypersensitivity reaction, as described by Stewart (1953).

Their work thus largely validated the earlier observation by Gordon (1948). Dargie and Allonby (1975) produced self-cure in sheep following experimental reinfection. They used abomasal losses of ^{15}Cr-labelled red cells as a parameter of worm activity. Their work has led further credence to the "immediate type hypersensitivity reaction" theory which is said to be triggered off by the antigenic materials released by newly acquired larvae during the third ecdysis (Stewart, 1953; Soulsby and Stewart, 1960). They differentiated the classical self-cure where reinfection was not followed by protection and another form of self-cure which was followed by protection. The immunologic theory was further supported by the occurrence of self-cure and expulsion of worms within 7-10 days of reinfection accompanied by raised antibody levels, increased peristalsis and oedema of the abomasum. Stewart (1953, 1955) observed that H. contortus will cause self-cure of infections with other small intestinal/

intestinal and stomach worms and ascribed this to the passage of antigenic materials produced in the stomach, into the small intestine. Altaif and Dargie (1978b) showed that self-cure was not related to the haemoglobin phenotype though Allonby and Urquhart (1976) and Preston and Allonby (1979b) produced evidence to suggest that such a relationship existed in experimentally and naturally infected merinos. Whether induced by larvae under experimental conditions or by grass, studies by Altaif and Dargie (1978a,b); Preston and Allonby (1978, 1979a,b) showed that self-cure was more closely associated with the breed than the haemoglobin type of the animals concerned.

Two different mechanisms appear to operate in explaining the different conditions under which self-cure operated. The 'anthelmintic factor' for field cases and the 'immunogenic theory' for experimentally produced self-cure. Regardless of the mechanism involved, the whole concept of self-cure opens up a wide field for further fundamental work on host-parasite relationships. If the reaction is truly immunogenic, reminiscent of an allergic reaction, is it accompanied by any of the manifestations of an allergic reaction, such as production of histamine or related compounds as postulated by Stewart (1953)? Could self-cure be merely a useful biological adaptation by the parasite to ensure a turnover in its population or to protect it against extermination by protecting the host against an overwhelming and fatal infestation? Whatever phenomenon is involved, the importance of self-cure in natural field cases of ovine haemonchosis in regard to control measures cannot be over-emphasised.

Gibbs (1968) showed that certain helminths were able to arrest their development inside the host in the face of adverse conditions and resume their development when such conditions improve. This inhibited development in H. contortus infections has been reported frequently from various parts of the world/

world (Muller, 1968; Connan, 1968, 1971; Hart, 1964; Blitz and Gibbs, 1972a,b; Ayalew and Gibbs, 1973; McKenna, 1973; Brundson, 1973; Fabiyi, 1973; Van Geldorp and Schillhorn, 1976; Ogunsusi and Eysker, 1979; Ogunsusi, 1979). The epidemiological implication of this arrested development is that, with the arrival of favourable conditions, i.e. spring in temperate countries and rains in the tropics, pasture contamination becomes rapid and lambs are exposed to heavy build-ups. This could result in acute haemonchosis. In tropical countries, termination of arrested development occurs at the beginning of the rains, i.e. April (Ogunsusi, 1979) and in spring in temperate countries (Blitz and Gibbs, 1971).

The factors responsible for resumption of development of inhibited larvae are yet to be determined. Soulsby (1965) and Dineen et al (1965) suggested host resistance as a primary cause for inhibited development but Waller and Thomas (1975) and Eysker and Ogunsusi (1979) could find no evidence to support this when they observed no significant differences in pattern of inhibition between ewes, permanent lambs and tracer lambs. Blitz and Gibbs (1972a) put forward the diapause phenomenon as suggested for Ostertagia ostertagi by Armour (1970) when they found low numbers of arrested larvae in spring and summer. The subsequent autumn increase was attributed to seasonal changes in the larvae resulting from decreasing temperature or photoperiod and that these developing larvae trigger a 'self-cure' like reaction in the host. This immunological response was said to be temporarily suppressed in lactating ewes allowing the worms to develop to patency, producing the characteristic rise in peripaturient faecal egg counts. Waller and Thomas (1975) working in North-east England, could find no evidence to support the diapause phenomenon when they recorded 100 per cent inhibition before any decline in temperature. They concluded that at least in that part of England, inhibition is the normal state, the parasite spending the major part of its life-cycle in the host and a short period on pasture, i.e. one generation per year. McKenna (1974) in New Zealand/

Zealand attributed differences in his results from that of Blitz and Gibbs (1972a) to the non-reproductive sheep used in his experiments, as opposed to the breeding ewes used by the Canadian workers. He also subscribed to the diapause phenomenon. Ogunsusi and Eysker (1979) in Nigeria and Connan (1975) suggested environmental stimuli as the factor responsible for an obligatory type of inhibition at the end of the rainy season enabling the parasite to survive the 6 months dry season. In view of these conflicting findings from different ecological zones, two possibilities need to be further examined. Firstly, the workers may be dealing with different strains of the parasite which have different generations per year and, therefore, different persistence intervals. Secondly, age resistance may be involved, as suggested by Waller and Thomas (1975).

Blitz and Gibbs (1971); Ogunsusi and Eysker (1979) noted that inhibited fourth stage larvae contained some characteristic rod-like crystals. These crystals were suggested to represent degenerative changes in the parasite which may be asoociated with retardation of development (Bird et al, 1978).

PATHOGENESIS.

The adult and immature stages of Haemonchus suck blood causing anaemia (Veglia, 1915; Bailey and Herlich, 1953). The pathogenesis of haemonchosis is centred on this blood loss anaemia. Various hypotheses have been advanced to explain the mode of pathogenesis. These range from the haemolytic anaemia postulated by Holman and Pattison (1941) and Schwartz(1921) to the aplastic anaemia noted by Neklyudov et al (1956) who propounded that substances produced by the parasite exerted a depressant effect on the central nervous system of the animal leading to a disturbance of the neuro-humoral regulation of haematopoiesis. However, the haemorrhagic anaemia concept first observed by Fourie (1931) and characterised by severe to chronic gastritis and subsequent anaemia appear to find most favour. This has largely been confirmed by the findings of Dargie and Allonby (1975) and other workers.

MORBIDITY AND MORTALITY.

The number of Haemonchus species required to produce illness or death under experimental conditions can be extremely variable. While Roberts (1957) was unable to produce haemonchosis in 3-4 month old worm-free calves infected with doses of larvae ranging from 50,000 to 200,000 given either singly or in spaced out intervals, Ross, Armour and Hart (1962) produced marked pathogenic effect following administration of 40,000 H. placei larvae given as a single dose to calves whose ages were not recorded. In sheep, Fourie (1931) produced anaemia with doses of larvae ranging from 3,000-20,600 and death when total number of larvae administered was 52,000. The ages of the sheep were not recorded. Andrews (1942) showed that 35,000 larvae given as a single dose to 3-month old lamb was fatal in 45 days and when dose of larvae was increased to 100,000, death occurred in 23 days. A single dose of 45,000 larvae given to 8 months old lambs did not prove fatal. This could be attributed to age resistance. Conway (1962) was able to prove that multiple doses of larvae given over a period of time produced a more severe reaction than a single dose.

Clinical/

Clinical Symptoms:

Patent infections produce similar clinical effects in both sheep and cattle though the disease is more common in sheep. Mayhew (1944) experimentally infected three to five month old calves with an unstated number of larvae and observed that there was loss of weight and anaemia. Fourie (1931) observed anaemia, tachypnoea, frequent pulse and pounding of the heart during a fatal haemonchosis in sheep while Gordon (1950) reported a fall in milk yield of ewes following the administration of 12,000 H. contortus larvae.

The classical acute haemonchosis was described by Soulsby (1965) as an anaemia and oedema caused by the rapid acquisition of larvae. Many workers have since drawn attention to a more widespread 'chronic syndrome' characterised by an insiduous loss of weight in which anaemia or oedema were not regular features (Allonby and Urquhart, 1975; Schillhorn van Veen, 1978; Eysker and Ogunsusi, 1980). A hyper-acute syndrome characterised by severe anaemia and dark coloured faeces prior to death has also been described (Allonby, 1973). Faecal egg counts vary from 0-40,000 in hyper-acute cases to 1,000 - 100,000 in acute cases and 200-2,000 in chronic cases (Allonby, 1973). Factors affecting egg production include:-

(a) resistance of sheep; (b) worm burden - larger worm burdens tend to have low rate of egg production;

(c) fasting - this decreases egg production; and

(d) anthelmintics (Gordon, 1950).

Haematology:

Haematological studies in Haemonchus infections has received a good deal of attention. The amount of blood each parasite removed from the abomasal mucosa was worked out by Martin and Clunies-Ross (1934); and Clark, Kies and Goby (1962) as 0.05 ml/worm/day. Assuming a worm burden of 2,000, Dargie (1973) pointed out this may be equivalent to 100 ml. of blood being lost daily into the abomasum.

Haematological values quoted by various workers differ from place to place. There is general agreement that the anaemia at one stage or the other is characterised by a fall in red blood cell numbers (RBC), packed cell volume (PCV) and haemoglobin concentration (Hb), while mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) showed an increase. There was considerable variation in white blood cell (WBC) and differential white cells values.

Values obtained from experimental infection of calves by Delaune and Mayhew (1943) revealed RBC values of 2.5×10^6 per cu.mm., and Hb values of 1.45 gm per cent. There was initial leucocytosis followed by a decrease to as low as 1.75×10^3 per cu.mm. Leucocyte differential counts did not follow any set pattern. Harness, Fitzsimmons and Sellwood (1970) gave three levels of infective larvae 50,000, 125,000 and 500,000 to calves and compared the observed blood picture with that of uninfected calves of similar age and breed. No changes were observed in 9-week old calves given 50,000 larvae and thus they concluded that calves of this age group can resist this level of infection. Those given heavier infections showed a normocytic macrocytic anaemia, the severity of which depending on the level of infection. The anaemia later became macrocytic and hypochromic which they attributed to increased demands on the erythropoietic tissues. Anosa (1978) observed the seasonal changes in the blood picture of White Fulani calves naturally exposed to helminthiasis caused mainly by Haemonchus. Lowest PCV values of 28.9 ± 5.7% was recorded in the late rainy season when helminth egg counts were maximal and the highest values of 35.8 ± 7.1% during the early rains when egg counts were moderate but pastures were at their optimum nutritionally. He also confirmed age resistance to the disease when he consistently obtained higher values in calves aged 0-3 month than in those between 3 and 12 months.

In/

In sheep the earliest experimental work on haematological changes was that of Fourie (1931) who infected sheep with H. contortus larvae and observed a progressive decrease in red cell count to as low as 1.6 x 10^6 per cu.mm., Hb from 82% to 10% and PCV from 34% to 5%. There was also marked bone marrow response leading him to characterise the anaemia as being haemorrhagic. Total and differential leucocyte counts followed no set pattern. Andrews (1942) largely confirmed the findings of Fourie but also recovered increasing amounts of blood from the faeces. He substituted jugular bleeding in an uninfected control to simulate the blood loss from the faeces and found the loss to be identical in both cases. The decrease in blood values was associated with increase in faecal egg count. He also confirmed the haemorrhagic nature of the anaemia produced on the basis of increased reticulocytosis. Charleston (1964) compared the haematology and bone marrow cytology of sheep experimentally infected with H. contortus with those of worm free sheep bled to simulate the blood loss of such an infection. He confirmed the earlier observation by Andrews (1942), though the anisocytosis of haemonchosis was less marked and bone marrow activity with distinct erythroid cells was greater in anaemic animals.

Ogunsusi (1978) and Anosa (1977) studied the haematological changes in Nigerian sheep of different age groups and suffering from acute and chronic haemonchosis. Blood values showed a rapid decline in RBC, PCV and Hb in those suffering the acute disease which was associated with a rapid increase in faecal egg count while these values declined slowly and steadily in those suffering the chronic form of the disease. By the third week, these values had dropped significantly in those suffering the acute disease while no significant drop was noticed by the seventh week in those suffering from chronic haemonchosis. Severe blood changes were observed in those of the 4-4½ and 5-6½mths.age group.Features of the anaemia included a high reticulocyte count in those suffering from severe anaemia, normochromic and normocytic/

normocytic response, age resistance as manifested by failure of 2 month old sheep to show severe symptoms and a positive correlation between worm burdens and severity of anaemia. Allonby and Urquhart (1975); Dargie and Allonby (1975) recorded similar findings in sheep grazing in an endemic area of Kenya. The latter workers combined radioisotopic, haematological and parasitological parameters to monitor simultaneously the pattern of erythrocyte loss and haemopoietic activity during the course of single and challenge infections with H. contortus. They infected 2 year old Merinos with 10,000 larvae and exposed them to a further challenge 7 to 8 weeks later. Marked haematological disturbances commenced between days 12-25 after infection with the PCV falling from 33% to 22% and subsequently remaining constant or increasing steadily. Haemorrhage was observed between days 7 to 10 after infection and increased in severity over the next 10-14 days. Despite this continuous haemorrhage, the PCV remained steady indicating an increased rate of red cell production. Evidence in support of this was obtained from the plasma iron turnover values which showed a rapid and progressive increase despite some depletion in the level of serum iron. The authors concluded that there were 3 stages in the pathogenesis of anaemia due to H. contortus. Stage 1 which was between days 7 and 25 post-infection and was characterised by a progressive and dramatic fall in PCV, low or negative faecal egg count and normal serum iron, was caused by the voracious blood sucking activity of the fifth stage larvae and the delay in the response of the host to the haemorrhage. During the second stage, egg counts may be significant, serum iron level was normal or slightly reduced while PCV values remained steady. The rate of red cell synthesis equal the rate of breakdown. The third stage was characterised by a dramatic fall in PCV with low serum iron concentrations due to frank iron deficiency. The rate of red cell breakdown exceeded the rate of synthesis. The authors finally concluded that PCV values and faecal egg counts were unreliable indices of the amount of blood being lost into the abomasum.

Following/

Following the observation of significant increases in plasma volume 2-3 weeks after infestation in sheep suffering from severe parasitism, Owens (1971) concluded that the infested sheep compensated for the reduction in red cell volume caused by parasitic blood loss by increasing the plasma volume.

Biochemistry:

Ross, Armour and Hart (1962) observed a fall in serum albumin levels 19 days after infection of calves with 40,000 larvae. Anderson, Gaff Hammond, Fitzgerald and Miner (1960) failed to observe any marked changes in serum protein in calves experimentally infected with 12,000 and 165,000 H. placei larvae. It is probable that the infection was not patent due to age resistance (ages not stated) or they used a less pathogenic strain of the parasite.

Evans, Blunt and Southcott (1963) administered a dose of 51,500 larvae to sheep 11 weeks after an initial dose of 4,000 larvae. The anaemia produced was characterised by a fall in PCV and Hb concentration followed by a marked rise in the concentration of potassium in the erythrocyte $\left[K_e^+\right]$ and a fall in the concentration of sodium $\left[Na_e^+\right]$; the total $\left[K_e^+ + Na_e^+\right]$ remained steady. Plasma potassium and sodium concentrations remained constant. They also produced evidence to show that sheep of haemoglobin type A harboured fewer worms than those with type B. Since young erythrocytes contain higher concentrations of potassium (Blunt and Evans, 1963) and the anaemia itself was characterised by a marked bone marrow response, the rise in $\left[K_e^+\right]$ observed could be explained by an influx of these young cells into the circulation (Evans et al, 1963; Blunt and Evans, 1963).

Christie, Brambell and Mapes (1967) reported a marked rise in abomasal pH in sheep which was associated with the emergence of H. contortus larvae from the mucosa 2-3 days after infection similar to the observation of Armour et al (1966)/

Armour et al (1966) and Jennings et al (1966)who observed a rise in abomasal pH due to heavy infestations with Ostertagia ostertagi. This rise in abomasal pH was also observed by Christie et al (1975) and Malczewski (1970). However, Christie et al (1978) using immunologically competent lambs investigated the effect of prolonged repeated (5 days a week) infection with 10,000 larvae of H. contortus for 6 months and found that at the end of the experiment all 7 sheep had acquired a high level of resistance which was not associated with an abnormally high gastric pH. The role of immunity to haemonchosis and development of elevated pH in the abomasum is not clear. Dargie (1973) stated that abomasal pH was not significantly elevated in haemonchosis but the experimental conditions did not include resistance to the disease. He also found that though plasma pepsinogen values may remain elevated as long as the animal is infected, the concentrations were not as high as those associated with ostertagiasis.

Body Weight:

Adverse nutritional conditions may influence acquired resistance to haemonchosis (Ross, 1932). Better fed animals are able to resist the disease (Ross and Gordon, 1936; Preston and Allonby, 1978) than their less well nourished colleagues. Whether this resistance is directed against worm establishment or to the effects of the disease remains to be determined.

Chronic haemonchosis causes an insidious weight loss (Allonby, 1973). There is a four-fold increase in albumin catabolic rate in animals suffering infections (Dargie, 1973). This increase causes mobilisation of tissue protein from areas such as the muscle to vital areas like the liver and bone marrow to maintain normal plasma proteins and haemoglobin. This mobilisation coupled with the failure to reabsorb all the nitrogen lost into the gut due to plasma leak were held responsible for the adverse effect of haemonchosis on body weight by Dargie (1973).

Immunity:/

Immunity:

This aspect of the pathogenesis of haemonchosis has received the most attention. Stoll (1929), while propounding the 'self-cure' theory, showed the development of an immunity to H. contortus in lambs exposed to infection over a period of time. Mayhew (1941) interpreted the sudden decrease in egg counts in calves giving varying doses of larvae over a considerable period as evidence of the development of an immune state, and stated 13 weeks as the lowest age at which immunity developed and 40 weeks as the maximum age.

Both Ross (1932) and Gordon (1950) in Australia observed that both aged ewes and wethers can be as heavily infected as lambs on the same pasture and while concluding that age resistance does not play any part in haemonchosis, nevertheless noted that lambs which had previous experience of infection were more resistant than lambs which had never encountered an infection. They then concluded that a temporary immunity was at play which soon disappeared in the absence of re-infection.

It would seem that immunological competence or responsiveness to haemonchosis is a function of age and the amount of functional antigen. Thus Manton et al (1962) gave 10,000 larvae to 2-4 months old Dorset Down lambs either as a single dose or over 60 days and failed to develop resistance to subsequent challenge, whereas the same regime of treatment conferred immunity in lambs 10-12 months old. Other works using X-irradiated larvae as immunising agents, have confirmed the immunological unresponsiveness of young sheep. Jarrett et al (1959; 1961); Urquhart et al (1966a) and Bitakaramire (1966) successfully immunised Scottish Blackface sheep aged 7-17 months with two doses of 10,000 X-irradiated larvae at 40 Kr. at one month intervals but attempts by Urquhart et al (1966b) to repeat this same technique in 5-12 week old lambs failed. Wilson and Samson (1974) were unsuccessful in using parenteral inoculations of larvae or somatic and metabolic extracts

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as antigens in sheep of $2\frac{1}{2}$-5 months of age, whereas Stoll (1956) reported high resistance in sheep of 7-12 months of age using the same type of antigens. Silvermann (1965) successfully used larval extracts to immunise 4-6 months old lambs but Nielsen (1975) could not. Reasons suggested for the failure to immunise younger sheep included interference with antigenic stimulation by colostrum acquired antibodies and immunogenic immaturity (Urquhart et al, 1966b).

Thus sheep become responsive at some time between 6-12 months of age but Lopez and Urquhart (1967) showed that this responsiveness can be jeopardised by infection acquired early in life. They successfully immunised adult two year old Merinos raised under worm-free conditions but not those raised in endemic areas. Dineen and Wagland (1966) showed that the unresponsiveness of those raised in endemic areas was not due to infection acquired early in life. They observed that the response of 7-8 months old Merinos and Border Leicester sheep given six sensitising infections 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 infections. When challenge was superimposed on existing infection, the challenge dose was established probably due to immunological exhaustion during the prolonged antigenic stimulation. Removal of the sensitising dose 8 days prior to challenge with an anthelmintic, led to recovery of immunological competence. Wagland and Dineen (1967) provided inconclusive evidence, using 3-4 months' old lambs, that the resistance was greater at 4 and 8 weeks after challenge than at 2 and 16 weeks after removal of sensitising dose suggesting an early immunologically latent period and rapid loss of resistance in the absence of reinfection. They thus suggested that anthelmintics should only be used on animals showing clinical signs of disease as they interfered with the development of resistance.

Donald/

Donald, Dineen and Adams (1969) could observe no detrimental effect of anthelmintics on resistance if the sensitising dose was allowed to persist for a considerable time (about 4 months) before challenge. Early removal of the sensitising dose (1½ months) led to a loss of immunity. They thus concluded that manifestations of resistance appear in a sequence which could be related to a product of larval dose and time and that strong resistance was associated with prolonged uninterrupted exposure.

The immunological unresponsiveness of lambs does not imply permanent susceptibility to haemonchosis for, in endemic areas, the disease is not so overwhelming that survivors do not exist. This suggests in part a degree of functional immunity. This was demonstrated by Christie et al (1964; 1966) who showed successful development of resistance in both 2 months and 7½ months old Scottish Blackface lambs when larval doses are interspersed with anthelmintics. Dineen et al (1965) also showed significant immunity in 2-3 months old Merinos by dosing them daily with 100 larvae for 30 days but if this dose of 3,000 was given singly no immunity was produced.

Mulligan et al (1961) showed surprisingly the development of solid immunity in Scottish Blackface lambs 7 months old given an initial dose of 2,000 attenuated larvae followed by a second dose of 5,000 whereas this vaccination regime failed to confer immunity in Merinos of the same age. This points to the existence of a fundamental difference in immunological competence of Merinos and Scottish Blackface sheep. This was further supported by the finding of Lopez and Urquhart (1967) who failed to protect 7 months old Merinos using the same schedule which protected Scottish Blackface sheep of similar ages. These findings imply a genetically determined resistance to haemonchosis. The reason for this unresponsiveness in lambs is unknown as lambs have been shown to develop both humoral and cell mediated responses/

responses to a wide variety of antigens (Silvermann and Prendergast, 1970; Cole and Morris, 1973) and are successfully immunised against a wide variety of bacterial diseases. Duncan, Smith and Dargie (1978) speculated that the unresponsiveness of young lambs may be due in part to a deficient production of mucus IgA and serum IgG antibodies when they failed to find significant levels of these antibodies in two months old lambs vaccinated and then challenged with 10,000 larvae. They concluded that young lambs may be incompetent in mounting a protective immune response against such complex nematode antigens or that the abomasum *is* slow to develop the capacity to produce IgA. Their work confirmed an earlier one by Smith (1977a and b) that adult sheep respond to H. contortus vaccination with the production of abomasal mucus IgA and serum IgG though their protective role was not confirmed. This association between mucus IgA and serum IgG antibodies and protection was subsequently demonstrated by Smith and Christie (1978; 1979). Globule leucocytes were found in large numbers in both resistant and susceptible sheep. They have been associated with prolonged exposure of the abomasum to parasites but their protective role has not been clarified.

The failure to resist infection following administration of anthelmintics, as proposed by Dineen and his colleagues (1966; 1967), was further supported by the works of Benitez-Usher et al (1977); and Altaif and Dargie(1978a,b)in Scotland, who showed that the successful immunisation schedule based on irradiated larvae broke down even in mature animals when the larvae were removed by the anthelmintic Thiabendazole at three weeks in between vaccinations. Similar observation was made by Ross (1963) in cattle infected with H. placei. They suggested that anthelmintics led to a cessation of antigenic stimulation leading to waning of immunity but disagreed with the recommendations of Dineen et al (1963) in that subclinical haemonchosis and reinfection on the field are equally important as the clinical disease (Allonby/

(Allonby, 1973), which Dineen et al failed to take into consideration. Recent work by Smith and Christie (1979) showed that the removal of vaccine worms one week prior to challenge with Levamisole did not affect the degree of protection conferred by immunisation, thus validating the earlier work of Donald, Dineen and Adams (1969) that strong resistance was associated with a prolonged period of uninterrupted exposure. Thus vaccine worms need a long period of uninterrupted exposure for resistance to be developed but once this immunity is obtained, the protective effects are dependent on other factors than a direct interaction between vaccine worms and challenge larvae. Factors such as the age of lambs at immunisation, which could produce a degree of immunological unresponsiveness, the relative nature of the sensitising infections and genetic influences could be important in this respect.

Genetic factors have been shown to operate in determining resistance between and within particular breeds of sheep enabling them to survive in areas where H. contortus is endemic (Evans, Blunt and Southcott, 1963; Evans and Whitlock, 1964; Jilek and Bradley, 1969). Heritable resistance to haemonchosis was also demonstrated by Whitlock (1958) and Ross, Lee and Armour (1959). If a genetic marker for host resistance could be determined, then breeding for host resistance could be important in the sheep industry of many tropical and sub-tropical countries where haemonchosis is a major problem. One such genetic marker is the haemoglobin genotype of which there are two different kinds, A and B, in the majority of normal adult sheep (Evans et al, 1956). The frequency is determined by two alleles which produce three phenotypes A, AB and B (Evans and Whitlock, 1964).

Evans/

Evans et al (1963) showed that HbA Merino sheep were more resistant than HbAB types under field conditions based on the lower egg counts and worm burden in the former than in the latter. No obvious differences in haematological indices were found. Evans and Whitlock (1964) linked the advantages of HbA sheep over HbAB and HbB types on the basis of their higher haematocrits in the face of infections. Jilek and Bradley (1969) used both haematological and egg count parameters to show that Hb type was implicated in both individual and breed resistance. The HbA sheep were more resistant than either the HbAB and HbB sheep and the higher survival of the Native Florida sheep than the imported Rambouillets was related to their higher frequency for HbA. Similar observations in Kenya by Allonby and Urquhart (1976) Preston and Allonby(1979a,b)based on parasitological evidence as well as on the frequency of self-cure demonstrated the advantages of HbA sheep over the other Hb types in an endemic area. The exact relationship between the Hb polymorphism and host parasite interactions is unknown but from the indirect evidence of variations in egg counts and haematological indices, the mechanism appeared to be related to variations in worm establishments. From the work documented, attempts to explain resistance to haemonchosis in terms of worm establishment have not been convincing. Thus Evans et al (1963) failed to record any haematological differences between the Hb types and even the worm burdens were recovered from animals which died of the infection and which must have lost a large proportion of their worm populations (Evans and Whitlock, 1964; Dargie and Allonby, 1975). Although Allonby and Urquhart (1975) and Preston and Allonby(1979a,b)linked 'self-cure' with immunological competence, they did not show if this competence also embraced reaction to reinfection. Radhakrishnan et al (1972), using standardised infections of H. contortus for comparing the response of animals with different Hb types, showed higher PCVs in HbA animals than in other types but egg counts and worm loads were actually/

actually higher in HbA than HbAB and HbB sheep. This led to widespread speculation of the exact importance of HbA. Its importance probably rests on its ability to limit persistence of infections, resist reinfection or enable the animals to withstand haemorrhagic and climatic stress.. However, HbA has been shown to confer certain advantages by Altaif and Dargie (1978a and b); Preston and Allonby (1979a,b), i.e.:

(a) greater affinity for oxygen, thus conferring a measure of physiological advantage;

(b) production of HbC in times of anaemic or climatic stress (Blunt and Evans, 1963). The HbC is a β-chain variant with an improved oxygen carrying capacity (Gabuzda et al, 1968);

(c) HbA sheep are heavier and maintain higher PCV, Hb and blood volumes than others (Evans and Whitlock, 1964; Allonby and Urquhart, 1975);

(d) the greater availability of oxygen and methionine to the parasite in HbB sheep, i.e. a direct Hb-parasite interaction as suggested by Evans et al (1963) and Maxwell and Baker (1970).

Though field observations strongly suggested that the Hb type and breed of the animal influenced infection by H. contortus, this had not been successfully corroborated experimentally nor had any rational explanation been provided until the works of Altaif and Dargie (1976; 1978a and b). The problem was in determining whether the advantages displayed by HbA sheep were due to a superior innate immunity to withstand effects or resist establishment of infection or both, or due to superior acquired immunity, i.e. superior ability to elicit an immune response. Allonby and Urquhart (1975); Dargie and Allonby (1975) showed that larval challenge was extremely variable and that differences in faecal egg counts and haematological indices were not necessarily consistent with parasite numbers. Hence these parameters

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were better indicators of acquired rather than innate immunity (Dargie, 1975) and disease resistance based on them are apt to be misleading. He therefore suggested the use of measurements of blood loss and their damage to the haemoglobin and plasma protein of the whole animal which are the direct effects of the parasite. Using radioisotopic criteria to measure blood loss, pool sizes and turnovers combined with haematological, biochemical and parasitological criteria, they compared these parameters in previously worm-free Blackface and Finn Dorset sheep exposed to single standardised H. contortus infections and showed conclusively that Hb type is related to resistance if the challenge is not massive (Altaif and Dargie, 1976; 1978a and b). They also provided explanations as to the role of HbC which they found to function mainly as an aid to survival playing no role in worm establishment either during primary infections or during reinfections. Also the direct Hb-parasite interaction, as proposed by Evans et al (1963), was shown not to explain the difference in worm burdens of animals with the same Hb type but of different breeds. The genetic resistance operated primarily against worm establishment and, barring the unlikely involvement of non-specific physiological factors such as weight, the resistance was controlled by the immune response elicited (Altaif and Dargie, 1978a and b; Preston and Allonby, 1979a,b).The nature of this response was unknown but appeared to operate only against the larval stages since it was first expressed on the parasite on the seventh to twelfth day of its development and the size and metabolic activities of the surviving worms were similar in all animals. The more frequent and effective self-cure shown by HbA types provided additional evidence of an immunological mechanism. The lack of correlation between Hb types and exposure to heavy challenge was attributed to delayed immune response arising from excessive amounts of antigen during the critical period of larval development.

Altaif/

Altaif and Dargie (1978a and b) concluded that, though the Hb type is a useful genetic marker for resistance against H. contortus, the degree of protection it afforded was influenced by yet undetermined breed characteristics.

Preston and Allonby (1978) showed that the higher resistance displayed by the indigenous Red Masai sheep was a product of natural selection in an environment where H. contortus was endemic and no routine anthelmintic practice was carried out. The Red Masai were shown to possess higher mean levels of anti-larval IgA than Merinos and these antibodies were shown by Smith and Christie (1979), Duncan, Smith and Dargie (1978) to be associated with resistance to haemonchosis. Preston and Allonby (1978a,b)and Altaif and Dargie (1978a and b) showed that differences in susceptibility to H. contortus are greater between breeds than between haemoglobin phenotypes within a breed, suggesting that the development of breed rather than strain differences in resistance to haemonchosis would be more useful in helminth control practices in particular areas.

Because hyperinfection using irradiated larval vaccines conferred protection against subsequent challenge in immunologically competent lambs, attempts were made to determine if immunisation of sheep with various types of larval antigens of H. contortus would produce the same effect. Scott et al (1971) were able to produce resistance to initial infection with H. contortus by the transfer of allogenic lymph nodes from a resistant ewe; infiltration of unrefined antigen into the abomasal mucosa; intramuscular injections of unrefined antigens plus adjuvant. Failing to observe resistance following the intravenous injection of allogenic globulins, they concluded that circulating antibodies could not be related to resistance. All the lambs were fed iron ad lib and, as shown by Dargie (1973), the massive turnover of body iron from the earliest onset of haemorrhage in haemonchosis can only be replenished from dietary sources; the resistance observed by Scott et al may be directed towards the effects of haemonchosis and not to infection. This is borne out

by their findings that feeding of oral iron supplement enabled lambs to survive normally fatal H. contortus infections. Smith (1977a) showed that, though larval antigens stimulated serum and mucus IgG antibodies, they were not protective. The protective effect of such antibodies has since been documented (Duncan, Smith and Dargie, 1978; Smith and Christie, 1979).

The finding that adult worms inhibit further development of the immature stages (Tetzlaff and Todd, 1973; Kennedy and Todd, 1975) has led to the concept of interaction between different age groups of haemonchosis and the additional role this plays in immunity to haemonchosis apart from the host immune responses. Evidence in support of this was provided by Kennedy and Todd (1975) who observed a four-fold decrease in egg production in premunised challenge exposed immunological competent lambs compared to premunised but not challenged controls. They concluded that the premunised controls would not have had such a tremendous egg production if the host alone was involved in resistance and ascribed the decrease in egg production in premunised challenged exposed lambs to age-group interaction effect, i.e. the adults inhibiting the development of the larvae. Tetzlaff and Todd (1973) also found that relatively less pathogenic adult worms could maintain themselves against massive challenge doses of a more pathogenic isolate of Haemonchus without 'self-cure' occurring, thus lending support to the age-group interaction phenomenon.

REFERENCES.

Adams, J.W. and McKay J. (1966),

Nature (Lond.), p. 216-217.

Allonby, E.W. (1973),

In 'Helminth Diseases of Cattle, Sheep and Horses in Europe'.

Ed. Urquhart, G.M. and Armour, J., p. 59-71, Glasgow University Press, Scotland.

Allonby, E.W. and Urquhart, G.M. (1973),

Parasitology, 66, 45-53.

Allonby E.W. and Urquhart, G.M. (1975),

Vet. Parasit., 1, 129-143.

Allonby, E.W. and Urquhart, G.M. (1976),

Res. Vet. Sci., 20, 212-214.

Altaif, K.I. and Dargie, J.D. (1976),

I.A.E.A., Vienna, 449-462.

Altaif, K.I. and Dargie, J.D. (1978a),

Parasitology, 77(2), 161-175.

Altaif, K.I. and Dargie, J.D. (1978b),

Parasitology, 77(2), 177-187.

Anderson, F.L., Graff, D.I., Hammond, D.M., Fitzgerald, P.R. and Miner, M.L. (1960),

J. Parasit., 46 (5, Sec. 2), 38-39.

Andrews, J.S. (1942),

J. Agric. Res., 38(10), 761-770.

Anosa, V.O. (1977),

Trop. Anim. Hlth. Prod., 9(11), 11-17.

Anosa, V.O. (1978),

Vet. Parasit., 4, 103-109.

Armour, J., Jarrett, W.F.H. and Jennings, F.W. (1966),
Am. J. Vet. Res., 27, (120), 1267-1268.

Armour, J. (1970),
Vet. Rec., 86, 184-190.

Ayalew, L. and Gibbs, H.C. (1973),
Can. J. Comp. Med., 37, 78-89.

Bailey, W.S. and Herlich, H. (1953),
Auburn Vet., 9, 105-110.

Benitez-Usher, C., Armour, J., Duncan, J.L., Urquhart, G.M. and Gettenby, G. (1977),
Vet. Parasit., 3(4), 327-342.

Bird, A.F., Waller, P.J., Dash, K.M. and Major, G. (1978),
Int. J. Parasitol., 8, 69-74.

Bitakaramire, P.K. (1966),
Parasitology, 56(4), 619-622.

Blitz, N.M. and Gibbs, H.C. (1971),
Can. J. Comp. Med., 35, 178-180.

Blitz, N.M. and Gibbs, H.C. (1972),
Int. J. Parasitol., 2, 5-12.

Blitz, N.M. and Gibbs, H.C. (1972),
Int. J. Parasitol., 2, 13-22.

Blunt, M.H. and Evans, J.V. (1963),
Nature, Lond., 200, 1215-1216.

Boag, B. and Thomas, R.J. (1971),
Res. Vet. Sci., 12, 132 -137.

Boag, B. and Thomas, R.J. (1973),
Res. Vet. Sci., 14, 11-30.

Brundson, R.V. (1973),

New Zealand Vet. J., 21, 125-126.

Charleston, W.A.G. (1964),

J. Comp. Path., 74(2), 223-240.

Christie, M G., Brambell, M.R. and Charleston, W.A.G. (1964),

J. Comp. Path., 74, 435-446.

Christie, M.G. and Brambell, M.R. (1966),

J. Comp. Path., 76, 207-216.

Christie, M.G., Brambell, M.R. and Mapes, C.J. (1967),

Vet. Rec., 80(5), 207-208.

Christie, M.G., Angus, K.W. and Hoston, I.K. (1975),

Int. J. Parasitol., 80, 89-100.

Christie, M.G., Hart, R., Angus, R.W., Devoy, J. and Patterson, J.E.(1978),

J. Comp. Path., 88, 157-165.

Clark, C.H., Kiesel, G.K. and Goby, C.H. (1962),

Am. J. Vet. Res., 23(96), 977-980.

Cole, G.J. and Morris, B. (1973),

Adv. Vet. Sci. Comp. Med., 17, 226-256.

Connan, R.M. (1968),

J. Helminth., 42, 9-28.

Connan, R.M. (1971),

Res. Vet. Sci., 12, 272-274.

Connan, R.M. (1975),

Parasitology, 71, 239-246.

Connan, R.M. (1976),

Vet. Rec., 99, 476-477.

Conway, D.P. (1962),

Diss., Abstr., 22(8), 2917.

Dargie, J.D. (1973),

In"Helminth Diseases of Cattle, Sheep and Horses In Europe",

Eds. Urquhart, G.M. and Armour, J., Glasgow, pp. 63-71.

Dargie, J.D. and Allonby, E.W. (1975),

Int. J. Parasitol., 5, 147-157.

Dalaune, E.T. and Mayhew, R.L. (1943),

Trans. Am. Microsc. Soc., 62(2), 179-193.

Dineen, J.K., Donald, A.D., Wagland, B.M. and Offner, J. (1965),

Parasitology, 55, 514-525.

Dineen, J.K. and Wagland, B.M. (1966),

Parasitology, 56, 665-677.

Donald, A.D., Dineen, J.K. and Adams, D.B. (1969),

Parasitology, 59, 497-503.

Donald, A.D. and Waller, P.J. (1973),

Int. J. Parasitol., 3, 219-233.

Duncan, J.L., Smith, W.D. and Dargie, J.D. (1978),

Vet. Parasit., 4, 21-27.

Edgar, G. (1933),

Aust. Vet. J., 9(4), 149-154.

Evans, J.V., King, J.W.B., Cohen, B.L., Harris, H. and Warren, F.L. (1956),

Nature (Lond.), 178, 849.

Evans, J.V., Blunt, H.M. and Southcott, W.H. (1963),

Aust. J. Agric. Res., 14(4), 549-558.

Evans, J.V. and Whitlock, J.H. (1964)

Science, 145, 1318.

Fabiyi, J.P. (1970),

Bull. Epizoot.Dis. Afr., 18, 29.

Fabiyi, J.P. (1970),

Bull. Epizoot. Dis. Afr., 21, 274-284.

Fitzsimmons, W.M. (1969),

Helm. Abs., 38, 157-190.

Fourie, P.J.J. (1931),

17th Rept. Div. Vet. Res. Animal Industry, S. Afr., 495.

Gabuzda, T.G., Schuman, M.A.A., Siliver, R.K. and Lewis, H.B. (1968)

J. Clin. Inves., 47, 1895-1904.

Gibbs, H.C. (1968),

Vet. Med. Rev. Leverkusen, p. 160-173.

Gibbs, H.C. (1973),

Can. J. Zool., 151, 281-288.

Gibbs, H.C. (1977),

Am. J. Vet. Res., 38(4), 533-535.

Gordon, H. McI. (1948a),

Aust. Vet. J., 24, 17 -45.

Gordon, H. McI. (1948b),

Qd. agric. J., 67, 33.

Gordon, H. McI. (1950),

Aust. Vet. J., 2, 14-21.

Hall, M.C. (1936)

cited by Fitzsimmons, W.M., 1969, Helm. Abs., 38, 157-190.

Harness, E., Fitzsimmons, W.M., Sellwood, S.A. (1970),

J. Comp. path. 80(2), 173-179.

Hart, J.A. (1969),

Brit. vet. J., 120, 87-95.

Holman, H.H. and Pattison, I.H. (1941),

Vet. Rec., 53(34), 491-498.

Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., Mulligan, W. and Sharp, N.C.C. (1959),

Am. J. Vet. Res., 20(76), 527-531.

Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., Mulligan, W. and Sharp, N.C.C. (1961),

Am. J. Vet. Res., 22(87), 186-188.

Jennings, F.W., Armour, J., Lawson, D.D. and Roberts, R. (1966),

Am. J. Vet. Res., 27(120), 1249-1257.

Jilek, A.F. and Bradley, R.E. (1969),

Am. J. Vet. Res., 30(10), 1773-1778.

Kennedy, T.J. and Todd, A.C. (1975),

Am. J. Vet. Res., 36(8), 1195-1198.

Lee, R.P. (1955),

Annual Report of Research in Vom, Nigeria, 1955-1956, p. 10.

Lee, R.P., Armour, J., and Ross, J.G. (1960),

Brit. Vet. J., 116, 34-42.

Lopez, V. and Urquhart, G.M (1967),

Adv. vet. Parasitol., 153-159.

Malczewski, A. (1970),

Acta. parasitologica polonica, 18, 399-415.

Manton, D., Silvermann, P.H. and Terry, R.J. (1962),

Res. Vet. Sci., 3(3), 308-314.

Maxwell, C. and Baker, C.M.A. (1970),
Molecular Biology and the origin of species Heterosis,
Protein polymorphism and Animal Breeding, p. 76-79,
London, Sidgwick and Jackson.

Martin, C.J and Ross, I.C. (1934),
J. Helminth., 12(5), 137-142.

Mayhew, R.L. (1941),
Am. J. Hyg., Sect. D., 33(3), 103-11.

Mayhew, R.L. (1944),
Cornell vet., 34(4), 299-307.

McKenna, P.B. (1973),
New Zealand Vet. J., 21, 98-102.

McKenna, P.B. (1974),
New Zealand Vet. J., 22, 214.

Michel, J.F. (1970),
Parasitology, 61, 435- 477.

Michel, J.F. (1974),
Adv. Parasitol., 12, 279-366.

Muller, G.L. (1968),
Onderstepoort, J. Vet. Res., 35, 159-194.

Mulligan, W., Gordon, H. McL., Stewart, D.F. and Wagland, B.M (1961),
Aust. J. Agric. Res., 12(6), 1175-1187.

Neklyudov, V.N., Bokhovitinov, D.V. and Sominski, Z.F. (1956)
cited by Fitzsimmons, W.M. (1969), Helm. Abst., 38, 157-190.

Nielson, J.T.M. (1975)
Int. J. Parasitol., 5, 427-430.

Ogunsusi, R.A. (1978)
Res. Vet. Sci., 25, 298-301.

Ogunsusi, R.A. and Eysker, M. (1979)
Res. vet. Sci., 26, 108-110.

Ogunsusi, R.A. (1979),

Res. Vet. Sci., 26, 189-192.

Okon, E.D. and Enyenihi, U.K. (1975),

Bull. Anim. Hlth. Prod. Afri., 23(2), 145-153.

Owen, N.C. (1971),

Jl. S. Afr. Vet. Med. Ass., 42(1), 9-12.

O'Sullivan, B.M. and Donald, A.D. (1970),

Parasitology, 61, 301-315.

Preston, J.M. and Allonby, E.W. (1978),

Vet. Rec., 103(23), 509-512.

Preston, J.M. and Allonby, E.W. (1979a),

Res. Vet. Sci., 26(2), 134-139.

Preston, J.M. and Allonby, E.W. (1979b),

Res. Vet. Sci., 26(2), 140-144.

Proctor, B.G. and Gibbs, H.C. (1968),

Can. J. Comp. Med., 32, 359-365.

Radhakrishnan, C.V., Bradley, R.E. and Loggins, P.E. (1972),

Amer. J Vet. Res., 33, 817-843.

Roberts, F.H.S. (1957),

Aust. J. Agric. Res., 8(6), 740-767.

Ross, I.C. and Gordon, H. McL. (1936),

The internal parasites and parasitic diseases of sheep.

Their treatment and control, Sydney, Angus and Robertson, p. 238.

Ross, J.G., Lee, R.P. and Armour, J. (1959),

Vet. Rec., 71(2), 27-31.

Ross, J.G., Armour, J. and Hart, J.A. (1962),

J. Parasit., 48(3), 496-503.

Ross, J.G. (1963),

Vet. Rec., 75, 129 - 132.

Schillhorn van Veen., T.W. (1973),

Nig. Vet. J., 1, 26-31.

Schillhorn van Veen, T.S. (1978),

Vet. Rec., 102, 364-365.

Schillhorn van Veen, T.W. and Ogunsusi, R.A. (1978),

Vet. Parasit., 4, 377-383.

Schwartz, B. (1921),

Parasitology, 7, 144-150.

Scott, H.L., Silvermann, P.H., Mansfield, M.E. and Levine, H S. (1971),

Am. J. Vet. Res., 32(2), 249-262.

Silvermann, P.H. (1965),

Am. Zoologist., 5, 153 - 163.

Silverstein, A.M. and Prendergast, R.A. (1970),

In 'Developmental Aspects of Antibody Formation and Structure',

Ed. J. Sterzyl and I. Rhida, Academic Press, New York, 69-77.

Skryabin, Shikhobalova and Shutts (1954),

cited by Fitzsimmons, W.M. (1969), Helm., Abst., 38, 157-190.

Smith, W.D. (1977a)

Res. Vet. Sci., 22, 128-129.

Smith, W.D. (1977b),

Res. vet. Sci., 22, 334-338.

Smith W.D. and Christie, M.G. (1978),

Int. J. Parasitol., 8, 219-223.

Smith, W.D. and Christie, M.G. (1979),

J. Comp. Path., 89, 141-149.

Soulsby, E.J.L. and Stewart, D.F. (1960),

Aust. J. Agric. Res., 11(4), 595-603.

Soulsby, E.J.L. (1965),

Textbook of Veterinary Clinical Parasitology, Volume I,

Helminths. Blackwell Scientific Publications, Oxford., p. 448.

Stewart, D.F. (1950a),

Aust. J. Agric. Res., 1(3), 301-321.

Stewart, D.F. (1950b),

Aust. J. Agric. Res., 1(4), 427-439.

Stewart, D.F. (1953),

Aust. J. Agric. Res., 4(1), 100-117.

Stewart D.F. (1955),

Nature, Lond., 175(449b), 1273-1274.

Stoll, N.R. (1929),

Am. J. Hyg., 10, 384-418.

Stoll, N.R. (1958),

Rice Inst. pamph., 45, 184-208.

Swan, R.A. (1970),

Aust. Vet. J., 46, 485-492.

Tetzlaff, R.D and Todd, A.C. (1973),

Am. J. Vet. Res., 34, 1549-1554.

Thomas, R.J. and Boag, B. (1972),

Res. Vet. Sci., 13, 61.

Thomas, R.J. and Boag, B. (1973),

Res. Vet. Sci., 15, 238-239.

Thomas, R.J. and Waller, P.J. (1979),

Res. Vet. Sci., 26, 209-212.

Urquhart, G.M., Jarrett, W.F.A., Jennings, F.W.,McIntyre, W.I.M.,

Mulligan, W. and Sharp, N.C.C. (1966a),

Am. J. Vet. Res., 27(121), 1641-1643.

Urquhart, G.M., Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M.,

and Mulligan, W. (1966b),

Am. J Vet. Res., 27(121), 1645-1648.

Van Geldorp, P.J.A. and Schillhorn van Veen, T.S. (1976),

Vet. Parasit., 1(3), 265-269.

Veglia, F. (1915),

3rd and 4th Ann. Rep. Dir. Vet. Res. Un. S. Afr., 347.

Wagland, B.M. and Dineen, J.K. (1967),

Parasitology, 57, 59-65.

Waller, P.J. and Thomas, R.J. (1975),

Parasitology, 71, 285-291.

Whitlock, J.H. (1958),

Cornell Vet., 48, 127-133.

Wilson, G.I. and Samson, K.S. (1974),

Res. Vet. Sci., 17, 390-394.

Evans, J.V., King, J.W.B., Cohen, B.L., Harris, H. and Warren, F.L. (1956),

Nature (lond.), 178, 849.

Ross, I.C. (1932),

Jl. Scient. ind. Res. Aust., 5(2), 73-80.

CHAPTER II

PATHOGENESIS OF BOVINE TRYPANOSOMIASIS WITH PARTICULAR REFERENCE TO HAEMATOLOGICAL CHANGES

PATHOGENESIS OF BOVINE TRYPANOSOMIASIS WITH PARTICULAR REFERENCE TO HAEMATOLOGICAL CHANGES

Introduction:

Trypanosomiasis is a group of acute to chronic infectious diseases of all domestic animals and man, except poultry, caused by trypanosomes which are flagellated unicellular protozoa belonging to the class zoomastigophora, order kinetoplastida. Most of the trypanosomes responsible for bovine trypanosomiasis are transmitted by tse-tse flies (Glossina spp.) which are the principal vectors. The disease is characterised generally by intermittent fever, anaemia, severe debility, weight loss and lymphadenitis. The trypanosomes undergo an obligatory development in the insect vector which could either be by posterior station or anterior station giving rise to stercorarian or salivarian trypanosomes respectively. Mechanical transmission by biting flies other than tse-tse flies is also possible. The salivarian trypanosomes are responsible for 'Nagana' (Zulu for depressed or low spirits), i.e. the tse-tse transmitted trypanosomiasis of cattle in Africa and the main species responsible are Trypanosoma vivax, Trypanosoma congolense and Trypanosoma brucei. Trypanosoma gambiense and Trypanosoma rhodesiense which cause sleeping sickness in man also fall within this group.

Trypanosomes as pathogens of cattle have been recognised as far back as 1894 when David Bruce sought for the aetiology of Nagana, a fatal disease of cattle in Zululand. Since then different species have been recognised which have been found to vary in virulence, infectivity and pathogenicity. Even among the same species different strains exist which have been found to differ in virulence (Desowitz, 1959). In West Africa T. vivax is generally considered most pathogenic, followed by T. congolense and T. brucei in that order (Isoun, 1975). In East Africa, however, T. congolense is usually considered more pathogenic than either T. vivax or T. brucei (Fiennes, 1970; Losos and Ikede, 1972).

Breed/

Breed differences also affect the course and degree of infection with trypanosomes. Thus certain West African humpless dwarf breeds such as the N'dama and the Muturu are known to exhibit greater tolerance to the disease than the humped Zebus (Desowitz, 1959; MacLennan, 1970; Chandler, 1958; Dargie et al., 1979a,b).

The problems caused by both animal and human trypanosomiasis in Africa are enormous. Firstly, tse-tse borne trypanosomiasis has profoundly affected the development of much of tropical Africa by rendering large areas of the continent detrimental to the raising of livestock except poultry. The areas affected cover a substantial proportion of sub-Saharan Africa and, according to Lumsden (1968), constitute more than one quarter of the land surface of the continent. This area was estimated in 1963 to be capable of supporting 125×10^6 cattle which was more than the total population in Africa at that time (Wilson et al., 1963). The disease is confined to lattitude 29°S. and 14°N. and paradoxically it exerts its greatest effect where it is not apparent in the loss of the vast grazing potential of the Savannah regions. This has led to serious nutritional and economic problems in these areas as many communities never had the opportunity to domesticate or utilize livestock except by hunting. There was thus lack of manure to fertilize land ; lack of draught animals to till the land thereby relegating man to the principal domestic power source and lack of protein in the diet causing a chronic protein shortage. Even livestock in the margins of fly-infested areas suffer high mortalities, while the cost of chemotherapy and prophylaxis adds to the economic loss suffered from the disease.

In West Africa, trypanosomiasis delimits the main cattle raising area to the sub-Saharan Sahel Savannah zone. This area which is tse-tse free with a scanty rainfall and low relative humidity is inhabited by nomadic Fulani who between them constitute the main cattle rearing community. Due to the absence of settled husbandry and lack of technical know-how this area is/

is always overstocked and overgrazed. With the advent of the dry season, these nomadic cattle men are therefore forced to trek their livestock southwards into known tse-tse infested bush for sustenance. There is thus a high mortality from the disease during this period. In contrast, settled herds experience high morbidities during periods of greatest fly activity, i.e. the rainy season. These nomads do not own the land on which their stock graze and the condition of land tenureship coupled with the force of tradition has denied them modern farming techniques and the advantages of a settled husbandry practice. In the southern areas where there is increased awareness of these benefits, trypanosomiasis has provided the major constraint to a comprehensive livestock farming. Most areas of tropical Africa are faced with this problem.

For many years research activities into this disease have centred mainly on chemotherapy and entomological control, i.e. measures aimed at combating natural infections (Murray, 1974). Studies on the pathogenesis of the disease, therefore, received little attention. With the advent of new techniques such as cryopreservation, serodiagnosis and use of laboratory animals for passaging and infectivity studies, there has been a shift in emphasis and most workers are now concerned with elucidating the pathogenesis of the disease. Most of the work with laboratory animals has been carried out with T. brucei and T. congolense which grow readily in these abnormal hosts. A review of the literature on some aspects of the pathogenesis of the disease, e.g. the pathology, the phenomenon of immunosuppression and antigenic variablity and, most importantly, haematological changes following infection is presented forthwith.

Pathology:

The descriptive pathology of bovine trypanosomiasis is still a subject of wide controversy. Much of the problem involves extrapolating results obtained in laboratory animals to natural cases of the disease, inability to follow/

follow sequentially the development of lesions as studies on pathology are invariably done on terminal cases and, most importantly, the problem of either regarding all trypanosomal infections as a single composite disease entity (Murray et al., 1974a; Fiennes, 1970) or as a group of diseases equal in number to the different species of pathogenic trypanosomes (Losos and Ikede, 1972).

Work carried out with laboratory animals (Goodwin, 1970, 1971; Murray, 1974a and b; Murray et al., 1974a; Losos and Ikede, 1972) showed that T. brucei in addition to infecting the plasma also infects the intercellular tissue fluids, especially of the connective tissue and fluids of body cavities. The pathological picture was therefore dependent on the areas of localisation. On this basis Murray et al. (1974a) studied the pathology of trypanosomiasis in rats infected with T. brucei and concluded that the disease process could be categorized into three, i.e.

(a) initial marked plasma cell hyperplasia in the immunological apparatus of the spleen, thymus and lymph nodes followed by depletion of these immunological cell types. There was increased activity of the mononuclear phagocytic system;

(b) production of haemolytic anaemia as evidenced by increased erythropoeisis in the spleen and bone marrow, reticulocytosis, haemosiderin deposits in various organs and marked erythrophagocytosis.

(c) specific organ damage most especially in the heart giving rise to myocarditis. This damage was attributed to an inflammatory reaction which led to necrosis and diffuse or focal infiltration by lymphocytes, histiocytes and trypanosomes.

Earlier Ikede and Losos(1972a,b)observed similar findings in cattle experimentally infected with T. brucei. The trypanosomes localised extravascularly and produced inflammatory lesions in the skin, testes, subcutaneous/

subcutaneous tissue, heart, the eye and central nervous system.

The pathology of T. congolense and T. vivax infections is not so straight-forward. Though Murray et al (1974a) recognised them as haemoflagellates, they were of the opinion that the pathology of the disease process caused by them was similar to their observations in rats infected with T. brucei. They supported their views with the finding of extensive depletion of the immunological apparatus of the lymph nodes and spleen, anaemia, as evidenced by haemosiderosis and erythrophagocytosis, and focal myocarditis in four East African cattle naturally infected with T. congolense and T. vivax. They further claimed that Maclennan in a personal communication found heart lesions in acute T. vivax infections of cattle in West Africa; and that three sheep in Glasgow experimentally infected with T. vivax had similar lesions as cattle, i.e. anaemia and myocarditis. Further support came from the work of Kaliner (1974) who observed mainly inflammatory and proliferative lesions in cattle experimentally infected with T. congolense and which were necropsied terminally. However, Losos and Ikede (1972); Naylor (1971c); Losos et al (1973) produced evidence in support of the early work of Hornby (1952) and Hornby and Bailey (1931) that at least T. congolense is strictly a plasma parasite and that the disease process was primarily the result of the anaemia. There was thus an absence of necrotic or inflammatory lesions. Lesions observed such as atrophy of skeletal muscles, mucoid degeneration of adipose tissue and central lobular necrosis in the liver were attributed to the result of the anaemia, while the focal polioencephalomalacia was ascribed to pressure necrosis caused by the oedema which resulted from the accumulation of trypanosomes in the capillaries and venules of the cerebral cortex and cerebellum. This ischaemic necrosis was also found in the heart and skeletal muscles. Earlier work with T. vivax

by/

by Lester (1932) and Fiennes (1952) also implied that this parasite could produce lesions similar to T. brucei, i.e. it is tissue invasive. In these reports neither was there complete supportive histological evidence nor were trypanosomes found outside the blood vessels. Losos and Ikede (1972) carried out routine histological examinations on cattle naturally infected for up to three months and could detect no trypanosomes outside the blood vessels. They therefore concluded that the disease caused by T. vivax appeared similar to that caused by T. congolense. These two parasites were consequently grouped as haematic, i.e. plasma parasites and T. brucei as humoral, i.e. plasma and tissue parasite. Mixed infections with more than one parasite are very common in natural cases of the disease (Losos and Ikede, 1972; Luckins, 1972). In the lesions described by Murray et al(1974a) from cattle in East and West Africa, mixed infections which included T. brucei probably contributed. T. brucei usually exhibits a low grade and transient parasitaemia in domestic animals often not detected routinely as it is easily masked by the higher parasitaemia of T. vivax and T. congolense. The possibility that the lesions in the Glasgow sheep experiment resulted from ischaemic necrosis rather than a necrosis sequel to inflammation cannot be ruled out.

There is a divergence of opinion as regards the mechanism by which these parasites cause their damage. Murray et al. (1974a) and Losos and Ikede (1972) proposed a possible involvement of cell-mediated immunologic responses based on the observed response by mononuclear cells. This explanation had support from the work of MacKenzie, Boreham and Facer (1972) who showed enhanced production of antibodies in rabbits infected with T. brucei and by Boreham and Kimber (1970) who had earlier demonstrated immune complexes in the kidneys of rabbits also infected with T. brucei.

During trypanosome infections vasoactive amines are released (Boreham, 1968; Richards, 1965). This led Goodwin (1971) to suggest that the vascular lesions seen in trypanosomiasis could be an immunological lesion mediated through the release of kinins by an antigen-antibody reaction.

Research/

Research activities are continuing aimed at clarifying the basic disease process caused by different species of trypanosomes and the underlying mechanisms responsible for the destructive damages caused by these parasites.

Haematological Changes:

There appears to be universal agreement that anaemia is the principal pathological and clinical feature of the tse-tse transmitted trypanosomiasis of cattle in Africa. Workers such as Hornby (1921, 1952) and Hornby and Bailey (1931) even concluded that the anaemia which followed infection was solely responsible for the disease process.

The course and degree of anaemia is dependent on various factors, e.g.

(a) the species and strain of the infecting trypanosome which may exhibit differences in virulence and hence infectivity;

(b) the status of the host with respect to age, health, nutrition, breed and previous exposure to the disease.

Thus Desowitz (1959) produced gradations in infection in three breeds of cattle (Muturu, N'dama and Zebu) infected with the same strain of T. vivax. The Muturu which had never been exposed suffered an acute disease, while the N'dama which had been raised in a trypanosomiasis endemic area suffered a transient asymptomatic infection and the Zebus suffered a chronic infection even though they had no previous experience of the disease. Similarly Dargie et al. (1979b) using a field strain of T. brucei isolated from a naturally infected N'dama cow showed gradations in infections between previously unexposed N'dama and Zebu cattle in that the anaemia produced was more severe in the latter. Such gradations have also been described in the two breeds when infected with T. congolense (Dargie et al. 1979a; Murray et al. 1979). This tolerance of the humpless N'dama and the Muturu over the Zebus has/

has been recognised for a long time (Fiennes, 1970; Maclennan, 1970; Roberts and Gray, 1973) and various reasons have been advanced for this. Fiennes (1970) postulated that the tolerance was acquired as a result of infection acquired early in life; Stewart (1951) incriminated the transfer of maternal antibodies; Desowitz (1959) suggested a low incidence of intercurrent disease and freedom from stresses of migration, while Roberts and Gray (1973) implicated genetic factors. But, as suggested by Dargie et al (1979b), the superiority of the N'dama over the Zebu probably lies in their greater capacity to limit the level and duration of parasitaemia rather than in mounting a more efficient erythropoietic response. The mechanism of this response was unknown but suspected to be immunological. The importance of age in the course and degree of the disease was shown by Fiennes et al. (1946); Valli and Mills (1980) who observed a milder though haemolytic anaemia in infected neonatal calves compared with 6-month old calves infected with T. congolense.

The principal features of the disease were described by Fiennes (1954) during infections of cattle with T. congolense and T. vivax. He found that the disease could be peracute, acute or chronic; that the number of red cells varied directly with the parasitaemia; that the anaemia of the acute stage was macrocytic while that of the chronic stage was microcytic and, most importantly, that the anaemia was non-haemolytic even though he incriminated a haemolytic factor in the haemolysis he observed in acute conditions. Subsequent work on the morphological characterization of the anaemia showed a macrocytic and normochromic response in T. congolense infected cattle (Naylor, 1971b; Valli and Mills, 1980); normocytic and normochromic also in T. congolense infections of cattle (Losos et al. 1973; Wellde et al. 1974).

Valli, Forsberg and McSherry (1978) observed a macrocytic normochromic response in acute cases and normocytic normochromic response in chronic T. congolense infections. Saror (1979) using electronic cell counting equipment/

equipment which offered greater accuracy over the manual evaluation used by the previous workers showed that the anaemia was basically normochromic and normocytic with a tendency towards being macrocytic and normochromic. Finding no evidence of iron deficiency in chronic cases, he concluded that iron deficiency was not significant in chronic trypanosomiasis, as suggested by Fiennes (1954). The onset of patency depends on the number of injected trypanosomes but usually ranges from 3-10 days (Wellde et al, 1974; Naylor, 1971a; Losos et al, 1973). The duration of the disease is, however, independent of this number. Following patency there is usually a progressive decrease in the packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell values (rbc) while the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values are increased. White blood cell (WBC) values are inconsistent. In natural infections of cattle with T. congolense Fiennes (1970) observed a fall in rbc value from 7.2 x 10^6/cmm^3 to 3.1 x 10^6/cmm^3 and Hb from 8.7 gm % to 5.2 gm %. Losos et al. (1973) recorded the haematological changes in cattle infected experimentally with T. congolense and which died as a result of the infection. PCV at time of death was 11.0± 3.3%; Hb 5.6± 1.1 gm %, rbc 1.9±3.3 x 10^6/cmm^3 and WBC 8.0 ± 4.1 x 10^3/cmm^3. Saror (1979) experimentally infected cattle with T. vivax and found that PCV fell from a normal of 38% to about 20% by the 12th day of patency, while Hb was 6gms/dl and rbc 3.5 x 10^6/cmm^3 at this time. There were increases in MCV and MCH indices. Maxie, Losos and Tabel (1976) compared the haematological aspects of the disease caused by T. congolense and T. vivax in cattle. They confirmed that the anaemia in both cases was initially normocytic and later macrocytic. The erythroid response was more marked in T. congolense while the T. vivax infected animals showed a consistently positive direct Coombs test, as opposed to the T. congolense infected group which reacted negatively. This led them to conclude that different mechanisms were involved in the pathogenesis of the haemolytic anaemia/

anaemia in the two conditions. The leucocytic response also differed markedly. While the leucopenia associated with T. congolense was observed not to change with chronicity, as previously noted by Naylor (1971b) and Wellde et al (1974), that due to T. vivax was shown to be transient, as previously documented by Vohradsky (1971); Saror (1976). In contrast, Losos et al (1973) in Uganda showed relative leucocytosis from about the 11th week of infection with T. congolense in cattle. Valli, Forsberg & Lumsden (1979) and Naylor (1971b) all recorded increased WBC counts from the 14th week of infection with T. congolense in cattle. It is noteworthy that the experiments of Maxie, Losos and Tabel (1976) had no surviving animals by the 11th week and this might have been responsible for the observed failure of WBC response rather than the conclusion of different pathogenesis being held accountable. One cannot rule out the possibility that different strains of the parasite or undetermined breed factors were involved as the cause of the observed differences, if any, was unknown. The leucopenia involved a decrease of lymphocytes and neutrophils (Losos et al, 1973; Anosa and Isoun, 1980). Anosa and Isoun (1980) attributed the lymphopenia to a probable depopulation of lymphoid nodules and transformation of lymphocytes into plasma cells, and the neutropenia to a part of the generalised bone marrow depression. Losos et al (1973) and Maxie, Losos and Tabel (1976) suggested that either leucocytes were attracted to trypanosomes located in the micro-circulation in the T. congolense infected animals or that the bone marrow erythropoiesis was so intense that erythroid cell production occurred preferentially to granulopoiesis. Comparable data in T. brucei infected cattle are few and far between but, as suggested by Dargie et al (1979a,b), clinical and pathophysiological changes in Zebu and N'dama cattle infected with T. brucei were similar to those infected with T. congolense. Similar haematological changes are therefore envisaged. Thus Anosa (1975) observed anaemia and leucopenia in T. brucei infected mice. Edwards et al (1956a) failed to observe any consistent pattern of leucocyte change in T. vivax infection of sheep, goats and cattle.

Reticulocyte/

Reticulocyte values have been inconsistent. Workers who demonstrated a regenerative anaemia with distinct erythroid hyperplasia have tended to observe reticulocytosis in peripheral blood smears. Thus Saror (1979) working with T. vivax infected cattle; MacKenzie and Cruickshank (1973) and Anosa and Isoun (1980) with T. vivax in sheep and and goats; Boreham (1967, 1968), Jenkins et al(1974a,b,1980) in T. brucei infection of rabbits; Murray (1974a), Anosa (1975), Jennings et al. (1974) in T. brucei infection of mice observed a reticulocytosis with an attendant macrocytosis which they held responsible for the increased MCH and MCV and decreased MCHC values. On the other hand, workers who failed to find increased reticulocyte counts irrespective of the observation of increased destruction of the red blood cells concluded that there was failure of compensatory stimulation of erythropoietic tissue (Naylor, 1971b; Boycott and Price Jones, 1913; Edward, Judd and Squire, 1956b; Losos et al, 1973). Erythrokinetic studies involving radioisotopes has provided evidence in support of the regenerative nature of the anaemia which is also accompanied by erythroid hyperplasia of the bone marrow (Holmes, 1976; Dargie et al, 1979a,b; Jennings et al, 1980).

Maxie, Losos and Tabel (1976); Valli and Mills (1980) observed that thrombocytopenia was a feature of T. congolense infection of both adult and young cattle. Naylor (1971) produced a transient thrombocytopenia which was said to soon return to normal values though the platelet data was not presented. Jenkins et al (1974) also reported thrombocytopenia in rabbits infected with T. brucei though Murray (1974a) could find no such evidence in mice infected with the same trypanosome specie.

Red cell abnormalities such as anisocytosis, spherocytosis, polychromasia, macrocytosis and crenation have been reported by/

by various workers in both domestic and laboratory hosts infected with either of the three pathogenic African trypanosomes (Boreham and Goodwin, 1967; Naylor, 1971a, b; Balber, 1974; Wellde et al, 1974; Saror, 1979; Anosa and Isoun, 1980; Jenkins et al, 1980).

There is general agreement in the literature that the anaemia is basically haemolytic, the haemolysis occurring extravascularly. This conclusion was based on the finding of:-

(a) increased levels of bilirubin in the blood (Hudson, 1944; Fiennes, 1954, 1970; Edwards, Judd and Squire, 1956b; Naylor, 1971c; Jenkins et al, 1974b). Increased levels of bilirubin was based on the consistently positive indirect Van den Bergh test in T. congolense infected cattle (Fiennes, 1954), while failure to find haemosiderin in the kidney tubules was interpreted as evidence of extravascular haemolysis (Valli, Forsberg and McSherry, 1978). Jenkins et al (1980) in a more recent study failed to observe increased bilirubin levels in T. brucei infected rabbits as they did in an earlier work (Jenkins et al, 1974a). They then concluded that the liver of rabbits was either more efficient in dealing with pigments or the haemolysis they produced in the second experiment was not severe enough to cause bilirubinaemia;

(b) erythrophagocytosis in various organs and tissues of different laboratory and domestic hosts infected with any of the three pathogenic trypanosomes. This has been observed in the spleen, liver, haemolymphs and lymph nodes of cattle infected with T. congolense or T. vivax (Fiennes, 1970; Naylor, 1971b; Murray et al, 1979b; Valli, Forsberg and McSherry, 1978; Losos et al, 1973; Wellde et al, 1974); in sheep and goats infected with T. congolense, T. vivax or T. brucei (Edwards, Judd and Squire, 1956a,b, 1959;

McKenzie/

McKenzie and Cruickshank, 1973; Van der Ingh et al, 1976; Saror, 1980) and in various laboratory rodents (Murray et al, 1974a,b; Boycott and Price-Jones, 1913; Jenkins et al, 1974a,1980; Isoun, 1975; Anosa, Jennings and Urquhart, 1977; Sadun et al, 1973). The spleen is the major site of erythrophagocytosis though in T. vivax infections in sheep the liver has been found to assume this major role if infections are severe (Anosa and Isoun, 1980). The fall in haematocrit resulted from the haemolysis and associated increase in erythrophagocytosis;

(c) Haemosiderin deposits in various organs and tissues except in the renal tubules (Boycott and Price-Jones, 1913; Fiennes, 1954; Edwards, Judd and Squire, 1959; Boreham, 1967; Naylor, 1971a; McKenzie and Cruickshank, 1973; Wellde et al, 1974; Losos et al, 1973; Jenkins et al, 1974a, 1980; Valli, Forsberg and McSherry, 1978; Murray et al, 1974a). Naylor (1971b) found evidence of intravascular haemolysis in some of his T. congolense infected cattle based on haemosiderin deposits in the renal tubules. The possibility of intercurrent protozoal disease such as acute Babesiosis cannot be ruled out in such cases;

(d) Erythroid hyperplasia of the bone marrow causing marked reticulocytosis (Fiennes, 1954; Boreham, 1967; Jennings et al, 1974; Jenkins et al, 1974a, b; Saror, 1979; Anosa and Isoun, 1980), decrease in the myeloid-erythroid ratio (Jennings et al, 1974; Sadun et al, 1973; Naylor, 1971b; Jenkins et al, 1974a,b) and normoblastic hyperplasia (Jennings et al, 1974). These showed that the anaemia was not megaloblastic but regenerative;

(e)/

(e) Findings from erythrokinetic studies using radioisotopic techniques. Such techniques permit a direct assessment of the kinetic aspect of trypanosomiasis as most of the measurements could be carried out concomitantly in the same animal. Observations based on these techniques during the course of T. vivax, T. congolense or T. brucei infections in cattle, sheep, goats and laboratory animals include a shortened half-life of ^{51}Cr-labelled red blood cells, accelerated urinary excretion of such label, retention of injected ^{59}Fe in the body despite a faster iron-turnover rate and rapid disappearance of the radio-iron from the plasma (Jennings et al, 1974; Jenkins et al, 1974a, 1980; Holmes and Jennings, 1976; Anosa and Isoun, 1980; Dargie et al, 1979a, b; Murray et al, 1979). These findings indicate a massive loss of red cells from the circulation often very early in the infection (about 10 days) which became greater as the disease progressed and that erythropoiesis was in no way impaired but rather enhanced.

Despite the overwhelming evidence that haemolysis is basically responsible for the anaemia of trypanosomiasis, the exact mechanism of the anaemia is largely controversial and various hypotheses have been postulated. Firstly, the haemolysis could be immunological in origin. Various immunological models have been postulated to account for the phagocytosis. Thus Herbert and Inglis (1973) demonstrated that trypanosome antigens can attach to the surface of red cells thereby rendering the cells susceptible to phagocytosis. This model was supported by the finding of Woo and Kabayashi (1975) who successfully eluted antibody from red cells of rabbit infected with T. brucei and demonstrated that the cells agglutinated trypanosomes in vitro. Woodruff (1973); Maruta and Mizuno (1971) showed a Coomb's positive anti-globulin reaction and the anaemia was consequently suggested to result from the phagocytosis of antibody coated red cells. Other workers have found it difficult to demonstrate a consistently positive

Coomb's antiglobulin reaction on cells from infected animals (Boreham and Facer, 1974; Kobayashi, Tizard and Woo, 1976; Ikede, Lule and Terry, 1977), though Dodd, Jenkins, Lincoln and McCrorie (1978) using the 'build-up' technique patented in their laboratory showed a consistently positive reaction throughout the duration of T. brucei infections in rabbits indicating that antibody attached to red cells was minimal. A model incorporating a role for complement was suggested by Ingram and Soltys (1960) and Woodruff et al (1973) based on the finding of high immuno-conglutinins which are antibodies produced against complement in laboratory animals infected with T. brucei. The trypanosome antigen-antibody reactions in the circulation could lead to production of complement which, on activation, adhere to the red cells and are consequently lysed or phagocytosized when immunoconglutinins are linked to the activated complement complex (Jennings, 1976).

Secondly, the haemolysis could result from the release of a haemolysin from trypanosomes. Thus Fiennes (1954) found that if red cells from normal bovines were incubated with infected plasma, the cells were haemolysed. Recently Huan et al (1975) observed haemolysis of mouse red cells following incubation with the supernate from a living suspension of T. brucei. The haemolytic factor was purified and characterized as a protein. Tizard et al (1977) and Tizard, Sheppard and Nielsen (1978) made similar observations with sheep red cells but attributed the haemolytic activity of the parasites to free fatty acids and suggested that the haemolytic factor described by Huan et al (1975) could have been a phospholipase. In vivo demonstration of this haemolytic factor mediated haemolysis has not been described in cattle. This mechanism is difficult to envisage in chronic trypanosomiasis when parasitaemias are scanty except, as suggested by Jenkins et al (1980), the haemolytic factor was released from dead trypanosomes.

The haemolysis was also postulated to result from disseminated intravascular coagulation (DIC). This followed the observation of thrombocytopenia/

thrombocytopenia (Jenkins et al,1974a,b,1980), increased production of clotting factors VIII and IX and fibrinogen in rabbits (Boulton, Jenkins and Lloyd, 1974; Boreham and Facer, 1974) infected with T. brucei; and of formation of fibrin thrombi in vessels (Isoun, 1968; Anosa and Isoun, 1980). DIC per se has only been observed to occur in human patients (Barrett-Connor, et al, 1973) and in rats (Davis et al, 1974).

Enhanced phagocytosis due to an altered red cell surface could result following competition for essential metabolites. Thus the hypoglycaemia encountered in terminal stages of trypanosomiasis (Hudson, 1944; French, 1938; Fiennes, Jones and Laws, 1946) was interpreted as a sequel of competition for glucose by both red cells and trypanosomes. If red cells are unable to regenerate ATP their surfaces will be altered and consequently subjected to phagocytosis (Maruta and Mizuno, 1971). This mechanism may not explain situations of low parasitaemia. Moreover, Jennings et al (1974a,b) Anosa (1975) showed that if cells from infected and anaemic donors are transferred to normal recipients, they have the same half-life implying that whatever alteration suffered by the latter was not permanent.

Holwill (1965) on observing the rapid and lashing type of motility exhibited by trypanosomes, suggested that the trauma caused to the red cells was strong enough to cause haemolysis. This hypothesis may not be significant in scanty parasitaemias.

Lastly, pyrexia which is often observed in trypanosome infected animals was considered as a factor likely to cause haemolysis (Karle, 1968a). Jenkins et al (1980) observed pyrexia in T. brucei infected rabbits but its role in haemolysis was not considered significant.

Other factors acting singly or in concert with haemolysis are thought to play a contributory role in the pathogenesis of bovine trypanosomiasis. Such factors include:-

(a) Haemodilution:/

(a) Haemoldilution:

This was first reported in T. congolense and T. vivax infections of cattle by Fiennes (1954). This observation was further supported by Clarkson (1968) and Naylor (1971b) using conventional plasma volume determination techniques. Radioisotopic measurement of plasma volume with ^{59}Fe-transferrin and circulating red cell volume with ^{51}Cr-labelled erythrocytes (Mamo and Holmes, 1975; Holmes and Mamo, 1975; Holmes, 1976; Holmes and Jennings, 1976; Anosa and Isoun, 1980) showed conclusively that haemodilution contributed to the fall in haematocrit of parasitized animals, especially during the early stages of infection. Holmes (1976) could find no evidence in support of the observation by Boreham and Goodwin (1967) that the high vascular permeability caused by elevated kinins in the tissues was responsible for the haemodilution. Reasons for the elevated plasma volume are unknown. Clarkson (1968) and Naylor (1971c) proposed that the osmotic effects of the increased γ-globulin fraction of plasma proteins found in trypanosomiasis could be responsible. Though albumin rather than globulins are recognized for their osmotic effects, the hyperglobulinaemia was of such magnitude (130%) that their osmotic effects could be considerable. Other probable reasons include changes in aldosterone levels, release of vasoactive peptides and release of metabolic products from trypanosomes (Holmes, 1976). Haemodilution was not a feature of T. brucei infection of mice on day 3 of infection (Jennings et al, 1974); of N'dama and Zebu cattle infected with T. brucei (Dargie et al, 1979a,b) and of T. congolense infection of cattle (Preston and Wellde, 1976). The discrepancy probably lies in the different techniques employed in estimating plasma and blood volumes.

(b) Dyshaemopoiesis:

This was regarded as an adjunct to haemolysis by workers who failed to observe reticulocytosis in infected animals (Fiennes, 1954; Edwards/

Edwards, Judd & Squire, 1956b; Naylor, 1971b; Parkin and Hornby, 1930). The poor reticulocyte response was interpreted as evidence of bone marrow depression. Work by Schnappauf et al (1967) showed poor reticulocyte response in cattle following the removal of large volumes of blood by phlebotomy implying that appreciable reticulocytosis can only be demonstrated in these animals under conditions of extreme anaemic stress. There is ample evidence in the literature that the bone marrow shows marked activity rather than depression in trypanosomiasis (Jenkins et al, 1980; Anosa and Isoun, 1980; Saror, 1979; McCrorie et al, 1980).

(c) Hypersplenism:

Splenomegaly is commonly observed in trypanosome infections (Wolbach and Binger, 1912; Hudson, 1944; Edwards, Judd and Squire, 1959; Isoun, 1968; Fiennes, 1970; Jennings et al, 1974; Jenkins et al, 1974b; Murray et al, 1974a; Losos and Ikede, 1972; McCrorie et al, 1980). The hyper-active spleen was postulated to account for the anaemia following the removal and destruction of the red cells by the organ. Factors in the splenic environment conducive to phagocytosis include:- a non-specific activation of splenic macrophages; damage to red cells due to increased osmotic and mechanical fragility or a decrease in K^+/Na^+ ratio (Richmond et al, 1967); damage to red cells due to low cholesterol and glucose levels and low pH due to lactic acid accumulation (Anosa, 1977). Hypersplenism may have a contributory role in the anaemia as splenectomy has been found to ameliorate the anaemia in T. brucei infected rabbits (Jenkins et al, 1974a; McCrorie et al, 1980), but not in T. vivax infections of sheep and goats (Anosa and Isoun, 1980). This role becomes less important during the terminal stages of the disease when the spleen either had returned to normal size or only slightly enlarged (Fiennes, 1954; Albright, Albright and Dusanic, 1977; McCrorie et al, 1980).

In/

In conclusion, all available evidence points to the anaemia as being haemolytic in origin but accompanied by haemodilution and hypersplenism. There was no evidence of dyshaemopoiesis from radioisotopic studies although iron-reutilisation from degraded erythrocytes was impaired as a result of blockage of reticuloendothelial iron release (Dargie et al, 1979a and b). The mechanism of the haemolysis remains largely unresolved. Immunological mechanisms have been demonstrated in abnormal laboratory hosts but not in vivo in domestic animals. Based on the close correlation of changes in haematological indices, circulating red cell and plasma volumes, erythropoiesis and red cell removal from the circulation with the presence of parasitaemia, and the rapidity with which these parameters returned to normal following treatment (Holmes and Jennings, 1976), the anaemia was considered not immune mediated (Fiennes, 1950; Huan et al, 1976; Holmes and Jennings, 1976). The pathogenesis of the anaemia in trypanosomiasis is still an open field and more fundamental work is continuing to be aimed at elucidating the precise mechanism(s) involved.

Changes in other plasma constituents were also investigated. Saror (1976) observed fluctuations in plasma copper levels during infections but the levels were within normal range for clinically healthy Zebu cattle used in the experiment. Serum iron levels and total iron binding capacities were unaltered during the course of infections using both conventional and radioisotopic criteria (Tartour and Idris, 1973; Holmes, 1976; Dargie et al, 1979a,b). Serum lipid levels declined in cattle and sheep suffering from T. vivax and T. congolense infections (Roberts, 1973). This was attributed partly to haemodilution and partly to a direct effect of the trypanosome on the metabolism of the host. Gamma globulinaemia, hypoalbuminaemia and an increase in total serum proteins have also been reported (Clarkson, 1968;

Edward/

Edward, Judd and Squire, 1959; Desowitz, 1959; Dar, Paris and Wilson, 1973; Anosa and Isoun, 1976). Haemodilution was responsible for the fall in albumin as albumin catabolism was unchanged (Holmes, 1976; Anosa and Isoun, 1976). The total intravascular pool of albumin was unaltered despite marked alteration in the distribution of albumin between the intravascular and extravascular pools and a reduction in the total body pool of albumin (Holmes, 1976). The total serum protein was unaltered due to a corresponding rise in the globulin fraction (Holmes, 1976) though early in the infection Wellde et al (1974) reported a fall which gradually returned to normal as infection progressed. Haemodilution, increased protein catabolism and disturbances with absorption or metabolism were suggested as probable causes of the observed fall. The excessive protein catabolism caused a slight elevation of blood urea nitrogen (Wellde et al, 1974).

Immunosuppression:

Circumstantial evidence linking trypanosomiasis with immunosuppression, i.e. the depressive effect of the disease on the immune responsiveness of the host to a wide range of antigens, has existed for years when severe streptococcal infection was observed in sleeping sickness patients (Greenwood, 1968). Experimental evidence of trypanosome induced immunosuppression was first produced by Goodwin (1970) and Goodwin et al, (1972) when they found that following infection of mice and rats with T. brucei the cell-mediated haemaglutinin response to sheep red blood cells(SRBC) was significantly reduced. Allt et al (1971) failed to develop experimental allergic neuritis in T. brucei infected rabbits and concluded that humoral antibody responses were also depressed. Several workers have now confirmed and extended these preliminary observations in laboratory animals using either T. brucei or T. congolense and a variety of antigens (Albright, Albright and Dusanic, 1977; Cox, 1975; Murray et al, 1973; Urquhart et al, 1973; Freeman/

Freeman et al, 1973; Mansfield and Wallace, 1974; Pearson et al, 1978a,b; Whitelaw et al, 1979; Roelants et al, 1979a,b). Extension of these investigations to large animals showed that Zebu cattle experimentally infected with T. congolense showed profound immunodepression to a polyvalent clostridial vaccine (Holmes et al, 1974), to Foot and Mouth disease and/or clostridial vaccine (Scott et al, 1977), to louping-ill virus vaccine (Whitelaw et al, 1979), and in sheep infected with T.congolense (Mackenzie et al, 1975), similar to the findings in laboratory animals. The mechanism underlying this phenomenon remains largely undefined.

The first probable mechanism implicates hyperglobulinaemia with immunodepression. Raised immunoglobulins (IgG and IgM), especially IgM, has been shown as a feature of the disease in laboratory and domestic animals (Mattern et al, 1961; Houba, Brown and Allison, 1969; Luckins, 1972a,b, 1974, 1976; Murray et al, 1974b; Clarkson and Penhale, 1973; Hudson et al, 1976; Pearson et al, 1978a,b; Kobayashi and Tizard, 1976; Greenwood, 1974). Murray et al (1974b) and Hudson et al (1976) observed high IgM levels only early in the infection while Ogilvie (1965) observed a post-acute persistance of high IgM levels which Brown (1967); Seed et al (1969) and Seed (1972) ascribed to the continuous antigenic variation exhibited by trypanosomes to which the host responded by producing new IgM antibodies. Malaria parasites exhibit similar antigenic variations but IgM levels are not excessively increased (Brown, 1971). However, hyperimmunoglobulinaemia appears dependent on the presence of the parasite as trypanocidal drugs cause a fall in the immunoglobulin levels (Luckins, 1974, 1976). The probable link was first suggested by Urquhart et al (1973) and later Hudson et al (1976), i.e. that trypanosomes contain a B-cell mitogen which caused polyclonal B cell stimulation giving rise to antibodies of many specifications (both specific/

specific and non-specific) and ultimately clonal exhaustion superseded and this caused the state of immunosuppression. They based their conclusions on the observation of reduced plaque forming cell response to SRBC as well as elevated background plaque forming cell responses to a variety of antigens in T. brucei infected mice. Corsini et al (1977) provided support when he showed high levels of IgM and IgG in spleen cells removed during the early phase of T. brucei infection in mice. The levels declined as infection progressed and stimulation by the B cell mitogen lipopolysaccharide (LPS) failed to elicit any further increase. The incorporation of 3H-labelled leucine by spleen cells was used as an index of immunoglobulin synthesis. The search for a putative in vivo B cell mitogen has so far been unsuccessful though Mansfield, Craig and Stelzer (1976) showed that trypanosomal extracts exerted a weak mitogenic effect and Clayton et al (1979), Esuruoso (1976), Greenwood and Odulojo (1978) attempted characterizing the nature of B cell mitogens but with limited success.

Chronically T. brucei infected mice suffered a reversal of immunosuppression within 48 hours of treatment with a trypanocidal drug (Murray et al, 1974b) implying that lymphocyte dysfunction was not solely responsible for immunosuppression as these mice were envisaged to be in a state of clonal exhaustion. Primi et al (1977) postulated that the stimulation of B cells by a mitogen might by-pass the normal regulatory functions of T-cell subpopulations (Helper and suppressor cells) on B cells and lead to the production of autoantibodies. These autoantibodies had earlier been demonstrated by Mackenzie and Boreham (1974), Mansfield and Kreier (1972), and Seed and Gam (1967). Mitry-Martin et al (1979) showed that these autoantibodies first appear 20-25 days after infection and therefore may not play any role in the immunosuppression seen in early trypanosomiasis/

trypanosomiasis usually from day 6 of infection. Though their production has been established, their *in vivo* effects and role in the severe lymphoid depletion seen in chronic infections have not been demonstrated. As previously stated, the T-cell subpopulations, i.e. helper cells and suppressor cells, are involved in the T-cell and B-cell immunoregulatory mechanisms. Any of these regulatory pathways can become defective. Following the immunosuppression produced with a variety of antigens by Hudson *et al* (1976) and Terry (1977), they concluded that an exaggeration of non-specific suppressor cell activity was responsible. This was supported by the finding of Jayawardena and Waksman (1977) that, following stimulation by a variety of antigens, the addition of T-lymphocytes from *T. brucei* infected mice inhibited the uptake of ^{3}H-labelled thymidine by normal lymphocytes *in vitro*. The same author and others in 1978 (Jayawardena *et al*, 1978) observed enhancement of antibody response to SRBC in early trypanosomiasis and incriminated helper cell activity while later infections exhibited diminished responses which they attributed to non-specific suppressor cell activity. Hyperimmunoglobulinaemia was attributed to the activation of helper T-cells which non-specifically stimulated B cells to produce IgM and IgG (polyclonal B cell activation) while immunodepression was explained on the basis of non-specific suppressor cell stimulation. Similar conclusions resulted from the works of Albright, Albright and Dusanic (1977); Roelants *et al* (1979 a,b) and Pearson *et al* (1978, 1979). T-cell failure rather than excessive T-cell activity was held responsible for immunosuppression and polyclonal B cell activation by Mansfield, Craig and Stelzer (1976) and Mansfield and Bagasra (1978). Though altered T-suppressor, T-helper, B-memory cell functions have been demonstrated (Corsini *et al*, 1977; Askonas *et al*, 1979) *in vitro*, the search for a probable trypanosome antigen which could cause these alterations in function *in vivo* remains elusive. Furthermore, Clayton, Ogilvie and Askonas (1979) showed that B cells are directly affected in trypanosomiasis when they demonstrated hyperimmunoglobulinaemia and

immunodepression/

immunodepression in nude mice which possess few, if any, T-cells. The role of each lymphocyte population or sub-population in immunodepression thus remains unclear.

Macrophages have also been implicated in immunosuppression by Corsini et al (1977) when they demonstrated that macrophages from the peritoneal cavity of T. brucei infected mice had suppressor properties. However, Goodwin (1970); Murray et al (1974b); Jayawardena et al (1978) stated that macrophages were not functionally impaired in trypanosomiasis. Trypanosomes are postulated to place a massive antigenic load on host immune system (Taussing and Lachman, 1972) which give rise to antigenic competition purportedly on macrophage receptor sites. When such sites are saturated, a state of immunodepression was produced.

The finding by Nielsen et al (1978) that cattle infected with T. congolense showed amongst other things reduced circulatory C_3 levels led them to suggest that C_3 reduction may contribute to immunosuppression. Complement (C_3) normally play a role in induction of immune responses. Hudson and Terry (1979) failed to find reduced C_3 levels in mice infected with a chronic strain of T. brucei which was attributed to the observed fact that mouse IgM antigen complexes were known to be poor activators of complement (Klaus et al, 1979) . The role of complement in immunodepression still remains undefined as immunosuppression and normal C_3 levels have been shown to co-exist (Hudson and Terry, 1979).

Certain polyunsaturated fatty acids (PUFA) have been found to depress the immune responses of mice (Mertin and Hughes, 1975). The observation that pathogenic T. congolense released free PUFA while the non-pathogenic T. lewisi did not (Tizard et al, 1977) led to the postulation that the distrubance of lipid metabolism may contribute to immunosuppression. Tai, Gibson and Terry (1979) recovered higher amounts of PUFA in T. congolense than in T. lewisi infections but could find no relationship/

relationship to immunodepression and actually found that serum levels of these PUFA were decreased. Increased immunoglobulin catabolism, as measured by a reduction in half-life of labelled immunoglobulins in cattle infected with T. congolense, was observed by Nielsen et al (1978) and this was postulated as contributing to the immunodepression observed in such infections, as previously reported by Jennings et al (1973).

Whether any of the different mechanisms reviewed acting singly or in concert or yet unknown mechanisms are involved in immunosuppression is a matter of conjecture. What is certain is that immunosuppression is a well recognised field phenomenon in bovine trypanosomiasis and its significance lies in its detrimental effect on the host. Such deleterious effects possibly include:-

(a) increased susceptibility to secondary infections (Holmes et al, 1974);

(b) interference with response to vaccination (Holmes et al, 1974), although Scott et al (1977) showed that the immunosuppression observed in T. congolense infected cattle in an endemic area of Ethiopia was unlikely to impede the development of protection against Foot and Mouth Disease and clostridial infections by vaccination since titres found were invariably above the levels necessary to give the required protection. Later work by Whitelaw et al (1979) showed that trypanocidal chemotherapy (diminazene aceturate) administered on the same day as vaccination largely restored the competence of the immune response of both mice and cattle infected with T. congolense and they advocated the use of such drugs during vaccinations against trypanosomiasis in endemic areas.

(c) interference with optimal immune responses to homologous parasites. Hudson et al (1976) observed such interference in mice infected with a sub-acute strain of T. brucei following the first wave of parasitaemia while Terry (1977) could observe no such interference in chronic trypanosome infections when animals are already immunodepressed.

The/

The nature of immunodepression may be different in T. brucei infections from the plasma parasites T. vivax and T. congolense as confirmed by Whitelaw et al (1979) when they observed 90% immunosuppression in T. congolense and T. vivax infected cattle to Louping-ill virus vaccine while the response in T. brucei infected cattle was not significantly reduced. This was attributed to the relatively light and transient parasitaemias exhibited by the T. brucei infected cattle. Findings from abnormal laboratory hosts may not be applicable to situations in normal hosts where parasitaemias are relatively low and chronic infections more common. More information is continually becoming available to unravel the complex phenomenon of immunodepression and the mechanisms involved.

Antigenic Variation:

Salivarian trypanosomes exhibit marked antigenic variants (Gray, 1965, and Vickermann, 1965) designed to evade host immunity. These variable antigens were first shown to be proteins (Brown and Williamson, 1962; Williamson and Brown, 1964) and later glycoproteins (Allsop and Njogu, 1974). The variation involved a change of the glycoprotein molecules constituting the cell surface coat of the trypanosome. Antibodies produced against the preceding variants were not reactive against the new variant (Salaman and Wedderburn, 1966; Barry, 1977). Although antigenic variation has been described in T. congolense (Wilson and Cunningham, 1972; Clarkson and Awan, 1969), and in T. vivax (Jones and Clarkson, 1972; De Gee, Shah and Doyle, 1979), only in T. brucei was the immunochemistry of the surface coat which appeared to be the site of the variable glycoprotein antigen (Cross, 1975; Rovis and Barbet, 1977) studied in great detail (Brown, 1977). Evidence of a protective response which transcends antigenic variation and is T-cell dependent has been shown in malaria infected hosts (Brown, 1976; Brown, Jarra and Hills, 1976). Similar protection studies in animals have been hampered by the inability of finding natural host-parasite/

host-parasite models. Limited evidence from game animals (Ashcroft, Burtt and Fairbairn, 1959) has shown that this protection may in fact occur though weak compared with malaria. The phenomenon of antigenic variation has thwarted vaccination efforts against African trypanosomiasis (Barry, Hajduk, Vickermann and Le Ray, 1979).

REFERENCES.

ALLBRIGHT, J.F., ALBRIGHT, J.W. and DUSANIC, D.G. (1977)
J. Reticuloendothelial Soc., 21, 21-31.

ALLSOPP, B.A. and NJOGU, A.R. (1974)
Parasitology, 69, 271-278.

ALLT, G., EVANS, E.M.E., EVANS, D.H.I. and TARGETT, G.A.T. (1971)
Nature, Lond., 233, 197-199.

ANOSA, V.O. (1975)
M.V.M. Thesis, University of Glasgow, Scotland.

ANOSA, V.O. (1977)
Cited by McCrorie, P., Jenkins, G.C., Brown, J.L. and Ramsay, C.E.(1980)
J. Comp. Path., 90, 123-137.

ANOSA, V.O. and ISOUN, T.T. (1976)
Trop. Anim. Hlth. Prod., 8, 14-19.

ANOSA, V.O. and ISOUN, T.T. (1980)
J. Comp. Path., 90, 155-168.

ANOSA, V.O., JENNINGS, F.W. and URQUHART, G.M. (1977)
J. Comp. Path., 87, 569-580.

ASHCROFT, M.I., BURTT, E. and FAIRBAIRN, H. (1959)
Ann. trop. Med. Parasit., 53, 147-161.

ASKONAS, B.A., CONSINI, A.C., CLAYTON, C.E. and OGILVIE, B.M. (1979)
Immunology, 36, 313 - 321.

BALBER, A.E. (1974)
Expt. parasit., 35, 209-218.

BARRETT-CONNOR, E., UGORETZ, R.J. and BRAUDE, A.I. (1973)
Arch. intern. Med., 131, 574-577.

BARRY, D.J. (1977).
J. Cell. Sci., 15, 1-16.

BARRY J.D., HAJDUK, S.L., VICKERMANN, K. and LE RAY, D. (1979)
Trans. Roy. Soc. trop. Med. Hyg., 73, 205 - 208.

BOREHAM, P.F.L. (1967)
Trans. Roy. Soc. trop. Med. Hyg., 61, 138.

BOREHAM, P.F.L. (1968)
Br. J. Pharmac. Chemother., 32, 493-504.

BOREHAM, P.F.L. and FACER, C.A. (1974)
Int. J. Parasitol., 4, 143-151.

BOREHAM, P.F.L. and GOODWIN, L.G. (1967)
Int. Scient. Com. Trypanosom., XI(100), 83.

BOREHAM, P.F.L. and KIMBER, C.D. (1970)
Trans. Roy. Soc. trop. Med. Hyg., 64, 168-169.

BOULTON, F.E., JENKINS, G.C. and LLOYD, M.J. (1974)
Trans. Roy. Soc. trop. Med. Hyg., 68, 153-154.

BOYCOTT, A.E. and PRICE-JONES, C. (1913)
J. Path. Bact., 17, 347-366.

BROWN, K.N.(1967)
in: "Immunologic Aspects of Parasitic Infections".
Pan American Health Organisation publication, No. 150.

BROWN, K.N. (1976)
Resistance to malaria. In: Immunologic Aspects of Parasitic Infections".
(Cohen, S. and Sadun, K. Eds.) 268-295. Blackwell London.

BROWN, K.N. (1977)
Immunity in Parasitic infections.
INSERM., 72, 59-70.

BROWN, K.N. (1971)
Nature, 230, 163-167.

BROWN, K.N., JARRA, W. and HILLS, L.A. (1976)
Inf. immun., 14, 858-871.

BROWN, K.N. and WILLIAMSON, J. (1962)
Nature (Lond.), 194, 1253-1255.

CHANDLER, R.L. (1958)
J. Comp. Path., 68, 253-260.

CLARKSON, M.J. (1968)
J. Comp. Path., 78, 189-193.

CLARKSON, M.J. and AWAN, M.A. (1969)
Ann. trop. Med. Parasit., 63, 515-527.

CLARKSON, M.J. and PENHALE, W.J. (1973)
Trans. Roy. Soc. trop. Med. Hyg., 67, 273.

CLAYTON, C.E., OGILVIE, B.M. and ASKONAS, B.A. (1979)
Parasit.Immunol., 1, 39-48.

CLAYTON, C.E., SACKS, D.L., OGILVIE, B.M. and ASKONAS, B.A. (1979)
Parasit. Immunol., 1, 39-48.

CORSINI, A.C., CLAYTON, C.E., ASKONAS, B.A. and OGILVIE, B.M. (1977)
Clin. exp. Immunol., 29, 122-131.

COX, F.E.G. (1975)
Nature, 258, 148-149.

CROSS, G.A.M. (1975)
Parasitology, 71, 393-417.

DAR, F.K., PARIS, J. and WILSON, A.J. (1973)
Trans. Roy. Soc. trop. Med. Hyg., 67, 287.

DARGIE, J.D., MURRAY, P.K., MURRAY, M. and McINTYRE, W.I.M. (1979a)
Res. vet. Sci., 26, 245-247.

DARGIE, J.D., MURRAY, P.K., MURRAY, M., GRIMSHAW, W.R.T. and McINTYRE, W.I.M. (1979b)
Parasitology, 78, 271-286.

DESOWITZ, R.S. (1959)
Ann. trop. Med. Parasit., 53, 293-313.

DAVIS, C.E., ROBBINS, R.E., WELLER, R.D. and BRAUDE, A.I. (1974)
J. Clin. Invest., 53, 1359-1367.

DODD, B.E., JENKINS, G.C., LINCOLN, P.J. and McCRORIE, P. (1978)
Trans. Roy. Soc. trop. Med. Hyg., 72, 501-505.

EDWARDS, E.E., JUDD, J.M. and SQUIRE, F.A. (1956a)
Ann. trop. Med. Parasit., 50, 233-240.

EDWARDS, E.E., JUDD, J.M. and SQUIRE, F.A. (1956b)
Ann. trop. Med. Parasit., 50, 242-251.

EDWARDS, E.E., JUDD, J.M. and SQUIRE, F.A. (1959)
W. Afr. Sci. Assoc. Journal, 5, 158-167.

ESURUOSO, G.O. (1976)
Clin. exp. Immunol., 23, 314-317.

FIENNES, R.N.T.W. (1950)
Ann. trop. Med. Parasit., 44, 42-54.

FIENNES, R.N.T.W. (1952)
Trans. Roy. Soc. trop. Med. Hyg., 46, 462-463.

FIENNES, R.N.T.W. (1954)
Vet. Rec., 66, 423-434.

FIENNES, R.T.W. (1970)
in: "The African Trypanosomiasis", Ed. W.H. Mulligan and W.H. Potts,
p. 279, George Allen and Unwin Ltd., London.

FIENNES, R.N.T.W., JONES, E.R. and LAWS, S.G. (1946)
J. Comp. Path. Ther., 56, 1-27.

FREEMAN, J., HUDSON, K.M., LONGSTAFFE ,J.A. and TERRY, R.J. (1973)
Parasitology, 67, 23.

FRENCH, M.H. (1938)
J. Comp. Path., 51, 269-281.

de GEE, A.L.W., SHAH, S.D. and DOYLE, J.J. (1979)
Exp. Parasit., 48, 352-358.

GOODWIN, L.G. (1970)
Trans. Roy. Soc. trop. Med. Hyg., 64, 797-817.

GOODWIN, L.G. (1971)
Trans. Roy. Soc. trop. Med. Hyg., 65, 82-88.

GOODWIN, L.G., GREEN, D.G., GUY, M.W. and VOLLER, A. (1972)
Brit. J. exp. Path., 53, 40-43.

GRAY, A.R. (1965)
J. Gen. Microbiol., 41, 195-214.

GREENWOOD, B.M. (1968)
Lancet, ii, 380-382.

GREENWOOD, B.M. (1974)
Lancet, i, 435-436.

GREENWOOD B.M. and ODULOJO, A.J. (1978)
Trans. Roy. Soc. trop. Med. Hyg., 72, 408-411.

HERBERT, W.J. and INGLIS, M.D. (1973)
Trans. Roy. Soc. trop. Med. Hyg., 67, 268.

HOLMES, P.H. (1976)
in: Nuclear Techniques in Animal Production and Health",
p. 463-474, I.A.E.A., Vienna.

HOLMES, P.H. and JENNINGS, F.W. (1976)
in: Pathophysiology of Parasitic Infections, Ed. E.J.L. Soulsby,
p. 149-210, Academic Press, N. York, San Francisco, London.

HOLMES, P.H. and MAMO, E. (1975)
Trans. Roy. Soc. trop. Med. Hyg., 69, 274.

HOLMES, P.H., MAMO, E., THOMSON, A., KNIGHT, P.A., LUCKEN, R.,
MURRAY, P.K., MURRAY, M., JENNINGS, F.W. and URQUHART, G.M. (1974)
Vet. Rec. 95, 86-87.

HOLWILL, M.E.J. (1965)
Expert. Cell. Res., 37, 306-311.

HORNBY, H.E. (1921)
J. Comp. Path., 34, 211-240.

HORNBY, H.E. (1952)
in: "Animal trypanosomiasis in Eastern Africa",
H.M.S.O., London.

HORNBY, H.E. and BAILEY, H.W. (1931)
Trans. Roy. Soc. trop. Med. Hyg., 24, 557-563.

HOUBA, V., BROWN, K.N. and ALLISON, A.C. (1969)
Clin. exp. Immun., 4, 113-123.

HUAN, C.N., WEBB, L., LAMBERT, P.H. and MIESCHER, P.A. (1975)
Schweizerische Medizinische Wochenschrift, 122, 299-325.

HUDSON, J.A. (1944)
J. Comp. Path., 54, 108-119.

HUDSON, K.M., BYNER, C., FREEMAN, J.and TERRY, R.J. (1976)
Nature (Lond.), 264, 256-258.

HUDSON, K.M. and TERRY, R.J. (1979), Immunology, 317-326.

IKEDE, B.O. and LOSOS, G.J. (1972a)
Vet. Path. 9, 278-289.

IKEDE, B.O. and LOSOS, G.J. (1972b)
Vet. Path., 9, 272-277.

IKEDE, B.O. and LOSOS, G.J. (1972c)
Bull. epizoot. Dis. Afr., 20, 212-228.

IKEDE, B.O., LULE, M. and TERRY, R.J. (1977)
Acta. Tropica., 34, 53-60.

INGRAM, D.G. and SOLTYS, M.A. (1960)
Parasitology, 50, 231-239.

ISOUN, T.T. (1968)
Ann. trop. Med. Parasit., 62, 188-192.

ISOUN, T.T. (1975)
Acta. Tropica., 32, 267-272.

JAYAWARDENA, A.N. and WAKSMAN, B.H. (1977)
Nature (Lond.), 265, 539-541.

JAYAWARDENA, A.N., WAKSMAN, B.H. and EARDLEY, D.D. (1978)
J. immunol., 121, 622-628.

JENKINS, G.C., FORSBERG, C.M., BROWN, J.L. and BOULTON, F.E. (1974a)
Trans. Roy. Soc. trop. Med. Hyg., 68, 154-155.

JENKINS, G.C., FORSBERG, C.M., BROWN, J.L. and PARR, C.W. (1974b)
Trans. Roy. Soc. trop. Med. Hyg., 68, 154.

JENKINS, G.C., McCRORIE, C., FORSBERG, C.M. and BROWN, J.L. (1980)
J. comp. Path., 90, 107-121.

JENNINGS, F.W. (1976)
in: "Pathophysiology of Parasitic Infections", eds. E.J.L. Soulsby, Academic Press Inc., N. York, San Francisco, London.

JENNINGS, F.W., MURRAY, P.K., MURRAY, M. and URQUHART, G.M. (1973)
Trans. Roy. Soc. trop. Med. Hyg., 67, 277.

JENNINGS, F.W., MURRAY, P.K., MURRAY, M. andURQUHART, G.M. (1974)
Res. vet. Sci., 16, 70-76.

JONES, T.W. and CLARKSON, M.J. (1972)
Ann. trop. Med. Parasit., 66, 203-212.

KALINER, G. (1974)
Expl. Parasit., 36, 20-26.

KARLE, H. (1968)
Acta. Med. Scandinavia, 183, 587 592.

KLAUS, G.B.B., PEPYS, M.B., KITAJIMA, K., ASKONAS, B.A. (1979)
Immunology, 38, 687-695.

KOBAYASHI, A., TIZARD, I.R. and WOO, P.T.K. (1976)
Am. J. trop. Med. Hyg., 25, 401-406.

LESTER, M.O. (1932)
Cited by Losos, G.J. and Ikede, B.O. (1972)
Vet. Path. Suppl. ad. 9, 1-71.

LOSOS, G.J. and IKEDE, B.O. (1970)
Canad. J. Comp. Med., 34, 209-212.

LOSOS, G.J. and IKEDE, B.O. (1972)
Suppl. ad. Vet. Path., 9, 1-71.

LOSOS, G.J., PARIS, J., WILSON, A.J. and DAR, F.K. (1973)
Bull. epizoot. Dis. Afr. 21, 239-244.

LUCKINS, A.G. (1972a)
Trans. Roy. Soc. trop. Med. Hyg., 66, 130-139.

LUCKINS, A.G. (1972b)
Br. Vet. J., 128, 523 528.

LUCKINS, A.G. (1974)
Trans. Roy. Soc. trop. Med. Hyg., 68, 148-149.

LUCKINS, A.G. (1976)
Ann. trop. Med. Parasitol., 70, 133.

LUMSDEN, W.H.R. (1968)
in: "Infectious Blood Diseases of Man and Animals", Vol. II,
eds. D. Weinman and M. Ristic, Academic Press, New York and London.

MacKENZIE, A.R. and BOREHAM, P.F.L. (1974)
Immunology, 26, 1225-1238.

MacKENZIE, A.R. BOREHAM, P.F.L. and FACER, C.A. (1972)
Trans. Roy. Soc. trop. Med. Hyg., 66, 344.

MacKENZIE, A.R., BOREHAM, P.F.L. and FACER, A (1973)
Trans. Roy. Soc. trop. Med. Hyg., 67, 268.

MacKENZIE, P.K.I., BOYT, W.P., EMSLIE, V.M., LANDER, K.P. and
SWANEPOEL, R. (1975)
Vet. Rec., 97, 452 453.

MacKENZIE, P.K. and CRUICKSHANK, J.G. (1973)
Res. vet. Sci., 15, 256-262.

MacLENNAN, K.J.A. (1970)
in: "The African Trypanosomiases", ed. W.H. Mulligan.
Allen and Unwin, London, 799-821.

MAMO, E. and HOLMES, P.H. (1975)
Res. Vet. Sci., 18, 105-106.

MANSFIELD, J.M. and BAGASRA, O. (1978)
J. immunol., 120, 759-765.

MANSFIELD, J.M. and KREIER, J.P. (1972)
Infect. Immun., 5, 648-656.

MANSFIELD, J.M. and WALLACE, J.H. (1974)
Infect. Immun., 10, 335-339.

MANSFIELD, J.M., CRAIG, S.A. and STELZER, G.T. (1976)
Infect. immun., 14, 976-981.

MARUTA, H. and MIZUNO, D. (1971)
Nature (Lond.), 234, 246 -252.

MATTERN, P., MASSEYEFF, R., MICHEL, R. and PERETTI, P. (1061)
Ann. Inst. Pasteur, 101, 382-388.

MAXIE, M.G., LOSOS, G.J. and TABEL, H. (1976)
in: "Pathophysiology of Parasitic Infections", ed. E.J.L. Soulsby, pp. 183-198.
Academic Press, N. York.

McCRORIE, P., JENKINS, G.C., BROWN, J.L. and RAMSEY, C.E. (1980)
J. Comp. Path., 90, 123-137.

MERTIN, J. and HUGHES, D. (1975)
Int. Archs. Allergy Appl. Immun., 48, 203-211.

MITRY-MARTIN, L., HUDSON, K.M. and TERRY, R.J. (1979)
Trans. Roy. Soc. trop. Med. Hyg., 73, 98-99.

MURRAY, P.K. (1974a)
Ph.D. Thesis, University of Glasgow, Scotland.

MURRAY, M. (1974b)
in: "Progress in Immunology, II", eds. L. Brent and J. Holbrow,
4, 181-192.

MURRAY, M., MURRAY, P.K., JENNINGS, F.W., FISHER, E.W. and URQUHART, G.M. (1974a)
Res. Vet. Sci., 16, 77-84.

MURRAY, P.K., JENNINGS, F.W., MURRAY, M. and URQUHART, G.M. (1974b)
Immunology, 27, 815-824.

MURRAY, P.K., MURRAY, M., WALLACE, M., MORRISON, W.I. and McINTYRE, W.I.M.(1979)
Int. Sci. Council for tryps. Research and Control, 15th Meeting.
The Gambia OAU/STRC.

MURRAY, P.K., URQUHART, G.M., MURRAY, M. and JENNINGS, F.W. (1973)
Trans. Roy. Soc. trop. Med. Hyg., 67, 267.

NAYLOR, D.C. (1971a)
Trop. Anim. Hlth. Prod., 3, 95-100.

NAYLOR, D.C. (1971b)
Trop. Anim. Hlth. Prod., 3, 159-168.

NAYLOR, D.C. (1971c)
Trop. Anim. Hlth. Prod., 3, 203-207.

NIELSEN, K., SHEPPARD, J., HOLMES, W. and TIZARD, I. (1978)
Immunology, 35, 811-816.

NIELSEN, K., SHEPPARD, J., HOLMES, W. and TIZARD, I. (1978)
Immunology, 35, 817.

OGILVIE, B.M. (1965)
Parasitology, 55, 325-335.

PARKINS, B.S. and HORNBY, H.E. (1930)
cited by Losos, G.J. and Ikede, B.O.,
Suppl. ad. Vet. Path, 9, 1-71.

PEARSON, T.W., ROELANTS, G.E., LUMSDEN, L.B. and MAYOR-WITHEY, K.S. (1978a)
Euro. J. Immunol., 8, 723-727.

PEARSON, T.W., ROELANTS, G.E., PINDER, M., LUNDIN, L.B. and MAYOR-WITHEY, K.S. (1978b)
Euro. J. Immunol., 9, 200-204.

PRESTON, J.M. and WELLDE, B.T. (1976)
in: "Walter Reed Army Institute of Research",
DAMD, 17-76-9412.

PRIMI, D., EDWARD-SMITH, C.I., HAMMARSTROM, L. and MOLLER, G. (1977)
Cell. immun., 32, 232-243.

RICHARDS, W.H.G. (1965)
Brit. J. pharmac. Chemother., 24, 124-131.

RICHMOND, J., DONALDSON, G.W.K., WILLIAMS, R., HAMILTON, P.J.S. and HUNT, M.S.R. (1967)
Br. J. Haem., 13, 348-363.

ROBERTS, C.J. (1973)
Trans. Roy. Soc. trop. Med. Hyg., 67, 11.

ROBERTS, C.J. and GRAY, A.R. (1973)
Trop. Anim. Hlth. Prod., 5, 211-213.

ROELANTS, G.E., PEARSON, T.W., TYRER, H.W., MAYOR-WITHEY, K.S. and LUNDIN, K.S. (1979a)
Europ. J. Immunol., 9, 195.

ROELANTS, G.E., PEARSON, T.W., MORRISON, W.I., MAYOR-WITHEY, K.S. and LUNDIN, L.B. (1979b)
Clin. exp. Immunol., 37, 457-469.

ROVIS, L. and BARBET, A.F. (1979)
in: Proc. 5th Int. Congress of Protozoology, ed. Hunter, S.H. p. 242.

SADUN, E.H., JOHNSON, A.J., NAGLE, R. and DUXBURY, R.E. (1973)
Amer. J. trop. Med. Hyg., 22, 323-330.

SALAMAN, M.H. and WEDDERBURN, W. (1966)
Immunology, 10, 445-458.

SAROR, D.I. (1976)
Vet. Rec., 98, 196.

SAROR, D.I. (1979)
Vet. Rec., 105, 96-98.

SCHNAPPAUF, H., STEIN, H.B., SIPE, C.R. and CRONKITE, E.P. (1967)
Amer. J. Vet. Res., 28, 275-278.

SCOTT, J.M., PEGRAM, R.G., HOLMES, P.H., PAY, T.W.F., KNIGHT, P.A., JENNINGS, F.W. and URQUHART, G.M. (1977)
Trop. Anim. Hlth. Prod., 9, 159-165.

SEED, J.R. (1969)
Exp. parasit., 26, 214-223.

SEED, J.R. (1972)
Exp. Parasit., 31, 98-108.

SEED, J.R. and GAM, A.A. (1967)
J. Parasit., 53, 946-950.

SEED, J.R., CORNILLE, R.L., RISBY, E.L. and GAM, A.A. (1969)
Parasitology, 59, 283-292.

STEWART, J.L. (1951)
Vet. Rec., 63, 454-457.

TAI, P.E., GIBSON, M.R. and TERRY, R.J. (1979)
Trans. Roy. Soc. trop. Med. Hyg., 73, 99.

TARTOUR, G. and IDRIS, O.F. (1973)
Res. vet. Sci., 15, 24-32.

TAUSSING, M.J. and LACHMANN, P.J. (1972)
Immunology, 22, 185-197.

TERRY, R.J. (1977)
In: "Les Colloques de l'Institut National de la Sante et de la Recherche Mediale", 72, 161-178.

TIZARD, I.R., NIELSEN, K., MELLORS, A. and ASSOKU, R.K. (1977)
Lancet, April, 750-751.

TIZARD, I.R., SHEPPARD, J. and NIELSEN, K. (1978)
Trans. Roy. Soc. trop. Med. Hyg., 92, 198-200.

URQUHART, G.M., MURRAY, M., MURRAY, P.K., JENNINGS, F.W. and BATES, E. (1973)
Trans. Roy. Soc. trop. Med. Hyg., 67, 528-535.

VALLI, V.E.O., FORSBERG, C.M. and LUMSDEN, J.H. (1979)
Vet. Path., 16, 96-107.

VALLI, V.E.O., FORSBERG, C.M. and McSHERRY, B.J. (1978)
Vet. Path., 15, 732-745.

VALLI, V.E.O. and MILLS, J.N. (1980)
Vet. Path., 16, 96-107.

VALLI, V.E.O. and MILLS, J.N. (1980)
Tropenmed. Parasit., 31, 215-231.

VAN den INGH, T.S.G.A.M., ZWART, D., SCHOTMAN, A.J.H., VAN MIERT, A.S.J.P.A.M. and VEENENDAHL, G.H. (1976)
Res. vet. Sci., 21, 264-270.

VICKERMANN, K. (1978)
Nature (Lond.), 273, 613-617.

VOHRADSKY, F. (1971)
Rev. Elev. Med. Vet. pays. trop., 24, 251-263.

WELLDE, R.L., DEINDL, G., SADUN, E., WILLIAMS, J. and WARUI, G. (1974)
Exp. Parasit., 36, 6-19.

WILLIAMSON, J. and BROWN, K.N. (1964)
Exp. Parasit., 15, 44-68.

WILSON, A.J. and CUNNINGHAM, M.P. (1972)
Exp. Parasit., 32, 165-173.

WILSON, S.G., MORRIS, K.R.S., LEWIS, I.J. and KROG, E. (1963)
Bull. Wld. Hlth. Org., 28, 595-603.

WOLBACH, S.B. and BINGER, C.A. (1912)
J. Med. Res., 27, 83-107.

WOO, P.K.T. and KOBAYASHI, A. (1975)
Ann. Soc. Belge. Med. trop., 55(1), 37-45.

WOODRUFF, A.W. (1959)
Trans. Roy. Soc. trop. Med. Hyg., 53, 327.

WOODRUFF, A.W. (1973)
Trans. Roy. Soc. trop. Med. Hyg., 67, 313-325.

WOODRUFF, A.W., ZEIGLER, J.L., HATHAWAY, A. and GWATA, T. (1973)
Trans. Roy. Soc. trop. Med. Hyg., 67, 329-337.

ZOUTENDYK, A. and GEAR, J. (1951)
S. Afr. Med. J., 25, 665.

TIZARD, I.R., SHEPPARD, J. and NIELSEN, K. (1978)
Trans. Roy. Soc. trop. Med. Hyg., 72, 198-200.

WHITELAW, D.D., SCOTT, J.M., REID, H.W., HOLMES, P.H., JENNINGS, F.W. and URQUHART, G.M. (1979)
Res. vet. Sci., 26, 102-107.

CHAPTER III

THE APPLICATION OF RADIOISOTOPES TO THE STUDY OF PARASITIC ANAEMIAS

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Introduction:

An animal is regarded as anaemic when the packed cell volume (PCV) and/or haemoglobin concentration (Hb) per unit volume of blood falls below the accepted normal range for that particular species (Schalm, 1965). The anaemia can result from one or a combination of three basic mechanisms, namely:- haemodilution; increased red cell loss or breakdown due to haemorrhage, haemolysis or increased erythrophagocytosis; and reduced red cell synthesis (Dargie, 1975).

Many parasitic infections are characterised by a concomittant anaemia. Helminth parasites causing anaemia by a direct haematophagia include the hookworms of man and animals, Necator americanus, Ancylostoma duodenale, A. caninum, Uncinaria stenocephala, Gaigeria pachyscelis, Bunostomum trigonocephalum, B. phlebotomum (Ortlepp, 1937, 1939; Roche et al, 1957; Miller, 1966), and the stomach worms Haemonchus placei in calves (Delaune and Mayhew, 1943) and H. contortus in sheep (Veglia, 1915; Fourie, 1931; Andrews, 1942; Whitlock, 1950; Richards et al, 1954; Clark, Kiesel, Goby, 1964; Dargie and Allonby, 1975). Anaemia was reported in infections with the stomach worm Ostertagia circumcinta in sheep by Horak and Clark (1964) and O. ostertagi Type II in calves by Martin et al (1957) and Anderson et al (1965, 1969). Other nematode parasites implicated in the production of anaemias include Oesophagostomum radiatum in calves (Bremner, 1969, 1970), O. columbrianum in sheep (Horak and Clark, 1966; Dobson, 1967; Bawden, 1969), Charbertia ovina in lambs (Wetzel, 1931; Ross, Dow and Purcell, 1969; Herd, 1971) and in calves (Ross and Todd, 1968) Trichostrongylus axei (Gibson, 1954; Kates and Turner, 1960), T. colubriformis (Horak, Clark and Gray, 1968; Barker, 1973), Strongyloides papillosus (Turner, 1959) and the redworm infections of the genus Strongylus/

in horses (Jennings, 1968). Anaemia was associated with chronic infections of Fasciola gigantica in cattle by Bitakaramire (1970) and Hammond (1973) and in acute and chronic F. hepatica infections in sheep and rabbits (Stephenson, 1947; Jennings, Mulligan and Urquhart, 1956; Todd and Ross, 1965; Symons and Boray, 1967, 1968; Holmes et al, 1968; Holmes, 1969; Holmes and Maclean, 1969; Holmes, Maclean and Mulligan, 1971). Heavy infections with other flukes such as F. magna, Dicroecoelium dendriticum and Paramphistomum cervi can produce anaemia (Soulsby, 1965). Acute outbreaks of Schistosoma bovis and S. mattheei with accompanying anaemia have been reported in cattle and sheep (Strydom, 1963; Dargie, 1980). Laboratory studies with S. mattheei in rabbits and sheep confirmed anaemia as one of the major signs of infection (Preston, Dargie and Maclean, 1973a, b).

Heavy infestations with arthropod ectoparasites such as lice, fleas and ticks can lead to anaemia. Thus the sucking lice of cattle Haematopinus eurysternus were reported to cause a severe anaemia by Shermanchuk, Hauffe and Thomson (1960) and fleas in sheep and goats by Obasaju and Otesile (1979). Ticks feed exclusively on blood and anaemia has been reported in infestations with Boophilus decoloratus in horses (Theiler, 1921), B. microplus in cattle (Riek, 1957; Little, 1963; O'Kelly and Seifort, 1970), Ixodes ricinus in sheep (Heath, 1951) and Dermacentor andersoni (Philip, Jellison and Wilkins, 1935; Jellison and Kohl, 1938).

Anaemia is recognised as a cardinal sign in a variety of protozoal diseases such as Anaplasmosis of sheep and cattle (Piercy, 1956; Baker, Osebold and Christenseen, 1961; Kreier, Ristic and Schroeder, 1964; Anthony and Roby, 1966; Ristic, 1968), Eperythrozoonosis (Neitz, 1937; Littlejohns, 1960, 1966; Foggie and Nisbet, 1964; Rouse and Johnson, 1966), Babesiosis (Zuckermann, 1964; Jennings, 1976), Theileriosis (Hooshmand-Rad and Hawa, 1973) and trypanosomiasis (Fiennes, 1954, 1970; Naylor, 1971; Losos and Ikede, 1972; Holmes and Mamo, 1975; Saror, 1979; Anosa and Isoun, 1980).

Despite the wide variety of parasites known to cause anaemia, only in a few cases have the underlying mechanisms been established. This is because the type and severity of anaemia due to any particular species varies and depends on such factors as number of parasites and stage of infection, the nutritional status, age, breed and even haemoglobin type of the host. Moreover, as pointed out by Dargie (1975), conventional methods of classification based on parameters such as PCV, Hb concentration and red cell counts give little reference to the cause of the anaemia or changes in the total body pool. A proper understanding of how parasites cause anaemia in their hosts should therefore include a description of the physiological and biochemical alterations in the host which arise directly or indirectly from the presence of the parasites, which can only be achieved by resorting to the methods of isotopic labelling. Radioisotopes have provided a knowledge of the underlying changes in metabolic pools and rates of addition and withdrawal from these pools by synthesis and catabolism respectively. Such studies have provided a better understanding of the mechanisms of the anaemias seen in some complex host-parasite systems such as Fascioliasis, Haemonchosis, Schistosomiasis and Trypanosomiasis on which this review is based.

Methods of Investigation:

Isotopes for in-vivo labelling of red cells or protein must attach firmly to these cells or proteins without damaging or altering their life spans. Furthermore, when the cells become effete or destroyed, the label should not be reutilized or reabsorbed but excreted quantitatively in the urine or in the alimentary tract if haemorrhage occurred. Unfortunately there is no one label which meets all these requirements but studies with ^{59}Fe and ^{51}Cr have been of considerable aid in elucidating the mechanism of anaemias seen in some parasitic diseases.

Studies/

Studies with ^{51}Cr-labelled erythrocytes:

Gray and Sterling (1950) first demonstrated the marked affinity of erythrocytes for ^{51}Cr obtained as sodium chromate. This property was subsequently confirmed by Nechelles, Weinstein and Leroy (1953) and Mollison and Veali (1955). The chemistry of the labelling depends on the fact that anionic hexavalent chromate penetrated the cells, was reduced to cationic trivalent chromium which then combined with intracellular protein. The procedure was that recommended by Gray and Sterling (1950) and consists of incubating whole blood or washed red cells to which radioactive chromium (^{51}Cr) of known specific activity had been added, for 30 minutes at 37°C. or one hour at room temperature. Unbound isotope was removed by washing the cells several times with physiological saline and the cells resuspended in the original plasma or in physiological saline for intravenous injection. For erythrokinetic studies a measured volume of the labelled cell suspension is injected through a jugular catheter into parasitized and control animals and a blood sample (5-10ml.) taken at 5-15 minutes after injection depending on the species. Further samples are collected at 20, 30, 60, 90, 120 and 180 minutes post-injection, and thereafter daily for 10-30 days. Aliquots of the blood and the plasma are diluted to a suitable volume (10 ml) with 0.01m NaOH for radioactivity determinations. The total daily output of urine and faeces are measured and suitable aliquots prepared for counting. Standards for each animal serving as corrections for radioactive decay and changes in the instrument are prepared from the injected red cell suspension. Radioactivity determinations are then made on the appropriate samples of blood, plasma, urine, faeces and the standards in a suitable scintillation counter. Haematocrit estimations are carried out on all blood samples.

Red/

Red cell turnover calculations are made and the following parameters determined.

(a) Circulating red cell volume: This is reliably determined by dividing the radioactivity in counts/min. of the total volume of injected tag by the radioactivity of the recovered tag after equilibration (usually the first blood sample obtained post-injection) using the dilution principle. This volume can also be determined by using any of the counts of the samples obtained in the 6-12 minutes post-injection interval or extrapolating to zero time and using the appropriate count. However, Wetterfors (1965) showed that there was no significant differences in red cell volume obtained by either of the three methods provided the ^{51}Cr was evenly distributed at equilibration and was not degraded or excreted within this period. The blood volume is calculated from the red cell volume by dividing by the haematocrit (expressed as a percentage). Hodgetts (1961); Gillet and Halmagi(1966); Zehr et al (1969) reported sheep blood volume of 66.4 mg./kg., 63.5 mg/kg. and 70.6 mg./kg of body weight respectively using ^{51}Cr-labelled cells while Giles et al (1977) reported 53.5 ml/kg. of body weight using ^{59}Fe labelling technique. These differences may not be significant due to blood volume variations between individual sheep on per unit weight basis and on the amount of adipose tissue in each sheep (Zehr et al, 1969).

(b) Apparent half-life of red cells ($t\frac{1}{2}$): This is calculated from the slope of the line obtained by a semi-logarithmic plot of daily blood activity against time as the time taken for the activity to fall by 50%. The blood activity disappearance curve has a clear slow exponential phase after the initial rapid decline in sheep (Sewell et al, 1968) and rabbits (Maclean et al, 1968) but not in cattle (Bitakaramire, 1970) which makes calculation of red cell $t\frac{1}{2}$ after extrapolation more reliable in sheep and rabbits than in cattle. Two assumptions are inherent in the/

the calculations. Firstly, that the labelled cells are made up of one population which, in reality, is false. Since labelling was done *in vitro*, the labelled cells will therefore consist of a random population of cells of various ages and the slope of the disappearance curve represents the function of rate of removal of old cells through senescence and rate of random destruction of younger cells. Secondly, that the ^{51}Cr remained firmly bound to the red cells without eluting. Again, considerable elution has been demonstrated, particularly in ruminants where 40 60 % of the original activity was lost during the first 3-5 days post-injection (Drury and Tucker, 1958; Tucker, 1963; Schnappauf, di Giacomo and Cronkite, 1965; McSherry, Van Dreumel and Robinson, 1966a, b; Todd and Ross, 1966; Bitakaramire, 1970; Giles *et al*, 1975; Gulliani et al, 1975). For these reasons 'apparent half-life' is used in red cell $t\frac{1}{2}$ estimations by the ^{51}Cr technique. The lower survival time due to elution can be avoided by using other isotopes which have been shown to remain firmly bound to the cells without eluting such as F di-isopropyl fluorophosphate, i.e. DF ^{32}P (Endie, Smith and Brown, 1960; Tucker, 1963) and ^{59}Fe (Giles et al, 1977) in red cell $t\frac{1}{2}$ determinations, though these methods have other disadvantages.

(c) Blood loss into the gut or urine: Owen, Bollman and Grindlay (1954) administered ^{51}Cr-labelled cells to human patients suffering from hookworm infections and recovered 90% of the activity in the faeces which led them to the conclusion that this isotope can be used to quantitate blood loss into the gut as only a negligible proportion was reabsorbed. A faecal clearance of blood and red cells was calculated for each 24-hr. collection period by dividing the total daily faecal radioactivity by the activity per ml. of red cells during the collection period. These values represent the/

the amount of blood, or more accurately red cells, which have to appear in the gut to account for the faecal radioactivity. Similar clearance figures can be obtained for the urine. More studies on blood loss using ^{51}Cr-labelled red cells have been reported by Miller (1966) in dogs, Roche et al (1957) in man, Holmes et al (1968) in sheep and Maclean et al (1968) in rabbits. A small percentage absorption of ^{51}Cr was reported by Clark, Kiesel and Goby (1962)in sheep which could be overcome if labelled cells are in circulation. It thus appeared as if the presence of chromium in the blood inhibited the uptake of more chromium from the gut and a slight underestimate of the true leak can be envisaged if no chromium was in circulation. Todd and Ross (1966) concluded that the elution suffered by this isotope precluded its use in reliably estimating gastrointestinal blood loss in cattle. Subsequent studies in F. gigantica infected cattle by Bitakaramire, (1970) showed that ^{51}Cr was reliable in estimating faecal red cell clearance. Moreover, radioisotope lost by elution is excreted in the urine, hence complications can only arise if the parasites cause intravascular haemolysis whereby the isotope is also excreted by the same route, or if there is significant contamination of the faeces with urine. As pointed out by Jennings (1968), estimates of red cell leak are needed for comparative studies in parasitological work and provided sufficient time is allowed for the blood radioactivity to fall to the exponential part of the disappearance curve, the faecal radioactivity can be reasonably and reliably related to the blood activity.

By analysing the pattern of ^{51}Cr loss from the body, the part played by elution, haemolysis or haemorrhage in any parasite anaemia can be determined as elution or haemolysis leads to elevated renal excretion of isotope whereas when labelled cells are/

are lost into the gut, the isotope appears quantitatively in the faeces (Ebaugh et al, 1958; Jennings, 1968; Mulligan, 1973; Dargie, 1975).

Studies with Radioiron (^{59}Fe):

Radioiron was one of the first isotopes used in vivo labelling techniques to obtain information concerning red cell production in man and animals. The early works of Huff et al (1950), Wasserman et al (1952) showed that iron circulating in the plasma as a complex with transferrin was largely carried to the bone marrow for haemoglobin synthesis with no appreciable returns, i.e. the loss of radioiron from the plasma was exponential. They then concluded that with a knowledge of the rate of removal of radioiron transferrin from the plasma and of the level of iron in the plasma at that time, the amount of iron passing through the plasma per unit time (the plasma iron turnover rate) can be calculated. Since after incorporation into haemoglobin, the labelled iron appears in the erythrocytes, the rate of red cell iron utilisation can also be calculated from the rate of appearance of ^{59}Fe in the erythrocytes. Certain variables were found to influence these measurements apart from erythropoiesis. Plasma iron turnover rate can be affected by:- dilution of isotope in enlarged iron stores, utilisation of iron for synthesis of myoglobin and iron containing enzymes, the early recycling of iron from rapidly destroyed red blood cells and the incorporation of ^{59}Fe into the molecule of haemoglobin before separation of the plasma from the red cells (London, Shemin and Rittenburg, 1948; Radin, Rittenberg and Shemin, 1950; Shemin, London/

London and Rittenberg, 1950; Bothwell et al, 1956). In the calculation of the rate of red cell iron utilisation, the reflux of the iron carried initially to non erythroid sources back to the plasma and thence to the bone marrow was not taken into consideration. Despite these limitations, these measurements have been shown to yield valuable comparative information on erythropoiesis (Weinstein and Beutler, 1962; Jennings, 1968; FAO/IAEA, 1970, 1980). For ferrokinetic investigations the labelled transferrin solution containing the ^{59}Fe as ferric sulphate in isotopic solution was injected intravenously into parasitised and control animals. Blood samples were collected at :- 5, 15, 30, 45, 60, 90, 120 and 180 minutes post-injection and further daily samples for the next 30 days. The total output of faeces and urine during each 24-hr. period was measured and samples taken for radioactivity determinations. Suitable samples of blood, plasma, faeces, urine and standards prepared from the injected plasma were assayed using a scintillation spectrometer. Venous blood haematocrit, haemoglobin concentration and serum iron concentration were also estimated by conventional analytical methods. The following ferrokinetic and erythrokinetic indices can be calculated from the results:-

(a) Erythrocyte Survival ($t\frac{1}{2}$). This is calculated from the slope of the line obtained by a semi-logarithmic plot of net radioactivity counts for each blood sample against time, as the time taken for the activity to fall by 50%. The isotope has been used for red cell survival studies in man (Williams et al, 1972), cattle (Kaneko, 1963), sheep (Tucker, 1963; Giles et al, 1977). The isotope does not elute from cells and hence appeared more reliable than ^{51}Cr in red cell survival estimations in normal animals (Giles et al, 1977)/

1977) but, as shown by Gibson et al (1947), the isotope was reutilised for new haemoglobin synthesis when haemolysis occurred in vivo, thereby giving relatively higher survival figures.

(b) Plasma iron turnover rate (PITR): This was calculated from the rate of disappearance of ^{59}Fe from the plasma expressed as a half life ($t\frac{1}{2}$) value, together with the serum iron and venous haematocrit at time of injection using the formula of Bothwell, Hurtado, Donohue and Finch (1957),

$$\text{PITR} = \frac{\text{serum iron (mg./100 ml.)} \times 0.693 \times 1440}{t\frac{1}{2}\text{ (min.)}} \times \frac{100\text{-Hv}}{100}$$

and expressed as mg/day/100 ml. blood or mg/kg/day of blood.

(c) The total plasma iron turnover was calculated from the PITR and the blood volume.

(d) Percentage red cell iron utilisation: This was obtained by a plot of activity per ml. RBC against time, calculated from the radioactivity and haematocrit of each blood sample using the formula:-

$$\text{utilisation\%} = \frac{\text{Blood volume} \times {}^{59}\text{Fe activity/ml. blood}}{\text{Total } {}^{59}\text{Fe activity injected}} \times 100$$

(e) The red cell iron incorporation rate is calculated as the product of percentage utilisation and PITR expressed as mg/day.

(f) Iron lost into the faeces daily was obtained from the blood and faecal radioactivities by the method of Roche, Perez-Giminez and Levy (1957) where

$$\text{Faecal iron loss} = \frac{\text{Hb(gm \%)} \times 3.34 \times {}^{59}\text{Fe blood clearance}}{100}$$

(g) Faecal and urinary clearances of red cells and blood can be obtained as previously described for ^{51}Cr.

From these results, the role played by dyshaemopoiesis or increased erythropoiesis in the anaemia can be defined as increased erythropoiesis is usually accompanied by low or normal serum iron, rapid/

rapid ^{59}Fe disappearance rate, high PITR and red blood cell iron turnover rate and increased red cell iron utilisation, while dyshaemopoiesis is characterised by high or normal serum iron, normal or reduced PITR and red cell iron turnover rate and low red cell iron utilisation.

Double Labelling with ^{59}Fe and ^{51}Cr:

This can yield valuable information on intestinal iron loss and possible reabsorption. The two isotopes are injected simultaneously (Roche, Perez Giminez and Levy, 1957) and radioactivity determinations carried out on suitable volumes of blood, plasma, urine and faeces collected at regular intervals over 10-30 days. ^{51}Cr lost into the gut is not significantly reabsorbed, therefore the blood lost into the gut can be determined from the blood haemoglobin concentration and faecal output of ^{51}Cr expressed as a clearance of whole blood. The ^{59}Fe activity in the faeces which denotes the amount of iron lost into the faeces is quantified from the ^{59}Fe faecal blood radioactivity and blood haemoglobin level. The difference between the amount of iron excreted in the faeces and that lost into the gut gives an estimate of Hb-iron reabsorption. Georgi (1964), Georgi and Whitlock (1965, 1967) calculated erythrocyte loss and restitution in ovine haemonchosis under the assumption of insignificant iron reabsorption. Subsequent work by Dargie (1973) in ovine haemonchosis and by Holmes and Maclean (1969), Dargie et al (1970), Dargie (1975) in ovine fascioliasis showed significant reabsorption under conditions of severe anaemic stress.

Other isotopes have been used in investigating different facets of red cell and iron kinetics. Havesay et al (1944) estimated blood volumes using ^{32}P labelled erythrocytes in man. Blood loss in rabbits was/

was estimated with ^{32}P labelled red cells and ^{131}I labelled serum albumin by Jennings et al (1954, 1955, 1956). Both isotopes were not firmly bound and also labelled other cells following degradation. Glycine-2-^{14}C was used in erythrocyte survival estimations in man by Shemin and Rittenburg (1946), swine by Bush et al (1955) and in sheep by Judd and Matrone (1962). The isotope was not reutilised following degradation, hence appeared superior to ^{59}Fe in erythrocyte survival determinations. Immature erythrocytes were also labelled in vitro with Glycine-2-^{14}C and used for red cell life span studies (London, Shemin, Rittenberg, 1948). These immature erythrocytes were subsequently shown to possess a shorter life span than mature cells by Neuberger and Niven (1951).

^{59}Fe and ^{51}Cr have featured largely in elucidating the mechanisms of the anaemias seen in fascioliasis, haemonchosis, schistosomiasis and trypanosomiasis.

The Anaemia of Fascioliasis.

Fascioliasis occurs in two distinct forms, the acute syndrome caused by the migratory and feeding habits of immature flukes in the liver which could be fatal (Taylor, 1964, Boray, 1968, Roberts, 1968) and the more common chronic form which is associated with the presence of adult flukes in the bile ducts. Anaemia is encountered in both forms of the disease which in field outbreaks is initially normocytic and normochromic, later becoming macrocytic and hypochromic (Dargie, 1973). The aetiology of the anaemia was for a long time controversial due to differences in opinion about the feeding habits and nutrition of the flukes. Workers such as Stephenson (1947), Todd and Ross (1964), Symons and Boray (1968) were of the opinion that the anaemia resulted from a direct blood loss due to the haematophagic activities of the flukes, while others have suggested other mechanisms such as dyshaemopoiesis (Obara, Sonoda and Watanabe, 1964, Sinclair, 1964) and release

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of toxins capable of depressing erythropoiesis or causing haemolysis (Lapage, 1962, Sinclair, 1967, Dawes and Hughes, 1970). Evidence from the simultaneous use of conventional and radioisotopic techniques has tended to favour the theory that the liver fluke is haematophagic. This conclusion was based on the consistent differences in behaviour of ^{51}Cr and ^{59}Fe labelled cells in fluke infected and normal hosts. When red cells labelled with ^{51}Cr was injected into hosts harbouring populations of adult F. hepatica (12 weeks old infection), the loss of isotope from the circulation as expressed by $t\frac{1}{2}$ values was in all cases faster than in normal animals and the rate of removal correlated closely with the number of parasites in the bile ducts (Holmes et al, 1968, Sewell et al, 1968, Dargie et al, 1970, Dargie, 1975). The rapid loss of isotope from the circulation and the shortened red cell survival in parasitised animals was associated with the presence of isotope in the faeces. Thus faecal clearance of red cells was 18 ml. in parasitised animals and only 0.7 ml. in normal animals, while urinary excretion of isotope was similar in both groups (Dargie, 1975). Comparative studies in F. gigantica infected cattle by Bitakaramire (1970) showed that the use of red cell $t\frac{1}{2}$ values as an index of erythrocyte loss from the circulation was unreliable in the animals due to rapid elution of the isotope. Faecal clearance of red cells were, however, significantly greater in fluke infected cattle. These findings can only be explained on the basis of loss of erythrocytes into the gut, presumably via flukes and bile. Furthermore, there was good correlation between fluke burden, apparent $t\frac{1}{2}$, PCV and enteric haemorrhage as animals with the highest fluke burdens generally had the lowest PCVs, shortest $t\frac{1}{2}$ and severest blood loss, indicating that adult flukes suck blood and that this was primarily responsible for the anaemia. The onset of this blood loss was found to coincide with the time of entry and subsequent development

of the flukes within the biliary system from the 8th week onwards (Dargie et al, 1970, Dargie and Mulligan, 1970, Holmes et al, 1971). A minor anaemia evidenced by a shortened red cell $t\frac{1}{2}$ and slight fall in PCV was observed during fluke migration (from the 5th to 8th week) but there was no increase in faecal radioactivity as haemorrhage was not occurring into the bile ducts (Dargie, 1975). This anaemia merely reflected the passage of erythrocytes into the migratory tracts left by young burrowing flukes, while failure to demonstrate increased urinary activity during this period was attributed to some trapping of liberated ^{51}Cr in the liver. Other data from fluke infected animals are in support of the haematophagic activity of the flukes. Firstly, Urquhart (1956), Ross, Todd and Dow (1966) demonstrated higher radioactivity in flukes relative to bile, the presence of intact red cells in the caeca of flukes and a positive benzidine reaction in bile recovered from infected animals. Secondly, Dargie et al (1968) observed the cessation of haemorrhage and enteric red cell leak with concurrent return of haematological indices to normal values following anthelmintic removal of the flukes. Thirdly, Symons and Boray (1967) showed that the mucosa was often missing and replaced by a blood clot at the site of fluke attachment. Fourthly, the features of the anaemia, i.e. an initial normocytic normochromic response tending to macrocytosis and hypochromia can be explained on the basis of haemorrhagic mechanism.

Ferrokinetic studies in F. hepatica infected sheep and rabbits and control uninfected hosts are characterised by three main features. Firstly the amount of iron carried from the plasma to the marrow for haemoglobin synthesis daily was increased roughly four times in the infected animals and/

and this increment was accomplished by an enhanced plasma ^{59}Fe clearance detectable from about the 6th week of infection (Symons and Boray, 1968). Secondly, the rate of iron incorporation into new cells was greatly increased (Symons and Boray, 1968, Dargie and Mulligan, 1970, Holmes et al, 1971) showing that erythropoiesis rather than being depressed was greatly accelerated in fascioliasis. This conclusion was supported by the presence of reticulocytes in the blood of fluke infected rabbits and sheep (Urquhart, 1956, Reid et al, 1970) and by erythroid marrow hyperplasia (Ross, Todd, Dow, 1966, Sewell et al, 1968). Thirdly, a ten-fold increase in the rate of disappearance of ^{59}Fe-labelled erythrocytes from the circulation was observed in infected animals (Dargie, 1973). Most of the iron derived from these cells was not reabsorbed from the gut except in the most anaemic animals (Holmes and Maclean, 1969, Maclean et al, 1968). Such losses together with a terminal inappetence in infected animals (Berry and Dargie, 1978) can bring about serious depletion of iron stores and inability to compensate for the haemorrhage. A frank iron deficiency with serum iron levels as low as 40 μg% supervenes and is rapidly fatal. This stage is a reflection of the effects of the blood loss and not the direct cause of the anaemia.

Three basic disturbances to erythrocyte metabolism were observed in the anaemia of fascioliasis, i.e. an increased rate of red cell destruction, a compensatory increase in red cell synthesis and a continuous drain on body iron stores due to the blood-sucking activities of the parasites. Haemolysis or dishaemopoiesis played no role in the anaemia. Similar pathogenic mechanisms probably operate in Fasciola magna and Dicroecoelium dendriticum infections but no kinetic information is as yet available.

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The Anaemia of Schistosomiasis:

The importance of schistosomiasis is well recognised in various parts of Africa (Pitchford, 1963; Dinnik and Dinnik, 1965; Dargie, 1980) and the clinical, parasitological and gross pathological features of the disease have been described (McCully and Kruger, 1969; Preston, Dargie and Maclean, 1973a; Warren, 1973; Saad et al, 1980). The pathology of the disease includes an anaemia which initially is normocytic and normochromic (Malherbe, 1970; Preston, Dargie and Maclean, 1973a) but with a tendency towards being macrocytic and hypochromic in severely affected animals. Various mechanisms such as haemodilution, haemolysis, dyshaemopoiesis and haemorrhage have been implicated in the aetiology of the anaemia (Jamra, Maspes and Meira, 1964; McCully and Kruger, 1969; Malherbe, 1970; Preston, Dargie and Maclean, 1973a, b). The precise role of each of these mechanisms in the aetiology of the anaemia was investigated by Preston et al (1963b), Preston and Dargie (1974), Dargie and Preston (1974), with the aid of red cells labelled with ^{51}Cr and ^{59}Fe and albumin labelled with ^{131}I in sheep with patent infections of Schistosoma mattheei. Erythrokinetic studies were then carried out before and after exposure to infection with metacercariae. A pronounced increase in blood and plasma volumes and a concomittant fall in red cell volume was observed from the 8th week after exposure coincidental with the inception of oviposition. Haemodilution was concluded as playing a significant part in the anaemia observed during this period. The mild anaemia which occurred between the 4th to 6th week was attributed to the combined effect of haemodilution and dyshaemopoiesis as red cell survival and intestinal leak were similar in both the infected and control animals. From the 8th week onwards there was a marked reduction in red cell lifespan, greatly accelerated rate of faecal clearance of red cells while urinary clearance of isotope remained similar/

similar in both groups. There was thus excessive disappearance of labelled cells from the circulation via the gut. There was also a close correlation between these observed changes and the onset of marked reductions in PCV and Hb, and animals with the lowest PCV generally had the lowest reduction in red cell lifespan and cell volume, and the highest enteric blood loss. Furthermore, with the return of faecal red cell clearance figures to values obtained in the controls, no further changes in PCV and red cell mass were recorded. The anaemia of this stage was essentially haemorrhagic in nature. Ferrokinetic studies revealed that between the 4th and 8th weeks of infection there was marked reduction in plasma iron turnover rate and red cell iron utilisation. Moreover, the reduction in red cell mass was shown to be greater than could be caused by haemorrhage alone and the deficit was explained by the degree of marrow depression observed. This was interpreted as evidence that dyshaemopoiesis was important in the anaemia which occurred between the 4th and 6th weeks of infection. With the onset of haemorrhage from the 8th week, the PITR and percentage red cell iron utilisation was found to be similar in both fluke infected and worm free controls, implying that the absence of marrow response greatly increased the severity of the anaemia. There was no evidence of iron deficiency, although this can be envisaged in severe or long-standing infections since no significant reabsorption of iron occurred in either the control or parasitised sheep.

What has emerged from these studies is the apparent complexity of schistosome anaemia with mechanisms such as haemodilution, dyshaemopoiesis and haemorrhage playing important parts at one stage or the other. This complexity was further highlighted by Holmes et al (1977) who employed similar techniques in studying the erythrokinetics of baboons experimentally infected with S. mansoni. With the onset of patency a mild/

mild anaemia developed which they showed to result from a combination of haemodilution and haemorrhage. There was no evidence of dyshaemopoiesis or haemolysis. Earlier work by Mahmound and Woodruff (1972) in S. mansoni infected mice showed that the anaemia was in part autoimmune caused by hepatosplenomegaly especially during the chronic stages. No splenomegaly was observed in the works of McCully and Kruger (1970) and Preston et al (1973a) in S. mattheei infected sheep even in long standing infections and this could have been responsible for the failure to demonstrate a haemolytic process in the anaemia.

The Anaemia of Haemonchosis:

The economic importance of Haemonchosis especially in sheep is well recognised in most tropical and sub-tropical countries (Dargie, 1973). The disease is characterised mainly by an anaemia which results from haemorrhage into the host abomasum due to the blood sucking activities of the immature and adult parasites (Veglia, 1915; Fourie, 1931; Andrews, 1942; Richards et al, 1954). Heavy infections produce a typical blood loss picture, i.e. a macrocytic hypochromic response whereas light infestations are characterised by a normocytic normochromic response which can also progress to the severe stage. Both acute and chronic infections are additionally marked by reductions in serum iron levels, percentage saturation of transferrin and depleted iron stores (Dargie and Allonby, 1975). Early kinetic studies involving the use of ^{59}Fe and ^{51}Cr labelled cells gave an estimate of 0.05 ml. blood loss per parasite and increased faecal clearance of red cells from 6-12 days after infection prior to the appearance of eggs in the faeces, i.e. confirmation of the haematophagic activity of the larval stages (Whitlock/

(Whitlock, 1950; Baker and Douglas, 1957, 1966; Clark, Kiesel and Goby, 1962; Georgi, 1962; Georgi and Whitlock, 1965). No attempts were made to monitor simultaneously the patterns of erythrocyte loss and haematopoietic response of sheep during the course of the infection and to correlate these with parasite egg production and changes in peripheral blood picture. Furthermore, the possible reabsorption of iron lost into the abomasum for haemoglobin synthesis was not investigated.

These deficiencies were remedied by Dargie and Allonby (1975) who combined both parasitological, haematological and radioisotopic techniques to study the red cell kinetics and iron metabolism of single and challenge infections of H. contortus in merino sheep in East Africa. Abomasal losses of red cells and iron were quantified using ^{51}Cr labelled cells and erythrocyte production/faecal iron excretions assessed by ^{59}Fe while measurements of egg output, PCV and Hb were carried out concurrently by conventional methods. Abomasal losses of ^{51}Cr labelled red cells were used as an index of worm activity in investigating the phenomenon of "self-cure". From their results, the anaemia of haemonchosis was concluded as occurring in three stages. The first which occurred during the first three weeks of infection (the pre-patent phase) was characterised by a progressive fall in PCV, low or negative faecal egg count and normal serum iron concentration. This early anaemia was attributed to the blood sucking activities of the immature and young adults of H. contortus. Blood loss was not as large as when the parasites were matured and the fall in PCV was caused by a delay in the response of the host erythropoietic system to supply red cells at the rate at which they are destroyed. The delay could be fatal when challenge is heavy.

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The second stage occurred in the early post-patent phase between the 3rd and 8th weeks of infection. Blood loss was at a maximum as indicated by $t\frac{1}{2}$ and ^{51}Cr faecal clearances, though no further drop in PCV was observed. They concluded that the haemopoietic system of the host was producing red cells at rates equal to their destruction (hyperactivity). This conclusion was confirmed by the finding of increased iron turnover rate, percentage red cell iron utilisation and elevated serum iron levels from ferrokinetic data. This phase is the chronic haemonchosis well recognised in field outbreaks of the disease (Allonby, 1973; Schillhorn van Veen, 1978; Ogunsusi and Eysker, 1979). Since the capacity of sheep infected with haemonchosis to reabsorb iron lost into the gut is severely limited (Dargie and Allonby, 1975), the body iron reserves become progressively depleted leading to the third stage of the anaemia which is characterised by a low serum iron and a further fall in PCV. The failure of erythropoiesis was due to a combined effect of iron deficiency and continuous loss of protein into the abomasum. When a challenge infection was superimposed during this terminal phase, a partial self-cure occurred but the renewed haemorrhage and the already existing fall in PCV and low serum iron completely overwhelmed the host synthetic machinery and death supervened. Various factors are known to influence the course and severity of the anaemia of H. contortus infections. Resistance to the disease was shown to be genetically determined, being related to such factors as the breed and haemoglobin type of the host. Jilek and Bradley (1969), Radhakrishnan et al (1972) showed that Florida Native ewes were more resistant than imported Rambouillet types and Altaif and Dargie (1976, 1978a, b) demonstrated similar advantages in Scottish Blackface sheep over Finn Dorsets. Sheep of haemoglobin type A were also shown to possess a higher resistance and to suffer a less severe anaemia than

Hb-B/

Hb-B types (Evans et al, 1963; Jilek and Bradley, 1969; Altaif and Dargie, 1978a, b). Altaif and Dargie (1976, 1978a, b) used abomasal losses of ^{51}Cr labelled cells as an index of worm activity to show that the differences in the severity of the anaemia observed in sheep of different breeds and Hb-types was due to an innate ability to resist the establishment of infection and not because of any physiological advantages which alleviated haemorrhagic stress,as previously suggested by Blunt and Evans (1963), Dawson and Evans (1962), Gabuzda et al (1968). Such abomasal losses of ^{51}Cr were also used to investigate the phenomenon of 'self-cure' by the same workers. Classical 'self-cure' was observed when a challenge infection was superimposed on an already existing infection, which led them to support the theory that self-cure under experimental conditions was mediated by an immediate type hypersensitive reaction in the abomasal mucosa, triggered off by antigenic materials released during the third ecolysis of the larvae, as previously suggested by Stewart (1953) and Soulsby and Stewart (1960). Self-cure under natural field conditions can be apparently non-immunogenic (Allonby and Urquhart, 1973).

The anaemia of haemonchosis is predominantly haemorrhagic due to the blood sucking activities of the immature and adult parasites but complicated by failure of erythropoiesis in terminal stages as a result of frank iron and globin deficiency.

The Anaemia of Trypanosomiasis:

The problem posed by both human and animal trypanosomiasis in tropical Africa are enormous and well documented (Lumsden, 1968). Bovine trypanosomiasis is caused by the three pathogenic African trypanosomes T. congolense, T. vivax and T. brucei, and the principal clinopathological feature of the disease is anaemia (Hornby, 1952; Fiennes, 1954; Edwards, Judd and Squire, 1956a,b; Losos and Ikede, 1972, Murray, 1974; Isoun, 1975; Saror, 1979). The anaemia is

usually/

usually normocytic and normochromic with a tendency towards macrocytosis and normochromia (Naylor, 1971b; Losos et al, 1973; Saror, 1979; Valli and Mills, 1980). Various mechanisms have been advanced in the pathogenesis of the anaemia. Haemolysis was suggested based on the observed increase in red cell destruction and manifested by (a) increased plasma bilirubin (Hudson, 1944; Fiennes, 1954, 1970; Jenkins et al, 1974), (b) erythrophagocytosis (Boycott and Price-Jones, 1913; Naylor, 1871a,b; Sadun et al, 1973; Mackenzie and Cruickshank, 1773; Murray et al, 1974; Jenkins et al, 1974, 1980), (c) haemosiderin deposits in tissues (Boycott and Price-Jones, 1913; Fiennes, 1954; Naylor, 1771a, Murray et al, 1974), (d) decreased red cell survival (Woodruff et al, 1973; Jennings et al, 1974; Jenkins et al, 1974, 1980), and (e) bone marrow erythroid hyperplasia (Sadun et al, 1973; Jenkins et al, 1974, 1980). Haemodilution was also implicated by Fiennes (1954), Clarkson (1968) and Naylor (1971c) and dyshaemopoiesis by Fiennes (1954, 1970) and Naylor (1971c).

The application of radioisotopic tracer techniques to the study of red cell kinetics and iron metabolism in parasitised animals has provided further insight into the mechanism of the anaemia. Using ^{51}Cr labelled cells it was found that, following infection with T. congolense or T. brucei in cattle, there was a massive loss of red cells from the circulation which was responsible for the anaemia. The half-life of the labelled cells was considerably shortened, the urinary excretion of such cells greatly accelerated without any significant faecal clearance of the cells (Mamo and Holmes, 1975; Holmes, 1976; Holmes and Jennings, 1976; Preston and Wellde, 1976; Dargie et al, 1979a,b). They concluded that the anaemia resulted from intravascular breakdown of red blood cells by erythrophagocytosis in the reticuloendothelial/

othelial system, especially in the spleen, as previously suggested in T. brucei infected mice by Murray et al (1973). Isotopic dilution studies using ^{59}Fe showed that haemodilution played a significant role in the anaemia especially during the early stages (Mamo and Holmes, 1975; Holmes, 1976). Dargie et al (1979a) could find no evidence of haemodilution using ^{131}I and ^{51}Cr in T. congolense infected cattle and attributed this to the difference in technique from those of the earlier workers. Studies with radioiron revealed a rapid disappearance of label from the plasma, a fast PITR retention of ^{59}Fe in the body and a four fold increase in erythropoietic index in infected animals (Holmes and Mamo, 1975; Dargie et al, 1979a,b) suggesting that dyshaemopoiesis was of no significance in trypanosome anaemia. Dargie et al (1979a) observed that red cell synthesis was less than expected from the degree of the anaemia and concluded that there was a defective iron reutilisation from degraded cells possibly due to reticuloendothelial blockage. They also showed that the superior resistance of the N'dama over the Zebu was due to their ability to control parasitaemia, and hence blood loss, and not to an innate ability to mount a more efficient erythropoietic response (Dargie et al, 1979a,b). Valli and Mills (1980) used radioisotopic criteria to show that neonatal calves suffered a milder haemolytic anaemia than those 6 months old. There was an inconclusive evidence of a mild and transient erythroid depression. Otherwise the anaemia was hyperproliferative in both age groups.

Trypanosome anaemia can be attributed to the combined effects of haemodilution and intravascular haemolysis. Haemorrhage and dyshaemopoiesis did not feature from radioisotopic studies.

REFERENCES.

Allonby, E.W. (1973)
In: 'Helminth Diseases of Cattle, Sheep and Horses in Europe', (eds. G.M. Urquhart and J. Armour), pp. 59-71, Maclehose & Co. Ltd., Glasgow.

Allonby, E.W. and Urquhart, G.M. (1973)
Parasitology, 66, 43-53.

Altaif, K.I. and Dargie, J.D. (1976)
I.A.E.A., Vienna, 449-462.

Altaif, K.I. and Dargie, J.D. (1978a)
Parasitology, 77, 161-175.

Altaif, K.I. and Dargie, J.D. (1978b)
Parasitology, 77, 177-187.

Anderson, N., Armour, J., Jarrett, W.F.H., Jennings, F.W., Ritchie, J. and Urquhart, G.M. (1965)
Veterinary Record, 77, 1196-1204.

Andrews, J.S. (1942)
Journal of Agricultural Research, 65, 1-18.

Anosa, V.O. and Isoun, T.T. (1980)
Journal of Comparative Pathology, 90, 155-168.

Anthony, D.W. and Roby, T.O. (1966)
American Journal of Veterinary Research, 27, 191-198.

Baker, N.F., Osebold, J.W. and Christenseen, J.K. (1961)
American Journal of Veterinary Research, 22, 590-596.

Baker, N.F. and Douglas, J.R. (1957)
American Journal of Veterinary Research, 18, 295-302.

Baker, N.F. and Douglas, J.R. (1966)
In: 'Biology of Parasites', (ed. E.J.L. Soulsby), pp. 155-183, Academic Press Inc., New York.

Barker, I.K. (1973)
International Journal of Parasitology, 3, 743-757.

Bawden, R.J. (1969)
Australian Journal of Agricultural Research, 20, 601-606.

Berry, C.I. and Dargie, J.D. (1978)
Veterinary Parasitology, 4, 327-339.

Bitakaramire, P.K. (1970)
In: 'Isotopes and Radiation in Parasitology', pp. 35-43. I.A.E.A., Vienna.

Blunt, M.H. and Evans, J.V. (1963)
Nature (Lond.), 200, 1215-1216.

Boender, C.A., Mulder, E., Phloem, J.E., De Wael, J., and Verloop, M.C. (1967)
Nature (Lond.), 213, 1236.

Boray, J.C. (1968)
Annals of Tropical Medicine and Parasitology, 61, 439-450.

Bothwell, T.H., Callender, S., Mallett, B. and Witts, L.J. (1956)
British Journal of Haematology, 12, 1-17.

Bothwell, T.H., Hurtado, A.V., Donohue, D.M. and Finch, C.A. (1957)
British Journal of Haematology, 12, 409-427.

Boycott, A.E. and Price-Jones, C. (1913)
Journal of Pathology and Bacteriology, 17, 347-366.

Bremner, K.C. (1969)
Experimental Parasitology, 24, 184-193.

Bremner, K.C. (1970)
Experimental Parasitology, 27, 236-245.

Bush, J.A., Berlin, N.I., Jenseen, W.V., Brill, A.B., Cartwright, C.E. and Wintrobe, M.M. (1955)
Journal of Experimental Medicine, 101, 451-462.

Clark, C.H., Kiesel, G.K. and Goby, C.H. (1962)
American Journal of Veterinary Research, 23, 977-980.

Clarkson, M.J. (1968)
Journal of Comparative Pathology, 78, 189-193.

Conrad, M.E. and Crosby, W.H. (1963)
Blood, 22, 406.

Dargie, J.D. (1973)
In: 'Helminth Diseases of Cattle, Sheep and Horses in Europe', (eds. G.M. Urquhart and J. Armour), pp. 59-71, 89-91, Robert Maclehose Ltd., Glasgow.

Dargie, J.D. (1975)
In: 'Pathogenic Processes in Parasitic Infections', (eds. A.E. Taylor and R. Muller), pp. 1-26. Blackwell Scientific Publications, Oxford, London, Melbourne.

Dargie, J.D. (1980)
Transactions of the Royal Society of tropical Medicine and Hygiene, 74, 5.

Dargie, J.D. and Allonby, E.W. (1975)
International Journal of Parasitology, 5, 147-157.

Dargie, J.D., Holmes, P.H., Maclean, J.M. and Mulligan, W. (1968)
Veterinary Record, 82, 360.

Dargie, J.D. and Mulligan, W. (1970)
Journal of Comparative Pathology, 80, 37-45.

Dargie, J.D. and Mulligan, W. (1971)
Journal of Comparative Pathology, 81, 187-202.

Dargie, J.D., Holmes, P.H., Maclean, J.M. and Mulligan, W. (1970)
Isotopes and Radiation in Parasitology, II, pp. 45-53. IAEA, Vienna.

Dargie, J.D., Murray, P.K., Murray, M. and McIntyre, W.I.M. (1979a)
Research in Veterinary Science, 26, 245-247.

Dargie, J.D., Murray, P.K., Murray, M., Grimshaw, W.R.T. and McIntyre, W.I.M. (1979b)
Parasitology, 78, 271-286.

Dargie, J.D. and Preston, J.M. (1974)
Journal of Comparative Pathology, 84, 83-91.

Dawes, B. and Hughes, D.L. (1970)
Advances in Parasitology, 8, 259-274.

Dawson, T.T. and Evans, J.V. (1962)
Australian Journal of Biological Science, 15, 371-378.

Delaune, E.T. and Mayhew, R.L. (1943)
Transactions of the American Microscopic Society, 62, 179.

Dinnik, J.A. and Dinnik, N.N. (1965)
Bulletin of Epizootic Diseases of Africa, 13, 341-359.

Dobson, C. (1967)
Australian Journal of Agricultural Research, 18, 523-538.

Drury, A.N. and Tucker, E.M. (1958)
Immunology, 1, 204-216.

Ebaugh, F.G., Clemens, T., Rodnan, G. and Paterson, R. (1958)
American Journal of Medicine, 25, 169-172.

Edwards, E.E., Judd, J.M. and Squire, F.A. (1956a)
Annals of Tropical Medicine and Parasitology, 50, 223-240.

Edwards, E.E., Judd, J.M. and Squire, F.A. (1956b)
Annals of Tropical Medicine and Parasitology, 50, 242-251.

Endie, G.S., Smith, W.M. and Brown, I.W.J. (1960)
Journal of General Physiology, 43, 825-839.

Evans, J.V., Blunt, M.H. and Southcott, W.H. (1963)
Australian Journal of Agricultural Research, 14, 549-558.

FAO/IAEA (1970)
Isotopes and Radiation in Parasitology, II,
I.A.E.A., Vienna.

FAO/IAEA
Isotopes and Radiation Research in Animal Diseases and their Vectors,
I.A.E.A., Vienna.

Fiennes, R.N.T.W. (1954)
Veterinary Record, 66, 423-434.

Fiennes, R.N.T.W. (1970)
In: 'The African Trypanosomiases' (ed. W.H. Mulligan and W.H. Potts), p. 729, George Allen & Unwin Ltd., London.

Foggie, A. and Nisbet, D.I. (1964)
Journal of Comparative Pathology, 74, 45-61.

Fourie, P.J.J. (1931)
17th Report of the Veterinary Science and Animal Industry of South Africa, 495-572.

Gabuzda, T.G., Schuman, M.A., Silver, R.K. and Lewis, H.B. (1968)
Journal of Clinical Investigation, 47, 1895 -1904.

Gallacher, C.H. (1963)
Australian Journal of Agricultural Research, 14, 349-363.

Gibson, T.E. (1954)
Journal of Comparative Pathology and Therapeutics, 64, 127-140.

Giles, R.C.Jnr., Berman, A., Hildebrandt, P.K. and McCaffrey, R.P. (1975)
Proceedings of the Society of Experimental Biological Medicine, 148, 795-798.

Giles, R.C.Jnr., Berman, A., Hildebrandt, P.K. and McCaffrey, R.P. (1977)
American Journal of Veterinary Research, 38, 535-537.

Gillet, D.J., Halmagi, D.F. (1966)
Journal of Surgical Research, 6, 211-214.

Georgi, J.R. (1964)
American Journal of Veterinary Research, 25, 952-954.

Georgi, J.R. and Whitlock, J.H. (1965)
American Journal of Veterinary Research, 26, 310-314.

Georgi, J.R. and Whitlock, J.H. (1967)
Cornell Veterinarian, 57, 43.

Gray, S.J. and Sterling, K. (1950)
Journal of Clinical Investigation, 29, 1604-1613.

Gulliani, G.L., Chanana, A.D., Cronkite, E.P., Joel, D.D., Laissue, J., Rai, K.R. (1975)
American Journal of Veterinary Research, 36, 1469-1471.

Hammond, J.A. (1973)
Tropical Animal Health and Production, 5, 22-26.

Heath, G.B.S. (1951)
Parasitology, 41, 209-233.

Herd, R.P. (1971)
International Journal of Parasitology, 1, 189-201.

Havesy, G., Koster, K.M., Sorensen, G., Warburg, E. and erahn, K. (1944)
Acta. Med. Scand., 116, 561-572.

Hodgetts, V.E. (1961)
Australian Journal of Experimental Biology, 39, 187-195.

Holmes, P.H. (1969)
Ph.D. Thesis, University of Glasgow.

Holmes, P.H. (1976)
In: 'Nuclear Techniques in Animal Production and Health', pp. 465-475, I.A.E.A., Vienna.

Holmes, P.H., Dargie, J.D., Maclean, J.M. and Mulligan, W. (1968)
Journal of Comparative Pathology, 78, 415-420.

Holmes, P.H., James, E.R., Maclean, J.M., Nelson, G.S., Taylor, M.G. (1977)
Journal of Helminthology, 51, 95-104.

Holmes, P.H. and Jennings, F.W. (1976)
In: 'Pathophysiology of Parasitic Invections' (ed. E.J.L. Soulsby), pp. 179-210, Academic Press, New York, San Francisco, London.

Holmes, P.H., Maclean, J.M. (1969)
Research in Veterinary Science, 10, 488-489.

Holmes, P.H., Maclean, J.M. and Mulligan, W. (1971)
In: 'Pathology of Parasitic Diseases', (ed. S.M. Gaafar), pp. 69-81, Purdue University Studies, Layfayette.

Holmes, P.H. and Mamo, E. (1975)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 69, 274.

Hooshmand-Rad, P., Hawa, N.J. (1973)
Tropical Animal Health and Production, 5, 97-102.

Horak, I.G., Clark, R. (1964)
Onderstepoort Journal of Veterinary Research, 31, 163-176.

Horak, I.G. and Clark, R. (1966)
Onderstepoort Journal of Veterinary Research, 33, 139-159.

Horak, I.G., Clark, R., Gray, R.S. (1968)
Onderstepoort Journal of Veterinary Research, 35, 195-224.

Hornby, H.E. (1952)
Animal trypanosomiasis in Eastern Africa, 1949,
H.M.S.D., London.

Hudson, J.R. (1944)
Journal of Comparative Pathology, 54, 108-119.

Huff, R.L., Henesy, T.G., Austin, R.E., Garcia, J.F., Roberts, B.M. and Lawrence, J.H. (1950)
Journal of Clinical Investigation, 29, 1041-1052.

Isoun, T.T. (1975)
Acta tropica, 32, 267-272.

Jamra, M., Maspes, V., Meira, D.A. (1964)
Revista, Institute of Tropical Medicine, S. Paolo, 6, 126-136.

Jellison, W.L. and Kohl, G.M. (1938)
Journal of Parasitology, 24, 143-154.

Jenkins, G.C., Forsberg, C.M., Brown, J.L. and Boulton, F.E. (1974)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 68, 154-155.

Jenkins, G.C., McCrorie, C., Forsberg, C.M., Brown, J.L. (1980)
Journal of Comparative Pathology, 90, 107-121.

Jennings, F.W. (1968)
In: 'Isotopes and Radiation in Parasitology',
I.A.E.A., Vienna, pp. 133-140.

Jennings, F.W. (1976)
In: 'Pathophysiology of Parasitic Infections', (ed. E.J.L.Soulsby),
Academic Press, New York.

Jennings, F.W., Lauder, I.M., Mulligan, W. and Urquhart, G.M. (1954)
Veterinary Record, 66, 155.

Jennings, F.W., Mulligan, W. and Urquhart, G.M. (1955)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 49, 305.

Jennings, F.W., Mulligan, W. and Urquhart, G.M. (1956)
Experimental Parasitology, 5, 458-468.

Jennings, F.W., Murray, P.K., Murray, M. and Urquhart, G.M. (1974)
Research in Veterinary Science, 16, 70-76.

Jilek, A.F. and Bradley, R.E. (1969)
American Journal of Veterinary Research, 30, 1173-1178.

Judd, J.T. and Matrone, F. (1962)
Journal of Nutrition, 77, 264-268.

Kaneko, J.J. (1963)
Annals of the New York Academy of Science, 104, 680-700.

Kates, K.C. and Turner, J.H. (19600
American Journal of Veterinary Research, 21, 254-261.

Kreier, J.P., Ristic, M., Schroeder, W.F. (1964)
American Journal of Veterinary Research, 25, 343-352.

Lapage, G. (1962)
Monning's Veterinary Helminthology and Entomology,
5th Edition, Bailliere, Tindall & Cox,London.

Little, D.A. (1963)
Australian Veterinary Journal, 29, 6-10.

Littlejohns, I.R. (1960)
Australian Veterinary Journal, 36, 260-265.

Littlejohns, I.R. (1966)
Australian Veterinary Journal, 42, 388-391.

Murray, P.K. (1974)
Ph.D. Thesis, University of Glasgow.

Murray, M., Murray, P.K., Jennings, F.W., Fisher, E.W. and Urquhart, G.M. (1974)
Research in Veterinary Science, 16, 77-84.

Murray, P.K., Urquhart, G.M., Murray, M. and Jennings, F.W. (1973)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 67, 267.

Naylor, D.C. (1971a)
Tropical Animal Health and Production, 3, 95-100.

Naylor, D.C. (1971b)
Tropical Animal Health and Production, 3, 159-168.

Naylor, D.C. (1971c)
Tropical Animal Health and Production, 3, 203-207.

Nechelles, T.F., Weinstein, I.M. and Leroy, G.V. (1953)
Journal of Laboratory Clinical Medicine, 42, 358 - 367.

Neitz, W.O. (1957)
Onderstepoort Journal of Veterinary Research, 9, 9-30.

Neuberger, A. and Niven, J.S.F. (1951)
Journal of Physiology, 112, 292-297.

Obara, J., Sonoda, W., Watanabe, S. (1964)
Experimental Parasitology, 15, 471-478.

Obasaju, M.F. and Otesile, E.B. (1979)
Tropical Animal Health & Production, 12, 116-118.

Ogunsusi, R.A. and Eysker, M. (1979)
Research in Veterinary Science, 26, 108-110.

O'Kelly, J.C. and Seifort, G.W. (1970)
Australian Journal of Biological Science, 23, 681-690.

Ortlepp, R.J. (1937)
Onderstepoort Journal of Veterinary Research, 8, 183-212.

Ortlepp, R.J. (1939)
Onderstepoort Journal of Veterinary Research, 12, 305-318.

Owen, C.A., Bellman, J.L. and Grindlay, J.H. (1954)
Journal of Laboratory and Clinical Medicine, 44, 238-245.

Philip, C.B., Jellison, W.L. and Wilkins, H.F. (1935)
Journal of the American Veterinary Medical Association, 86, 726-744.

Piercy, P.L. (1956)
Annals of New York Academy of Science, 64, 40.

Pitchford, R.J. (1956)
Journal of South African Veterinary Medical Association, 34, 613-618.

Preston, J.M., Dargie, J.D. and Maclean, J.M. (1973b)
Journal of Comparative Pathology, 83, 417-428.

Preston, J.M., Dargie, J.D. and Maclean, J.M. (1973a)
Journal of Comparative Pathology, 83, 401-415.

London, I.M., Shemin, D. and Rittenburg, D. (1948)
Journal of Biological Chemistry, 173, 797-823.

Losos, G.J. and Ikede, B.O. (1972)
Veterinary Pathology suppl. ad. 9, 1-71.

Losos, G.J., Paris, J., Wilson, A.J. and Dar, F.K. (1973)
Bulletin of Epizootic Diseases of Africa, 21, 239-244.

Lumsden, W.H.R. (1968)
In: 'Infectious blood diseases of Man and Animals, Vol. II',
(ed. Weinman, D. and Ristic, M.),
Academic Press, New York, London.

Mackenzie, P.K. and Cruickshank, J.G. (1973)
Research in Veterinary Science, 15, 256-262.

Maclean, J.M., Holmes, P.H., Dargie, J.D. and Mulligan, W. (1968)
In: 'Isotopes and Radiation in Parasitology', pp. 117-123,
I.A.E.A., Vienna.

Mahmound, A.A.F. and Woodruff, A.W. (1972)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 66, 75.

Malherbe, W.D. (1970)
Onderstepoort Journal of Veterinary Research, 37, 37-44.

Mamo, E. and Holmes, P.H. (1975)
Research in Veterinary Science, 18, 105-106.

Martin, W.B., Thomas, B.A.C. and Urquhart, G.M. (1957)
Veterinary Record, 69, 736-739.

McCully, R.M. and Kruger, S.P. (1969)
Onderstepoort Journal of Veterinary Research, 36, 129 -161.

McLeod, J. (1933)
Transactions of the Highland Agricultural Society of Scotland, pp. 1-15.

McSherry, B.J., Van Dreumel, A.A. and Robinson, G.A. (1966a)
Canadian Veterinary Journal, 7, 176-179.

McSherry, B.J., Van Druumel, A.A. and Robinson, G.A. (1966b)
Canadian Veterinary Journal, 7, 204 - 206.

Miller, T.A. (1966)
Journal of Parasitology, 52, 844-855.

Mollison, P.L. and Veali, N. (1955)
British Journal of Haematology, 1, 62 -66.

Mulligan, W. (1973)
In: 'Helminth Diseases of Cattle, Sheep and Horses in Europe',
(eds. G.M. Urquhart and J. Armour),
Robert Maclehose & Co. Ltd., Glasgow.

Journal of Comparative Pathology, 84, 73-81.

Preston, J.M. and Wellde, B.T. (1976)
Final Report to Department of the Army, Walter Reed Army Institute of Research, DAAD, 17-76-9412.

Radhakrishnan, C.V., Bradley, R.E. and Loggins, A.E. (1972)
American Journal of Veterinary Research, 33, 817-833.

Radin, N.S., Rittenburg, D. and Shemin, D. (1950)
Journal of Biological Chemistry, 184, 745 - 751.

Reid, J.F.S., Armour, J., Urquhart, G.M. and Jennings, F.W. (1970)
Veterinary Record, 86, 242-249.

Richards, R.M., Shumard, R.F., Pope, A.L., Philips, P.H. and Herrick, C.A. (1954)
Journal of Animal Science, 13, 274-282.

Riek, R.F. (1957)
Australian Journal of Agricultural Research, 8, 209-214.

Ristic, M. (1968)
Infectious blood diseases of man and animals, Vol. II, Chpt. 23 (eds. Weinman and Ristic), Academic Press, New York, London.

Roberts, H.E. (1968)
British Veterinary Journal, 124, 433-450.

Roche, M., Perez-Giminez, M.E. and Levy, A. (1957)
Nature (Lond.), 162, 1278-1279.

Roche, M., Perez-Giminez, M.E., Layrisse, M. and DiPrisco, E. (1957)
Journal of Clinical Investigation, 36, 1183-1192.

Ross, J.G., Todd, J.R. and Dow, C. (1966)
Journal of Comparative Pathology, 76, 67 - 81.

Ross, J.G., Dow, C. and Purcell, D.A. (1969)
British Veterinary Journal, 125, 136 -144.

Rouse, B.T. and Johnson, R.H. (1966)
Veterinary Record, 79, 223-224.

Saad, A.M., Hussein, M.F., Dargie, J.D., Taylor, M.G. and Nelson, G.S. (1980)
Research in Veterinary Science, 28, 105-111.

Sadun, E.H., Johnson, A.J., Nagle, R., Duxbury, R.E. (1973)
American Journal of Tropical Medicine and Hygiene, 22, 323 - 330.

Saror, D.I. (1979)
Veterinary Record, 105, 96-98.

Schalm, O.W. (1965)
Veterinary Haematology, 2nd Edition, Lea and Febiger, Philadelphia.

Schillhorn van Veen, T.W.S. (1978)
Veterinary Record, 102, 364-365.

Schnappauf, H., Di Giacom , R. and Cronkite, E.P. (1965)
American Journal of Veterinary Research, 26, 1212-1214.

Sewell, M.N.H., Hammond, J.R., Dineing, D.C. (1968)
British Veterinary Journal, 124, 160-170.

Schemin, D., London, I.M. and Rittenburg, D. (1950)
Journal of Biological Chemistry, 183, 757.

Shemin, D. and Rittenburg, D. (1946)
Journal of Biological Chemistry, 166, 627-632.

Shermanchuk, J.H., Haufe, W.O., Thomson, C.O.M. (1960)
Canadian Journal of Comparative Medicine, 24, 158-161.

Sinclair, K.B. (1964)
British Veterinary Journal, 120, 212-222.

Sinclair, K.B. (1967)
Helminthological Abstracts, 36, 115-134.

Soulsby, E.J.L. (1965)
Textbook of Veterinary Clinical Parasitology,
Blackwell, Edinburgh.

Soulsby, E.J.L., Stewart, D.F. (1950)
Australian Journal of Agricultural Research, 11, 595-603.

Stephenson, W. (1947)
Parasitology, 38, 123-127.

Stewart, D.F. (1953)
Australian Journal of Agricultural Research, 4, 100-117.

Strydom, H.F. (1963)
Journal of South African Veterinary Medical Association, 34, 69 - 72.

Symons, L. E.A., Boray, J.C. (1967)
Veterinary Record, 81, 63.

Symons, L.E.A., Boray, J.C. (1968)
Z. tropenmed. Parasitology, 19, 451 - 472.

Taylor, E.L. (1964)
F.A.O. Agricultural Studies, 64, 221-234.

Theiler, A. (1921)
Journal of the Department of Agriculture of South Africa, 2, 153.

Todd, J.R. and Ross, J.G. (1965)
Experimental Parasitology, 19, 151-154.

Todd, J.R. and Ross, J.G. (1966)
Clinica chem. acta., 14, 22 - 27.

Tucker, E.M. (1962)
Research in Veterinary Science, 4, 11-23.

Turner, J.H. (1959)
American Journal of Veterinary Research, 20, 102-110.

Urquhart, G.M. (1956) cited by Holmes, P.H. (1969)
Ph.D. Thesis, University of Glasgow.

Valli, V.E.O., Forsberg, C.M. and McSherry, B.J. (1978)
Veterinary Pathology, 15, 732-745.

Valli, V.E.O. and Mills, J.N. (1980)
Tropenmed. Parasitology, 31, 215-231.

Veglia, F. (1915)
3rd and 4th Report of the Division of Veterinary Science and Animal Industry of South Africa, 347-500.

Warren, K.S. (1972)
Helminthological Abstracts, 42, 591-633.

Wasserman, L.R., Rashkoff, I.A., Leavitt, D., Mayer, J. and Port, S. (1952)
Journal of Clinical Investigation, 31, 32.

Weinstein, I.M. and Beutler, E. (1962)
Mechanisms of Anaemia,
McGraw-Hill Book Co. Inc., London.

Wellde, R.L., Deindl, G., Sadun, E., Williams, J. and Warui, G. (1974)
Experimental Parasitology, 36, 1974.

Wetterfors, J. (1965)
Acta medica Scandinavia Supplementary, 177, 243-256.

Wetzel, R. (1931)
North American Veterinary, 12, 25-28.

Whitlock, J.H. (1950)
Cornell Veterinarian, 40, 288-299.

Williams, W.J., Beutler, E., Ersley, A.J. and Rundles, R.W. (1972)
Haematology, McGraw-Hill Book Comp. Inc., New York,
pp. 152-162, 1386-1392.

Woodruff, A.W., Zeigler, J.L., Hathaway, A. and Gwata, T. (1973)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 67, 329 - 337.

Zehr, J.E., Johnson, J.A. and Moore, W.W. (1969)
American Journal of Veterinary Research, 30, 1699-1703.

Zuckermann, A. (1964)
Experimental Parasitology, 15, 138-183.

CHAPTER IV

THE APPLICATION OF RADIOISOTOPES TO THE STUDY OF PARASITIC HYPOPROTEINAEMIAS

THE APPLICATION OF RADIOISOTOPES TO THE STUDY OF PARASITIC HYPOPROTEINAEMIAS.

INTRODUCTION.

Most parasitic infections are characterised by changes in the plasma protein composition and distribution of the hosts, which usually take the form of depression in albumin and elevation in one or more of the globulins (Urquhart and Armour, 1973). Gastrointestinal loss of proteins as a cause of hypoproteinaemia was demonstrated in certain diseases of man by Citrin et al (1957) and later confirmed by Schwartz and Jarnum (1962), Jeffries and Sleisinger (1962). These diseases were referred to as protein-losing-gastroenteropathies and their discovery was facilitated by the introduction of radioisotopes into clinical investigations. Employing similar techniques in animals, certain parasitic infections have been shown to be analogous to diseases of man where intestinal loss of proteins have been demonstrated. Such losses have been observed in trichostrongylid infections of cattle, swine, sheep (Cornelius et al, 1962; Mulligan et al, 1963; Nielsen, 1966; Dargie, 1973) and in trematode infections of sheep by Holmes, 1969; Holmes et al, 1968; Maclean et al, 1968; Dargie, Maclean and Preston, 1973), hence these diseases could be regarded as true protein losing gastroenteropathies.

The plasma proteins are maintained in a state of dynamic equilibrium between the intravascular and extravascular pools by continuous synthesis and catabolism. A change in concentration may indicate altered distribution or changes in the rates of synthesis and catabolism. Conventional analytical methods provide static parameters which are of limited use. A proper understanding of how these changes are brought about necessitates a study of protein metabolism involving the measurement of pool sizes and turnover rates. Such measurements can only be carried out by radioisotopic techniques.

METHODS/

METHODS OF INVESTIGATION.

Early attempts at labelling proteins for metabolic studies utilised ^{14}C and ^{38}S and though these isotopes were not denatured, they were reutilised for new protein synthesis after catabolism (Margen and Tarver, 1956). Moreover, Holmes (1969) pointed out that the technique was time-consuming and the yield of labelled proteins poor.

Labelling of proteins, especially albumin and immunoglobulins, in vitro with the isotopes of iodine (^{132}I and ^{125}I) was shown to be a valuable tool in the study of protein metabolism by Sterling (1951), McFarlane (1956, 1958) and Holmes (1969). These workers observed that the labelled albumin behaved metabolically like the animals' own unlabelled proteins, remaining firmly bound under physiological conditions until the albumin was degraded whereupon the label was not reutilized for new protein synthesis but excreted largely in the urine if the thyroid had been blocked by the administration of excess ordinary iodine. Commerically obtained immunoglobulin is usually impure hence the different IgG subclasses are usually separated by anion exchange chromatography prior to labelling to achieve metabolic homogeneity (Nielsen and Nansen, 1968). This is not usually a problem with albumin.

In the study of parasitic hypoproteinaemias the iodine monochloride method of McFarlane (1958) is commonly used to label proteins in vitro with radioiodine. The protein in slightly alkaline medium was treated with iodine monochloride to which the radioactive iodine had been added as carrier-free iodide. The chemistry of the radioiodination involved the substitution of iodine for hydrogen in the tyrosine groups of albumin, the buffer aiding the conversion of iodine monochloride to hypoiodite which appeared necessary for the substitution to occur. Precautionary measures aimed at preventing denaturation were outlined by Freeman (1967). Firstly, over-iodination occurred frequently, hence the/

the specific activity of the labelled protein was reduced by the addition of carrier protein. Denaturation was also shown to be a function of time (Nielsen and Nansen, 1968), hence the protein should be used immediately after labelling. Finally, the activity was ensured to be bound to the protein being iodinated. The labelled protein was finally dialysed for 48 hours at 5°C. against two changes of large volumes of physiological saline and centrifuged before injection.

A measured volume of the labelled proteins was injected intravenously into infected and control animals. Heparinized blood samples were taken 5-10 minutes after injection and further samples taken at suitably spaced out intervals over the next two weeks. Serum protein estimations were carried out on blood samples obtained prior to the injections and during the study. The total daily outputs of urine and faeces were measured and radioactivity determinations made on suitable samples of plasma, urine, faeces and standards prepared from the injected albumin suspension. The data was analysed and the following indices calculated:-

(a) The plasma volume (Vp). Plasma volumes were estimated by the dilution principle from the radioactivity of the first plasma sample taken after injection using the formula

$$Vp(ml) = \frac{\text{Total injected radioactivity}}{\text{Radioactivity/ml. of 5 or 10 min. sample}}$$

For comparison, the volumes were either expressed as absolute values or on a body weight basis.

(b) Intravascular pool of albumin (CA). This was obtained by multiplying the plasma volume by the plasma albumin concentration determined by chemical analysis or electrophoresis.

(c)/

(c) Total body albumin (TA). This was calculated by either employing the 'equilibrium time' method described by Campbell et al (1956) or the extrapolation method of Sterling (1951). The plasma radioactivity disappearance curve has an initial rapid decline attributed to distribution throughout the total body pool, followed by a more gradual exponential fall due to catabolism. Making the assumption that no further redistribution occurred during the 'linear' phase, Sterling (1951) proposed that the intercept C obtained by extrapolating the exponential part of the curve to the ordinate indicated the fraction of the total amount of albumin that would have been present in the plasma at zero time if the label had equilibrated instantaneously with all the albumin compartments. Hence:-

$$TA\ (gm/kg) = \frac{CA\ (gm/kg)}{C_1}$$

Two assumptions are inherent in this calculation, i.e. that protein synthesis and catabolism occurred both extravascularly and intravascularly and that no significant redistribution of labelled proteins occurred after the initial equilibration hence all body compartments have equal specific activities. Anderson (1964) pointed out that catabolism was largely intravascular, hence after the preliminary distribution period, the specific activity of the plasma will always be lower than that in the extravascular compartments, resulting in a net transfer of protein of higher specific activity from the extravascular pools back into the plasma. This net transfer decreases the slope of the plasma activity curve making C_1 very low and, as suggested by Mathews (1966), the values obtained for total protein and extravascular protein tended to be slightly overestimated. The equilibrium-time method of Campbell et al (1956) depended on the daily excreted activity in the faeces and urine. The extravascular activity was obtained as the difference between the cumulative activity excreted in the urine and faeces (Q_R) and the plasma activity (Q_P). At equilibrium time, the/

the extravascular activity was maximal hence the ratio between the extravascular and intravascular activities was assumed equal to the ratio of their pool masses, i.e.

$$\frac{QE}{Q_P} = \frac{EA}{CA}$$

and since TA = CA + EA

$$TA\ (gm/kg) = \frac{CA\ (gm/kg)\ (Q_P + Q_E)}{Q_P}$$

This method also assumed intravascular catabolism of protein and rapid excretion of degradation products. However iodine is primarily distributed in the iodine pool of the body before ultimate excretion (Nielsen and Nansen, 1968) and in situations of increased protein, breakdown as in most parasitic infections, the significant retention of iodine will occur leading to overestimation of QE and of TA.

(d) The extravascular pool of albumin (EA) was obtained as the difference between TA and CA.

(e) Catabolic rate. This was indirectly estimated by analysis of the plasma activity curve using the methods of Mathews (1957) or Nosslin (1966) or directly from measurements of excreted radioactivity as described by Campbell et al (1956) or Pearson et al (1958). In the Mathews' method where protein degradation was assumed as occurring intravascularly, the apparent half-life ($t\frac{1}{2}$) was calculated from the linear part of the curve as the time taken for the radioactivity to fall by 50%. The slope constant of the curve was calculated by the equation:-

$$b_1 = \frac{0.693}{t\frac{1}{2}(days)}$$

The extrapolated portion of the curve was subtracted from the original curve to yield a new curve with slope constant b_2 and intercept with the ordinate C_2. This technique was continued until subtraction resulted in one single, linear curve. The exponential part of each curve was characterised by a slope constant (b_1, b_2 etc.) and an intercept

(C_1/

C_1, C_2 etc.). It was shown mathematically that the catabolic rate (K) as a fraction of the intravascular pool broken down per day can be calculated from the equation:-

$$K = \frac{1}{\frac{C_1}{b_1} + \frac{C_2}{b_2} \ldots\ldots \frac{C_n}{b_n}}$$

The slope b_1 and intercept C_1 of the final exponential was usually accurately determined while b_2 and C_2 were more subject to errors since they were difference measurements. The absolute amounts of albumin degraded per day was obtained by multiplying K by the intravascular pool of albumin. In the Campbell et al (1956) method the excreted isotope in urine and faeces was assumed proportional to the amount of labelled albumin in the intravascular pool where degradation occurred. The fraction of the plasma pool broken down each day was then determined from the daily excreted activity and the average activity present in plasma within the same time:

$$K = \frac{\text{Total excreted activity}}{\text{Mean plasma activity}}$$

(f) Faecal clearance of plasma. This was obtained by dividing the total radioactivity in each daily faecal collection by the activity per ml. of plasma taken at the beginning of the collection period. The figure represents the amount of plasma which would have to appear in the gut to account for the faecal radioactivity.

The fractional catabolic rate or turnover rate determined by either methods described above serve as indices of metabolism and not as absolute values as computation of absolute values require 'steady-state' conditions (synthesis = breakdown) which rarely holds true in parasitized animals (Mulligan, 1973; Dargie, 1975). Moreover, the discrepancy between the observed and true catabolic rates was greatest when K was calculated from excreted activity in faeces and urine (Campbell's method). In subjects

where/

where quantitative collection of urine and feaces is difficult, turnover data are best calculated from the plasma radioactivity curve using the methods of Mathews (1957) or Nosslin (1966). A high turnover rate occurring concomittantly with normal or increased intravascular albumin pools indicate greatly accelerated synthesis. If the hypercatabolism exceeds the animal's capacity to manufacture albumin, the result is a hyper catabolic hypoalbuminaemia.

Loss of protein into the gastrointestinal tract may be the most important factor responsible for reduced $t\frac{1}{2}$ and increased catabolic rates. Such losses cannot be quantitated with iodine labelled plasma proteins due to the substantial breakdown and reabsorption of the label from the gut. A synthetic macromolecule which was not subjected to enzyme breakdown, e.g. ^{131}I or ^{125}I-labelled polyvinylpyrollidone (PVP) or a plasma protein label which was not reabsorbed, e.g. ^{51}Cr labelled albumin was used for this purpose. Gordon (1969) first demonstrated the use of ^{131}I-PVP for the measurement of protein loss from the plasma into the gut. The label was neither degraded nor absorbed. Later works by Jarnum (1961, 1963) demonstrated significant renal excretion of the label which affected protein leak estimates. Irrespective of this, a good correlation was found between faecal excretion of PVP and gastrointestinal loss by Holmes (1969) and Mulligan et al (1973) and the main objection to its use appeared to be its dissimilarity as a molecule to plasma proteins (Mulligan, 1973). ^{51}Chromic Chloride was used to label albumin by Gray and Sterling (1950) but it was Waldman(1961) who introduced the isotope as a quantitative tool for diagnosing intestinal protein loss. The label was unreliable in albumin turnover studies as it was observed to label the immunoglobulins after degradation of the albumin (Van Tongeron and Majoor, 1966) and also led to denaturation of the protein molecules thus giving a very short plasma $t\frac{1}{2}$ (Hofer, Schatz and Thumb, 1968). It

remained/

remained valuable for detecting enteric protein leak. Van Tongeron and Majoor (1966) found that the rate of disappearance of ^{51}Cr from the plasma was the same whether the isotope was administered as ^{51}Chromic Chloride, ^{51}Cr-albumin or ^{51}Cr Serum. For convenience carrier-free ^{51}Cr Chloride was injected *in vivo* and labelling of proteins allowed to occur. This method was adopted for subsequent studies. Following injections of either ^{131}I-labelled PVP or ^{51}Cr intravenously, complete 24 hour collections of faeces were made and plasma samples also collected daily. Serum albumin concentration was determined from a blood sample collected prior to the injection. Faecal clearances were calculated as previously described and the quantity of albumin lost into the gut obtained by multiplying the serum albumin concentration by the plasma clearance. Other isotopes used for quantifying enteric plasma leak include ^{95}Niobium-labelled albumin by Jeejeebhoy et al (1964), ^{67}Copper labelled ceruloplasmin by Waldman and Wochner (1965) and ^{59}Fe-labelled iron dextran (Jarnum *et al*, 1968). The ^{95}Nb-labelled albumin was unsuitable for turnover studies while the high cost and short half-life of ^{67}Cu-ceruloplasmin has precluded its routine use. The ^{59}Fe-iron dextran was shown to be the most promising as there was no observed urinary excretion and also gave a better correlation between faecal excretion and turnover rate.

In studies of protein metabolism in parasitic hypoproteinaemias, ^{131}I or ^{125}I is routinely employed for turnover studies while ^{131}I-PVP or $^{51}CrCl_3$ is used for quantifying protein losses into the gut from the plasma. The results of such studies have helped in elucidating the mechanism of hypoproteinaemia in some parasitic diseases which are reviewed forthwith.

HYPOPROTEINAEMIA/

HYPOPROTEINAEMIA OF FASCIOLIASIS.

Chronic fascioliasis is associated with marked changes in the plasma protein profile, the total protein levels usually remaining within normal range while albumin levels are depressed and the globulins elevated (Secretan and Bickel, 1960; Sinclair, 1962; Thorpe, 1965; Ross, Todd and Dew, 1966; Simensen et al, 1968). The aetiology of the hypoalbuminaemia remained controversial for a long time. Thus Thorpe (1965) suggested liver damage as the primary factor interfering with albumin synthesis, while Sinclair (1962) concluded that losses of albumin occurred in the inflammatory exudates in the liver.

Studies of experimental fascioliasis involving ^{131}I-albumin, ^{131}I-PVP or $^{51}CrCl_3$ in rabbits, sheep and cattle with patent F. hepatica infections have revealed the following features (Dargie et al, 1967, 1968, 1970; Holmes et al, 1968; Holmes, 1969; Maclean et al, 1968; Nansen et al, 1968; Nansen, 1971; Dargie and Mulligan, 1971; Dargie, 1975; Holmes et al, 1971).

(a) The mild hypoproteinaemia which occurred during the migratory phase of the flukes (3-6 weeks post infection) was attributed to liver damage which caused a decreased synthesis and increased degradation of albumin;

(b) Though infected animals were all hypoalbuminaemic, the reductions in the intravascular pool of albumin were lower than the extravascular pool reductions. Since a continuous exchange occurs between these two comportments, a net transfer of albumin from extravascular sites such as muscle and skin back into the plasma must have been taking place in order to keep the plasma albumin concentration within normal limits;

(c) The fractional catabolic rate (k) was markedly increased. In sheep with patent infections in the bile ducts (12-14 weeks old infection) k was 0.140 and only 0.054 in controls (Holmes, 1969), while in rabbits the equivalent figures were 0.433 and 0.191 respectively (Dargie et al, 1968). That the hypercatabolism did not give rise to a severe hypoalbuminaemia/

hypoalbuminaemia, was explained by the fact that synthesis must have been almost keeping pace with catabolism, the rate being faster in infected than in control animals. An insignificant difference in catabolic rate between infected and control cattle was observed in F. gigantica infections by Bitakaramire (1970). This was attributed to the low fluke burdens in the infected calves which failed to cause enough plasma loss to increase the albumin catabolism. A two-three fold increase in immunoglobulin (especially IgG_2) turnover rate was demonstrated in sheep and cattle using $^{131}I IgG_2$ by Holmes (1969); Holmes et al, 1968; Nansen et al, 1968; Nansen and Nielsen, 1968). The hypercatabolism was attributed to increased biliary loss. Thus a loss of both albumin and immun globulins was occurring into the gut of parasitized animals. Electrophoresis revealed a hyperimmunoglobulinaemia and hypoalbuminaemia. This implied that IgG synthesis must have been proceeding at such an increased rate that normal or even elevated serum levels were obtained. A direct correlation was found between the plasma concentrations of albumin and immunoglobulin G and their catabolic rates in mice and sheep (Gordon et al, 1959; Montgomery et al, 1962; Fahey and Robinson, 1963; Nielsen, 1966, 1970). Hence the high catabolic rate of IgG could have been caused by a combined effect of high serum level of IgG per se and the biliary loss. The capacity of fluke infected animals to synthesize plasma proteins appears variable with IgG synthesis taking precedence over albumin synthesis. This was confirmed by Nansen (1971) who showed that the synthesis rate of albumin was normal or slightly decreased while IgG_2 synthesis was greatly accelerated in cattle. The reduced synthesis of albumin probably resulted from liver damage since the liver is the only site for albumin production.

(d) The faecal excretion of ^{131}I-PVP or $^{51}CrCl_3$ was greater in infected animals showing that there was a loss of protein through the bile ducts into the gut. This was attributed to the haematophagic activities of the

parasites/

parasites. Using simultaneous labelling of red cells with ^{51}Cr and plasma proteins with ^{95}Nb, Dargie et al (1968, 1970); Maclean et al (1968) and Holmes (1969) presented evidence that the macromolecule leakage was greater than can be accounted for entirely by blood loss. Histology of the bile ducts of fluke infected animals revealed enlargement of the surface area due to epithelial hyperplasia and such lesions were thought to contribute to the additional plasma leakage (Murray, 1974; Nansen, 1970). Such leakages are probably secondary to the direct loss caused by flukes (Dargie, 1975).

(e) A marked rise in serum albumin levels and a fall in catabolic rate was observed following anthelmintic removal of the fulkes (Maclean et al, 1968; Holmes, 1969). The drop in catabolism was attributed primarily to the removal of the flukes which led to a cessation of whole blood loss, and secondarily to a reduced synthesis of albumin which was possibly mediated by a regulatory mechanism preventing serum albumin concentration from rising above the normal level. Furthermore, the liver damage might have been worsened by the fasciolicide thereby causing an impairment of function.

HYPOPROTEINAEMIA OF SCHISTOSOMIASIS.

Marked serum protein changes characterised by a fall in albumin concentration and an elevation of total protein and gammaglobulin concentrations have been reported in various laboratory animals, sheep, cattle and man infected with either Schistosoma japonicum, S. mattheei, S. mansoni or S. bovis (Evans and Stirewalt, 1957; Sadun and Walton, 1958; Ramirez et al, 1961; Smithers and Walker, 1961; Sadun and Williams, 1966; Von Lichtenberg et al, 1971; Malherbe, 1970; Preston, Dargie & Maclean, 1973a; Saad et al, 1980). Various reasons have been advanced for these observed changes. Fayez and Aziz (1963) attributed the reduced albumin synthesis to liver dysfunction while Smithers and

Walker/

Walker (1961) concluded that increased catabolism of albumin in monkeys resulted from losses of plasma into the gut. Furthermore, as S. mansoni infections of man led to portal hypertension with a consequent ascites and fluid retention, Fiorillo et al (1954) were of the opinion that the hypoalbuminaemia merely reflected a pooling of albumin in the extravascular tissue spaces.

More insight into the pathophysiological processes responsible for these changes came from the radioisotopic turnover studies with ^{125}I-labelled albumin by Dargie, Maclean and Preston (1972, 1973) in S. mattheei infected sheep and by Smithers and Walker (1961) working with S. mansoni infected monkeys. No significant alterations in protein metabolism occurred during the first four weeks of infection indicating that immature flukes have little or no effect on the protein status of the hosts. With the onset of oviposition (from the 4th week hypoalbuminaemia and hypergammaglobulinaemia became a feature of infection. Despite the hypoalbuminaemia, the intravascular mass of albumin remained virtually unchanged which was taken as evidence that the pronounced plasma volume expansion observed during the same period (6th to 12th week) was responsible for the hypoalbuminaemia. The fall in extravascular-intravascular ratio was attributed to a net transfer of protein from extravascular sites into the plasma as part of the normal homeostatic response for the maintenance of constant plasma protein concentrations. The catabolic rate in infected sheep was markedly increased being 0.113 as compared to 0.075 in the controls. A close correlation was observed between the fractional catabolic rate, the severity of the hypoalbuminaemia and the fall in total exchangeable albumin. From faecal clearance figures, the cause of the hypercatabolism was/

was attributed to loss of plasma into the gut. Clearance figures using ^{131}I-labelled albumin with its attendant unreliability due to breakdown and reabsorption of the albumin in the gut, still showed a value of 57.3 ml. for the infected sheep and only 9.1 ml./day for the controls (Dargie, Maclean and Preston, 1973). Studies with ^{51}Cr-labelled red cells in schistosome infected sheep by Preston and Dargie (1973) when compared transtedially with the ^{131}I-labelled albumin studies by the same workers revealed that high catabolic rates were still being observed at a time when faecal clearance of red cells had returned to values found in control animals. An additional loss of plasma over and above that lost by haemorrhage thus occurred which was linked with either increased vascular permeability or leakage of lymph through the obstructed, dilated or eroded lymphatics. Since the hypoalbuminaemia was not so severe despite the high catabolic rate, they concluded that synthesis must have been proceeding at a faster rate in the infected animals. An estimate of synthesis can be obtained by multiplying k by the total body pool of albumin and this was found to be 22% greater in infected relative to control animals from the 4th week onwards. Infected animals were also anorexic at about this period and this was thought to cause an impairment of protein synthesis due to reduced dietary intake. Thus the catabolic rate and synthesis rate observed in later stages of the disease between the 8th and 12th weeks were reduced compared with the preceding four week period. They concluded that infected animals compensated for the reduced protein intake by a reduction in protein synthesis and catabolism as was observed in malnourished children (Cohen and Hensen, 1962) and rabbits (Hoffenberg, Black and Brock, 1966).

The hypoproteinaemia of schistosomiasis, at least in sheep, resulted from a combination of haemodilution, redistribution, hypercatabolism due to enteric loss and possibly reduced synthesis due to terminal inappetence.

Haemonchosis/

HAEMONCHOSIS:

There appears to be general agreement that the three major clinical features of haemonchosis, i.e. anaemia, hypoproteinaemia and weight loss, result directly from the blood sucking activities of both the larval and adult stages of the parasite.

Investigations into the hypoproteinaemia have been limited to a study of the hypoalbuminaemia. ^{131}Iodine-labelled albumin and ^{131}I-labelled PVP were used in measurements of albumin turnover and enteric leak respectively in Merino sheep by Dargie (1973). Hypoalbuminaemia was confirmed in infected animals but the drain on extravascular protein was greater than the intravascular pool which led to a lowered ratio of extravascular to intravascular protein. A net transfer of albumin into the plasma thus occurred. A high rate of albumin degradation expressed by a Shortened $t_{\frac{1}{2}}$ and high fractional catabolic rate was a feature of the disease from the 1st to 3rd weeks of infection. These values were 245 hrs and 0.136 in parasitized animals compared with 502 hrs and 0.069 respectively in the controls. The total body pool of albumin and serum protein concentration was not so seriously lowered in the infected animals compared with the controls indicating that synthesis was also going on at a faster rate. Faecal clearance figures using ^{131}I or ^{131}I-PVP revealed a substantial leakage of plasma macromolecules into the gut, which correlated well with the worm burden. The hypercatabolic hypoalbuminaemia was attributed to the blood sucking activities of the parasites and eventual passage of the constituents into the gut. Dargie (1975) also suggested that amounts of plasma in excess of those expected from losses due to haemorrhage probably passed into the gut as was observed in fascioliasis by Dargie *et al* (1968). The hypercatabolic hypoalbuminaemia eventually led to diversion of amino-acids from low priority sites such as muscle to vital organs. This diversion coupled with the failure of infected sheep to reabsorb substantial amounts of the nitrogen lost into the gut was thought to cause the pronounced fall of body weight.

Albumin turnover studies were also carried out in Merino sheep re-infected with 10,000 larvae of H. contortus in order to induce and hence examine the phenomenon of self-cure (Dargie, 1973). When self-cure occurred, the rate of albumin breakdown and faecal clearance of plasma progressively declined to control values while serum albumin concentration increased progressively. Those animals which failed to self-cure, i.e. those overwhelmed by the challenge infection, exhibited a marked increase in albumin breakdown and enteric plasma leak which was progressive to death. Still others were able to maintain a relatively close to normal serum albumin concentration in the face of the continued hypereatabolism and enteric plasma leakage. He concluded that self-cure was apparently immunogenic and that the pathogenicity of the disease was related to the immune status of the host. Altaif and Dargie (1976, 1978) later used observations from albumin turnover studies to show that genetic resistance to haemonchosis was influenced by the breed and haemoglobin type of the hosts. Thus Finn Dorset sheep had higher fractional catabolic rates, more severe hypoalbuminaemia and higher faecal plasma clearances than Scottish Blackface sheep. Similar disadvantages were displayed by sheep of HbB compared to HbA within each breed of animal. The severity of the albumin depletion was found to be directly related to the severity of the catabolic stress.

The hypoproteinaemia of haemonchosis is of the hypercatabolic type and appears to result directly from the feeding activities of the parasites and eventual passage of the constituents into the gut.

HYPOPROTEINAEMIA OF OSTERTAGIASIS AND OTHER TRICHOSTRONGYLES.

Hypoproteinaemia, especially hypoalbuminaemia, has been reported in Type II or chronic ostertagiasis of cattle (Anderson et al, 1965;/

1965; Martin et al, 1957; Nielsen and Aalund, 1961; Mulligan, Dalton and Anderson, N., 1963) and in sheep suffering from acute O. circumcincta infections (Horak and Clark, 1964; Holmes and Maclean, 1971). Following the discovery of the 'syndrome of protein losing gastroenteropathy' in man by Citrin et al (1957) in which radioisotopic tracer techniques were used to demonstrate gastric leakage of plasma proteins in some gastrointestinal disorders of man, attempts were made to examine if similar pathophysiological abnormality also occurred in animals suffering from gastrointestinal diseases. Ostertagiasis was the first disease entity to be studied and the first reports of such studies came from the works of Mulligan et al (1963). Using ^{131}I-labelled albumin these workers showed conclusively that heifers with ostertagiasis had a marked hypercatabolic hypoalbuminaemia. This was confirmed in subsequent studies in cattle by Nielsen (1966a), Halliday et al (1968), Nielsen (1968), and in sheep by Holmes and Maclean (1971). These authors showed that the hypoalbuminaemia was due to an intestinal loss of albumin when they recorded higher faecal radioactivities in infected animals relative to controls using either ^{131}I-albumin or $^{51}CrCl_3$. In sheep, Holmes and Maclean (1971) showed that the high fractional catabolic rate and increased faecal radioactivity was limited to the acute stages of the disease, i.e. between the 1st and 3rd weeks of infection. The route of this excessive leakage was thought to be through the junctional complexes of the hyperplastic abomasal mucosa which were observed to open up in the parasitized abomasa thereby permitting macromolecules to pass through (Nielsen, 1968; Murray, 1969; Murray et al, 1970). However, Nielsen (1966a) was of the opinion that the functional state of the gastrointestinal tract might be more important in the pathogenesis of the protein leakage when he recorded high fractional catabolic rates and high/

high faecal excretion of ^{131}I only during the diarrhoeic phases of the disease in cattle. These values were normal in quiescent infected animals. Furthermore, no significant changes were observed in albumin turnover and faecal excretion of radioactivity in two sows infected with Hyostrongylus rubidus which failed to scour even though the abomasal mucosa was hyperplastic (Nielsen, 1966b). Studies in sheep by Holmes and Maclean (1971) showed that albumin turnover values were higher in diarrhoeic than non-diarrhoeic animals but faecal clearance values were similar and high turnover and increased faecal radioactivity occurred in the absence of diarrhoea. Perhaps some losses of plasma from increased endogenous catabolism into perivascular spaces (not into the gut) occurred in the severely affected animals as suggested by Holmes and Maclean (1971) or diarrhoea per se was important in precipitating the protein leakage (Nielsen, 1966a).

Hyostrongylus rubidus infection in pigs also causes an abomasitis not unlike the lesions of O. ostertagi in cattle. Studies with $^{51}CrCl_3$, ^{131}I-labelled albumin and ^{131}I-PVP by Dey-Hezra et al (1972), Titchener et al (1974) confirmed a hypercatabolic hypoalbuminaemia with greatly increased albumin turnover and faecal excretion rates. These values were higher in diarrhoeic pigs. As mentioned earlier, Nielsen (1966b) studied two cases with ^{131}I-albumin and failed to find evidence of hypercatabolism. This was attributed to the absence of scouring in the pigs. Strongylus ransomi was also shown by Dey-Hezra et al (1972) to cause a gastrointestinal loss of protein in pigs while Bremner (1969) demonstrated similar losses in cattle infected with Oesophagostomum radiatum and plasma albumin turnover rate and loss into the intestine were also shown to be markedly increased in guinea-pigs infected with Trichostrongylus/

Trichostrongylus colubriformis by Symons, Jones and Steel (1974) although hypoproteinaemia had been observed in Trichostrongylus spp. infections of ruminants as early as 1939 by Andrews and later by Horak, Clark and Gray (1968).

In conclusion, evidence from radioisotopic studies have shown that the hypoproteinaemia associated with the parasitic infections reviewed was hypercatabolic in nature. This was based on the observed apparent reductions in the plasma albumin concentrations and in the size of the intravascular pools of albumin and the very marked increases in albumin catabolic rate. As pointed out by Mulligan (1972) and Dargie (1975), the distinction between absolute and fractional rates of catabolism should be recognised in interpreting the results and, furthermore, a rapid turnover of a smaller pool of albumin could occur when animals are severely hypoproteinaemic in which case the absolute amount of albumin degraded per day may not be in excess of normal. The hypercatabolism was subsequently shown to result from losses of plasma into the gut. In all the diseases reviewed, the loss of plasma into the gut failed to give rise to a severe hypoalbuminaemia, a finding which was interpreted as evidence that synthesis was almost keeping pace with catabolism and also proceeding at a faster rate in infected animals. The excessive loss of plasma into the gut was suggested as the stimulus for the increased rate of protein synthesis by Symons, Jones and Steel (1974). This increased rate of essential protein synthesis apparently occurred at the expense of other productive processes such as muscle protein synthesis, an assertion borne out by the finding of reduced synthesis and increased catabolism of muscle protein in mice and guinea-pigs suffering from gastrointestinal nematode infections (Symons and Steel, 1971, 1972). The effect was more pronounced in anorexic animals and since most of the parasitic conditions reviewed are characterised by anorexia, a marked rechannelling of amino acids/

acids away from productive processes into synthesis of essential proteins necessary for survival is envisaged. The net effect is an impairment in productivity (Dargie, 1975). Other implications of the macromolecular leakage include a possible deleterious effect on nitrogen balance of the hosts. Increased loss of nitrogen in the urine has been reported in most of these diseases but significant reabsorption of the nitrogen also occurred, except in cases where the lower gastrointestinal tract was affected. The disturbance in nitrogen balance was thought to result from a failure of the host to conserve the amino acids derived from excessive breakdown of tissue and blood proteins (Dargie, 1975). Where the parasites cause damage to the lower gastrointestinal tract where no significant reabsorption occurs as in Oesophagostomum columb ianum infection of cattle (Bremner, 1969), the adverse effect on nitrogen balance was attributed solely to impaired absorption which could also be worsened by inappetence. Finally, in situations where the macromolecular leakage embraced all the plasma proteins, e.g. fascioliasis, the loss of immunoglobulins may affect the development and course of immunity although this has not been proven conclusively.

REFERENCES.

Altaif, K.I. and Dargie, J.D. (1976)
I.A.E.A., Vienna, pp. 449-462.

Altaif, K.I. and Dargie, J.D. (1978)
Parasitology, 77, 161-175.

Andersen, S.B. (1964)
I.A.E.A., Vienna, pp. 63-69.

Anderson, N., Armour, J., Jarrett, W.F.H., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M. (1965)
Veterinary Record, 77, 1196-1204.

Andrews, J.S. (1939)
Journal of Agricultural Research, 58, 761-770.

Bitakaramire, P.K. (1970)
I.A.E.A., Vienna, pp. 35-43.

Bremner, K.C. (1969)
Experimental Parasitology, 25, 382-394.

Campbell, R.M., Cuthbertson, D.P., Mathews, C.M.E. and Macfarlane, J.S. (1956)
International Journal of Applied Radio Isotopes, 1, 66-84.

Citrin, Y., Sterling, K., Halstead, J.A. (1957)
New England Journal of Medicine, 257, 906-912.

Cohen, S. and Hansen, J.D.L. (1962)
Clinical Science, 23, 351.

Cornelius, C.E., Baker, N.F., Kaneko, J.J. and Douglas, J.R. (1962)
American Journal of Veterinary Research, 23, 837-842.

Dargie, J.D. (1973)
In: Helminth Diseases of Cattle, Sheep and Horses in Europe, (eds. G.M. Urquhart and J. Armour), Glasgow: Robert Maclehose & Co. Ltd., pp. 63-71.

Dargie, J.D. (1975)
In: Pathogenic processes in Parasitic Infections, (eds. E.R. Taylor and R. Muller), Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne, pp. 1-26.

Dargie, J.D. (1980)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 74, 5.

Dargie, J.D. and Mulligan, W. (1971)
Journal of Comparative Pathology, 81, 187-202.

Dargie, J.D., Holmes, P.H., Maclean, J.M. and Mulligan, W. (1967)
Veterinary Record, 80, 390.

Dargie, J.D., Holmes, P.H., Maclean, J.M. and Mulligan, W. (1968)
Journal of Comparative Pathology, 78, 101-105.

Dargie, J.D., Holmes, P.H., Maclean, J.M. and Mulligan, W. (1970)
I.A.E.A., Vienna.

Dargie, J.D., Maclean, J.M., Preston, J.M. (1972)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 66, 546.

Dargie, J.D., Maclean, J.M. and Preston, J.M. (1973)
Journal of Comparative Pathology, 83, 543-557.

Dey-Hezra, A., Giese, W., Enigk, K. (1972)
Deutsche tierarztl. Wochenschrift, 79, 421.

Evans, A.S. and Stirewalt, M.A. (1957)
Experimental Parasitology, 6, 8-17.

Fahey, J.L. and Robinson, A.G. (1963)
Journal of Experimental Medicine, 118, 845-868.

FAO/IAEA (1970)
Proceedings of the 2nd Research and Coordination Meeting, Vienna, 1969.

Fayez, M. and Aziz, S. (1963)
Gaz. Kasr. El. Aini., 31, 193.

Fiorillo, A.M., Jamra, M., Eston, V.R., Eston, T.A. and Pagano, C. (1954)
Review of the Ass. Med. Bras., 1, 173.

Freeman, T. (1967)
In: Handbook of Experimental Immunology (ed. D.M. Weir), Oxford, Edinburgh, Blackwell Publications, pp. 597-607.

Gray, S.J. and Sterling, K. (1950)
Journal of Clinical Investigation, 29, 1604 - 1613.

Gordon, R.S. Jnr. (1959)
Lancet, 1, 325 - 326.

Gordon, R.S. Jnr., Bartter, F.C. and Waldman, T.A. (1959)
American Journal of Medicine, 51, 553-561.

Halliday, G.J., Mulligan, W. and Dalton, R.G. (1968)
Research in Veterinary Science, 9, 224-227.

Hofer, R., Schwatz, H. and Thumb, B. (1968)
Clinica. Chim. Acta, 22, 135-143.

Hoffenberg, R., Black, E. and Brock, J.F. (1966)
Journal of Clinical Investigation, 46, 2064 - 2066.

Holmes, P.H. (1969)
Ph.D. Thesis, University of Glasgow.

Holmes, P.H., Dargie, J.D., Maclean, J.M. and Mulligan, W. (1968)
Veterinary Record, 83, 227-228.

Holmes, P.H. and Maclean, J.M. (1971)
Research in Veterinary Science, 12, 265-271.

Holmes, P.H., Maclean, J.M. and Mulligan, W. (1971)
In: Pathology of Parasitic Diseases (ed. S.M. Gaafar),
pp. 69-81, Lafayette, Purdue University Studies.

Horak, I.G. and Clark, R. (1964)
Onderstepoort Journal of Veterinary Research, 31, 163 - 176.

Horak, I.G., Clark, R. and Gray, R.S. (1968)
Onderstepoort Journal of Veterinary Research, 35, 195-224.

Jarnum, S. (1961)
Scandinavian Journal of Clinical Laboratory Investigation,
13, 447-461.

Jarnum, S. (1963)
In: Protein losing gastroenteropathy, pp. 232,
Blackwell Scientific Publications, Oxford.

Jarnum, S., Westergaad, H., Yssing, N. and Jensen, H. (1968)
Gastroenterology, 55, 229 - 241.

Jeejeebhoy, K.N. (1965)
Technical Report Series 45, 169, I.A.E.A., Vienna.

Jeejeebhoy, K.N., Singh, B., Mani, R.S. and Sanjana, S.N. (1964)
In: Physiology and pathophysiology of plasma protein metabolism,
Hane Huber Publications, Berne and Stuttgart, pp. 61-67.

Jeffries, G.H. and Sleisinger, M.H. (1962)
In: Plasma proteins and gastrointestinal tract in health and
diseases (eds. M. Schwartz and P. Vesin), Munksgard,
Copenhagen p. 108.

Maclean, J.M., Holmes, P.H., Dargie, J.D. and Mulligan, W. (1968)
I.A.E.A., Vienna, pp. 117-123.

Malherbe, W.D. (1970)
Onderstepoort Journal of Veterinary Research, 37, 37-43.

Margen, S. and Tarver, H. (1956)
Journal of Clinical Investigation, 35, 1161-1172.

Martin, W.B., Thomas, B.A.C. and Urquhart, G.M. (1957)
Veterinary Record, 69, 736 - 737.

Mathews, C.M.E. (1957)
Physiological Medical Biology, 2, 36-53.

Mathews, C.M.E. (1966)
Journal of Nuclear Biological Medicine, 10, 3.

Macfarlane, A.S. (1950)
Biochemistry Journal, 62, 135.

Macfarlane, A.S. (1958)
Nature (Lond.), 53, 182.

Montgomery, D.A.D., Neill, D.W. and Dowdle, E.B.D. (1962)
Clinical Science, 22, 141.

Mulligan, W. (1972)
Proceedings of the Nutrition Society, 31, 47-51.

Mulligan, W. (1973)
In: Helminth Diseases of Cattle, Sheep and Horses in Europe, (eds. G.M. Urquhart and J. Armour), R. Maclehose & Co. Ltd., University of Glasgow, pp. 127-131.

Mulligan, W., Dalton, R.G. and Anderson, N. (1963)
Veterinary Record, 75, 1014.

Murray, M. (1969)
Gastroenterology, 56, 763-772.

Murray, M. (1974)
In: Helminth Diseases of Cattle, Sheep and Horses in Europe, (eds. G.M. Urquhart and J. Armour), R. Maclehose & Co. Ltd., Glasgow, pp. 92-96.

Murray, M., Jennings, F.W. and Armour, J. (1970)
Research in Veterinary Science, 11, 417-427.

Nansen, P. (1971)
Acta. Vet. Scandin., 12, 335-343.

Nansen, P., Eriksen, L., Simesen, M.G. and Nielsen, K. (1968)
Nord. Vet. Med., 20, 651-656.

Nielsen, K. (1966)
Gastrointestinal protein loss in cattle: A clinical and pathophysiological study (ed. C.F. Mortensen), Copenhagen.

Nielsen, K. (1968)
Isotopes and Radiation in Parasitology (Panel Proc. Series), I.A.E.A., Vienna, p. 125-132.

Nielsen, K. and Aalund, O. (1961)
Nord. Vet. Med., 13, 388 - 409.

Nielsen, K. and Nansen, P. (1968)
Isotopes and Radiation in Parasitology,
I.A.E.A., Vienna, pp. 63-69.

Nosslin, B. (1966)
Journal of Nuclear Biological Medicine, 10, 3-30.

Pearson, J.D., Veali, N., Vetter, H. (1958) cited by Nielsen, K. (1970)
Isotopes and Radiation in Parasitology,
I.A.E.A., Vienna, pp. 23-40.

Preston, J.M. and Dargie, J.D. (1974)
Journal of Comparative Pathology, 84, 73-81.

Ramirez, E.A., Riverade Sala., Serrano, D. and Cancio, M. (1961)
American Journal of Tropical Medicine and Hygiene, 10, 530-534.

Ross, J.G., Todd, J.R. and Dow, C. (1966)
Journal of Comparative Pathology and Therapeutics, 76, 67-81.

Saad, A.M., Hussein, M.F., Dargie, J.D., Taylor, M.G. and Nelson, G.S. (1980)
Research in Veterinary Science, 28, 105-111.

Sadun, E.H.,and Walton, B.C. (1958)
American Journal of Tropical Medicine and Hygiene, 7, 500-504.

Secretan, P.H. and Bickel, G. (1960)
Ach. mal. Appar. dis., 49, 642.

Simesen, M.G., Eriksen, L., Nansen, P., Andersen, S. and Nielsen, K. (1968)
Nord. Vet. Med., 20, 638-649.

Sinclair, K.B. (1962)
British Veterinary Journal, 118, 37-53.

Schwartz, M. and Jarnum, S. (1962)
In: Plasma proteins and gastrointestinal tract in health and disease (ed. M. Schwartz and P. Vesin), Munksgard, Copenhagen, p. 116.

Smithers, S.R. and Walker, P.J. (1961)
Experimental Parasitology, 11, 39-49.

Sterling, K. (1951)
Journal of Clinical Investigation, 30, 1238-1242.

Symons, L.E.A. and Jones, W.O. (1971)
Experimental Parasitology, 29, 230-241.

Symons, L.E.A. and Jones, W.O. (1972)
Experimental Parasitology, 32, 335-342.

Symons, L.E.A., Jones, W.O. and Steel, J.W. (1974)
Experimental Parasitology, 35, 492-502.

Thorpe, E. (1965)
Research in Veterinary Science, 6, 498-509.

Titchener, R.N., Herbert, I.V. and Probert, A.J. (1974)
Journal of Comparative Pathology, 84, 399-407.

Urquhart, G.M. and Armour, J.(1973)
In: Helminth Diseases of Cattle, Sheep and Horses in Europe,
R. Maclehose & Co. Ltd., University of Glasgow Press.

Van Tongeron, J.H. and Majoor, C.L.H. (1966)
Clinica. Chim. Acta., 14, 31-41.

Von Lichtenberg, F., Sadun, E.H., Cheever, A.W., Erikson, D.G.,
Johnson, A.J. and Boyce, H.W. (1971)
American Journal of Tropical Medicine and Hygiene, 20, 850-862.

Waldman, T.A. (1961)
Lancet, 2, 121-123.

Waldman, T.A. and Wochner, R.D. (1965)
Technical Report Series 45, 171,
I.A.E.A., Vienna.

CHAPTER V

THE INFLUENCE OF NUTRITION ON THE PATHOGENESIS OF PARASITIC INFECTIONS

THE INFLUENCE OF NUTRITION ON THE PATHOGENESIS OF PARASITIC INFECTIONS

Nutrition in common with variables such as rate of larval dose, the species of parasite, the immune status of the host and the breed and age of the host exert a profound influence on the pathogenic effect of parasites. Most studies on the influence of nutrition on the relationships between parasites and their hosts in domestic and laboratory animals have been carried out on gastrointestinal and liver parasites. These parasites cause some derangement of function which ultimately gives rise to metabolic disturbances and poor productivity of the hosts. The impaired productivity is usually manifested primarily by loss of appetite, impaired digestion or malabsorption, curtailed deposition of certain minerals, abnormal losses of endogenous metabolites into the gut and by reductions in liveweight gains or actual weight losses. These changes are non-specific and are influenced by the same variables irrespective of the aetiological agent involved.

Though parasites affect the nutritional physiology of their hosts, the plane of nutrition offered to the host also influences the occurrence and severity of parasitisms. Gordon (1964) defined two effects of nutrition on the pathogenesis of parasitic infections:- (1) the indirect effect which finds expression through the amount and nature of forage available or through the grazing habits of the animal and invariably affects the epidemiology of the parasites by influencing the availability of the free-living stages of the life cycle. Little attention was focussed on this aspect though reports are in agreement that the seasonal availability of the pasture and free-living stages of the parasites are usually associated with increased occurrence of overt/

overt disease during favourable seasons (Drurie, 1962).

(2) The direct effect is associated with the ability of the host to resist infections. In this regard Hunter (1953) pointed out that diet *per se* had little or no effect on the parasites themselves. Most studies attribute the effects of nutrition to alterations in the resistance of the host to their parasites though only in a few reports have individual dietary deficiencies been related to the immunological competence of the hosts. Thus Jackson (1925) showed that malnutrition caused atrophy of the thymus while Wells (1963) demonstrated that low protein diets depressed the cellular reactions of rats infected with *Nippostrongylus brasiliensis*. These early reports have lately been confirmed by Dineen and Adams (1971) in *Trichostrongylus colubriformis* infections of the guinea-pig and by Dobson and Bawden (1974) in *Oesophagostomum columbianum* infections of sheep. Resistance to parasitic infections can either be directed against establishment of the infection or to the effects of an already established infection. Though there are reports of resistance to the establishment of an infection (Chandler, 1953; Geiman, 1958) most reports are agreed that nutrition has no effect on the establishment of infections but rather modifies the effects of an established infection (Kates and Wilson, 1955; Gibson, 1963; Bawden, 1969).

The association between particular nutritional deficiencies and increased susceptibility to parasitic infections was first made by Ackert and Beach (1953) from their studies on *Ascaridia galli* infections in chickens whereby a specific deficiency of dietary protein was incriminated. Later works have shown that the vitamin A content (Hunter, 1953), the protein level and quantity of the diet (Vegors *et al*, 1956) are the most important nutritional factors in modifying reactions to parasitic infections.

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In this review, the influence of nutrition on the relationship between gastrointestinal and liver parasites and their hosts is considered along two lines. Firstly, the impairment of production due to disturbances of the nutritional physiology of the hosts by parasites and secondly, how nutritional planes modify these disturbances at the various sites of parasitism.

A. Parasites and the Nutritional Physiology of their Hosts:

Most gastrointestinal and liver parasites are associated with impairment of growth and/or loss of weight of their hosts. The check in growth rate resulted in poor productivity though factors such as the higher water content of infected animals (Berry and Dargie, 1976; Sykes and Coop, 1976) curtailed deposition of fat, protein, calcium and phosphorous (Sykes and Coop, 1976, 1977) also lowered productivity by affecting carcase quality. Production losses can also be manifested by reductions in wool growth and up to 40% reductions have been described in infected animals (Southcott, Heath and Langlands, 1967; Roseby, 1970; Barger, Southcott and Williams, 1973; Edwards et al, 1976). Various reasons have been advanced in an attempt to explain these effects:-

(1) Derangement of basic cellular functions arising out of pathological lesions caused by the parasites. Such lesions range from destruction of gastric glands during growth and emergence of the immature forms from the gastric mucosa in Ostertagia circumcincta and T. axei infections (Armour, Jarrett and Jennings, 1966; Urquhart and Armour, 1973); mutilation of the mucosa and haematophagic activities of Haemonchus contortus (Fourie, 1931) to mucosal and villous atrophy or flattening with sparse stunted microvilli in T. colubriformis infections; ulceration and haemorrhage in O. columbianum and Chabertia ovina infections (Bawden, 1969; Herd, 1971; Barker, 1973); hepatic necrosis/

necrosis and haemorrhage during fluke migration with biliary erosion when infections are patent in the bile ducts (Murray and Rushton, 1975) and erosions and haemorrhage in S. mattheei infections (Preston, Dargie and Maclean, 1973). These lesions, depending on their severity, were postulated to make an animal disinclined to eat and consequently affected body weight and growth changes.

(2) Reductions in Feed Intake - This is a common finding in parasitisms and, when such reductions are lower than amounts consumed by the uninfected counterpart, lead to serious impairment of wool growth and bodyweight gains. Reductions in voluntary feed intake have tended to be transient in infections produced by a single large dose of infective larvae, e.g. in Ostertagiasis of sheep and calves (Jennings et al, 1966; Holmes and Maclean, 1971) and in T. colubriformis infections of sheep (Steel, 1972; Roseby, 1973; Reveron et al, 1974). Where experimental infections have been achieved by daily dosing with less numbers of infective larvae to simulate the natural cases, reductions in appetite have been consistent and persistent due possibly to continual presence of immature stages in the gastrointestinal mucosa, e.g. ostertagiasis (Coop, Sykes and Angus, 1976; Sykes and Coop, 1977), T. colubriformis infections (Coop, Sykes and Angus, 1976; Sykes and Coop, 1976). However, persistent reductions in appetite were reported in F. hepatica infections irrespective of the method of dosing (Sinclair, 1975; Berry and Dargie, 1976; Sykes, Coop and Rushton, 1980) and in single heavy infections of O. columbianum (Gordon, 1958; Horak and Clark, 1966; Dobson, 1967) and S. mattheei (Berry, Dargie and Preston, 1973). Only in H. contortus (Dargie, 1973), mixed populations of nematodes (Southcott et al, 1967) and Cooperia spp. (Andrews, 1939; Andrews et al, 1944; Coop, Sykes and Angus (in press) were infections not characterised by reductions in food intake.

Though/

Though various reports are in agreement that the reductions in food intake accounted for a significant proportion of the weight differences between infected and ad libitum fed controls, the aetiology of the reduced intake remains unresolved. Among the reasons postulated are the pathological lesions produced by the parasites and the physical limitations on the capacity of the gut to transport digesta (Grovum and Philips, 1978). Dargie (1980) was of the opinion that, though the lesions could make an animal disinclined to eat, the reduction in intake often fell progressively as infection progressed, especially if the food was of poor quality, implying that the persistent anorexia could not be attributed solely to the lesions produced initially. He therefore concluded that the clinical condition of the animal, as manifested by blood and serum protein changes which fell progressively with increasing duration of infection, were more important determinants of the reduced intake. Parasites also lower the efficiency of feed conversion into growth independent of the reduced food intake (Sykes and Coop, 1976, 1977) though such effects are often overshadowed by the latter.

(3) Anaemia and plasma protein changes - Poor production has also been blamed on the production of anaemia, hypoalbuminaemia and hyperglobulinaemia by these parasites. Where information is available, the application of conventional and radioisotopic techniques have proved valuable in elucidating the mechanism of the anaemia and hypoproteinaemia. Thus in O. circumcincta (Holmes and Maclen, 1971), H. contortus (Dargie, 1973), T. colubriformis (Barker, 1973), O. radiatum (Bremner, 1969), C. ovina (Herd, 1971), F. hepatica (Berry and Dargie/

and Dargie, 1978; Dargie and Berry, 1979) and S. mattheei (Dargie, Maclean and Preston, 1973; Preston and Dargie, 1974) infections, the anaemia and hypoalbuminaemia were associated with high fractional rates of removal of red cells and albumin coincidental with the observed weight loss or poor weight gains of the animals. In addition, large volumes of blood and plasma were being lost into the gastrointestinal tract and the site of loss was referrable to the predilection site of the parasites except in fascioliasis where the loss occurred via bile into the intestines. The endogenous loss of albumins was also attended by increased synthesis in the liver and, in the case of immunoglobulins, the hypercatabolism was accompanied by an even more pronounced synthesis (Dargie, 1975). The increased synthesis of essential proteins, coupled with the loss of proteins into the gut, invariably produced reductions in weight or curtailed weight gains. These effects occurred independently of reduced feed intake and Dargie (1980) therefore concluded that the lowered production of parasitised animals could be explained partly by reduced feed intake and partly by their higher rates of blood protein turnovers.

(4) Mineral metabolism - Lowered productivity has been associated with curtailed deposition of Ca, P, Mg, reductions in bone size and density of bone matrix and its degree of mineralisation in O. circumcincta and T. colubriformis infections of sheep (Barger, 1973; Reveron et al, 1974; Sykes, Coop and Angus, 1975, 1977; Sykes and Coop, 1976; 1977). In T. colubriformis infections hypophosphataemia was a constant finding while in O. circumcincta infections serum levels of Ca and P were normal. In both infections the reductions in bone size and/or circulating P could not be accounted for solely by reductions in food intake. Reduced digestibilities of either both Ca and P (Sykes and Coop, 1976, 1977; Sykes/

Sykes, Coop and Angus, 1977) or of P alone (Reveron et al, 1974) were reasons adduced for the bone abnormalities. These workers concluded that in trichostrongylosis there was an induced Ca and P deficiency while in ostertagiasis an induced energy or protein deficiency probably occurred. However, no attempts were made to show that P digestibilities was reduced more than Ca nor to determine the relative contributions of dietary and endogenous losses to the faecal mineral losses. Moreover, energy and protein intake in infected animals was actually lowered by 20%. Because of these shortcomings Dargie (1980) was of the opinion that the observed differences between the two infections merely reflected differences in degree of infection. The other parasites do not appear to affect mineral metabolism directly though reductions in food intake per se could manifest in reductions in skeletal size as was observed in fascioliasis by Sykes, Coop and Rushton (1980).

(5) Alterations of gut and liver functions - Evidence incriminating alterations in function of the sites of parasitism which could account for poor productivity is equivocal.

On observing decreased flow rate of digesta from the rumen relative to the other parts of the gut and the finding of increased amounts of liquid and solid materials in the abomasum and small intestine relative to the rumen in T. colubriformis infected sheep, Roseby (1977) concluded that the parasite altered gut motility and digesta flow. However, there was no indication whether the sheep were diarrhoeic and the volume and flow rates were obtained from different animals. Also the flow-rate of particulate matter was not measured hence his conclusions failed to gain universal agreement. Similarly Bawden (1970) who observed decreased flow rate of digesta in non-diarrhoeic sheep infected with O. columbianum did not use pair-fed controls. Even where conclusive evidence was obtained that parasites altered gut activity

based/

based on electrical activity of the gut wall of sheep infected with T. axei (Bueno et al, 1975), such sheep were diarrhoeic. Diarrhoea is at best transient or even absent in most of the parasitic conditions discussed and such alterations in gut motility, if at all, can only be localised in certain regions of the gut and not generalised. The contribution of altered gut motility or digesta flow to lowered productivity remains doubtful.

Impairment of hormones and digestive secretions has also received some attention. In calves fitted with abomasal cannula and infected with O. ostertagi, marked changes in abomasal secretions such as elevated pH, Na concentration and viable bacteria coupled with reductions in K, Cl and peptic activity were recorded. These defective secretions were concluded to interfere with protein digestion possibly through the presence in the duodenum of digesta with elevated pH which could reduce the secretions of the pancreas and the intestine. However, Ben-Ghedalia et al (1976) found that defective abomasal secretions did not interfere with the handling of proteins by the gut as a whole when large amounts of protein infused directly into the ileum were completely digested and some of the N retained. Titchen and Anderson (1977) with the aid of a cannula in the abomasum and another in a separate fundic pouch compared secretions in the pouch and the parasitized abomasum (O. circumcincta). There was a profound hypergastrinaemia and a doubling of secretions despite marked reductions in food intake. Earlier work by Walsh (1975) had shown that gastrin increased water and electrolyte secretion by the liver, pancreas and ileum and the hypergastrinaemia was therefore implicated in promoting diarrhoea, dehydration and weight loss. Evidences from other studies appear at variance with this conclusion. Cohen and Sadun (1976) observed that Pentogastrin, a synthetic analogue of gastrin inhibited reticulo-ruminal/

reticulo-ruminal and abdominal activity in sheep thus tending to reduce digesta flow and counteracting any net movement of fluids. Moreover, diarrhoea is not a regular feature of abomasal parasitism even though gastrin levels are elevated and in cases where diarrhoea features prominently, e.g. in T. colubriformis infections, elevated gastrin levels have not been reported (Urquhart and Armour, 1973).

Impaired digestibility or absorption of certain nutrients arising out of the pathological leasions caused by the parasites have also been incriminated in poor productivity. Reductions in nitrogen digestibility have been reported in sheep infected with O. circumcincta (Horak and Clark, 1964; Holmes and Maclean, 1971; Parkins, Holmes and Bremner, 1973; Sykes and Coop, 1977) and H. contortus (Dargie, 1980). There are also reports of reductions in digestibility in T. colubriformis infections (Franklin et al, 1946; Shumard et al, 1957; Barger, 1973) but the majority of studies have shown neither protein nor energy digestibilities to be affected (Andrews et al, 1944; Roseby, 1973; Reveron et al, 1974; Sykes and Coop, 1976). Reductions in N digestibility have also been reported in O. columbianum infected sheep (Gordon, 1958; Horak and Clark, 1966; Dobson, 1967) and O. radiatum infections in calves (Bremner, 1969). In F. hepatica infections achieved by daily-dosing with 5-14 metacercariae for 98 days to simulate field conditions, no reductions in N and dry matter digestibilities were noticed (Sykes, Coop, Rushton, 1980), whereas in single large experimental infections of up to 5,000 metacercariae some workers observed reductions in digestibility (Duwell et al, 1972; Canale et al, 1973; Dargie, Berry and Parkins, 1979) while others have failed to observe any reductions (Sinclar, 1975; Hawkins and Morris, 1978). Berry, Dargie and Preston (1975) also/

also reported reductions in N and dry matter digestibility in sheep infected with S. mattheei. Interpretation of these observations are complicated by various factors. Where reductions in digestibility have been observed (H. contortus; F. hepatica; T. colubriformis; O. radiatum), such reductions can be explained by the excessive gut leakage of plasma. Moreover, where the loss was occurring near the sites of digestion or absorption of nitrogenous materials, the extra protein appear processed whereas in large intestinal parasitism where no significant reabsorption occurs, the extra protein leak was being lost wholly as protein in the faeces. For these reasons poor digestion or malabsorption cannot be said to be operating. Even in ostertagiasis where Sykes and Coop (1977) showed that the reductions in digestibility could not initially be explained by gut leakage alone, the digestibility coefficient subsequently returned to values explainable by the gut leakage and Dargie (1980) was of the opinion that this phenomenon could have been responsible in the first place. Also in schistosomiasis the reductions in digestibility were complicated by the occurrence of diarrhoea. It would thus appear as if parasites per se do not affect N digestibilities and, even if they do, their contribution to lowered productivity remains questionable.

(6) Disturbances of post-absorptive metabolism - Poor nitrogen retention caused mainly by excessive urinary excretion has been demonstrated in T. colubriformis, O. circumcincta and S. mattheei infections of sheep (Sykes and Coop, 1976, 1977; Parkins, Holmes and Bremner, 1973; Berry, Dargie and Preston, 1975). The increased urinary losses of N was blamed on reduced digestibility of nutrients. But as earlier shown such conclusions are questionable and at best a lowered efficiency of utilisation of apparently digested N seems to be more acceptable in explaining the urinary losses. Following the finding of elevated levels of urea in the plasma of sheep infected with T. colubriformis or O. circumcincta relative to pair-fed controls

(Parkins/

(Parkins et al, 1973; Roseby and Leng, 1974), the excessive urinary N loss was concluded to result from urea metabolism. However, the source of the urea remained controversial with possible sources being from the gut leaked proteins or from catabolism of muscle proteins. Various other observations are in favour of the latter. Thus, the studies of 14-C-L-leucine incorporation into liver and muscle of guinea pigs and sheep infected with T. colubriformis by Symons and Jones (1975, 1978) have revealed a reduction in muscle protein synthesis, while liver protein synthesis showed an increase. The increased liver synthesis was related to protein leakage in the gut rather than to reductions in appetite and also occurred in the membrane-bound ribosomes which produced the circulating plasma proteins (Symons, Jones and Steel, 1974; Symons and Jones, 1978). Furthermore, the differences in N metabolism between infected and control animals occur at periods when the animal is losing proteins into the gut as well as synthesising these proteins in the liver (Bremner, 1969; Dargie, 1975; Roseby, 1977; Dargie et al, 1979) implying that amino-acid degradation must be occurring elsewhere. Whatever the source of the amino acids, the net effect is a loss of weight due to loss of proteins in the gut coupled with increased catabolism of body and tissue proteins. Thus the distribution of synthesis between and the extent of catabolism within the various body pools of proteins differ in parasitised and normal animals with much of the available amino acids in the former being diverted to organs involved with the synthesis of those amino acids essential for survival. The mechanism responsible for such inter-tissue transfer of amino acids remain unclear but a probable endocrine control has been postulated based on the finding of high plasma corticosteroid and lower thyroxine levels in T. colubriformis infected sheep relative

to/

to pair-fed controls (Pritchard et al, 1974). It is noteworthy that Coop et al (1976) were of the opinion that the amino acid responsible for the increased urinary loss of N could have originated from the gut through increased degradation of amino acids in the caecum and increased absorption of the resultant N as ammonia.

Parasites also lower the efficiency with which apparently digested or metabolised energies are utilised for growth or maintenance. Up to 50% reductions in efficiency have been noted by Sykes and Coop (1976) which confirmed earlier observations by Andrews et al (1944) and Reveron et al (1974) in T. colubriformis infected sheep though Steel (1972) could observe no such effects. Similar reductions have been reported in ostertagiasis of sheep by Holmes and Maclean (1971), Sykes and Coop (1977) and in fascioliasis by Berry and Dargie (1976) and Sykes, Coop and Rushton (1980). The lowered effieiency of feed utilisation was held responsible for the poor growth of the parasitized animals over and above that caused by the lowered feed intake. The main cause of this lowered efficiency was traced to the high cost of blood protein synthesis in terms of energy consumption (Dargie, 1980).

In conclusion, parasites affect the nutritional physiology of their hosts primarily through poor feed conversion efficiency which has been shown to result from disturbances in food intake, post-absorptive N and energy metabolism. The last two disturbances being traceable to the production of immunoglobulins and other essential amino acids and the leakage of blood and proteins into the gastro-intestinal tract.

B./

B. The Influence of Nutrition on the Relationships between Parasites and their Hosts:

1 - Abomasal Parasites.

Early attempts at defining the influence of nutrition on the pathogenesis of abomasal parasites utilised the feeding of diets of different crude protein contents and examined effects such as worm establishment based on worm recovery at necropsy and faecal egg counts. Higher numbers of parasites were reported in animals kept on the poor nutritional plane (Fraser et al, 1938; Fraser and Robertson, 1933; Taylor, 1943; Kates and Wilson, 1955; Gibson (1954a,b) & a case was therefore made that nutritional plane affected worm establishment. Brundson (1964) working with a mixed population of trichostrongyles used similar criteria to show that nutritional plane had no effect on already established infections even though when his infected experimental animals previously kept in pens were moved to infected pastures, those on the previously low nutritional plane had lowered resistance to the establishment of reinfection. In these early studies no pair-fed controls were utilised, hence it was difficult to separate the effects of reduced feed intake from those due to the nutritional plane and, moreover, the possibility existed that resistance to the effects of parasitism was being considered rather than resistance to the establishment of the infection. As pointed out by Sykes (1978) indices such as worm recovery at necropsy or egg counts do not necessarily quantitate worm burdens or the metabolic impact of such burdens.

Studies employing pair-fed controls coupled with assessment of worm burdens have tended to show that nutritional plane has no effect on worm establishment in abomasal parasitisms (Goldberg, 1965; Scroggs, 1968; Downey et al, 1972; Sykes and Coop, 1977). However, Preston and/

and Allonby (1978) showed that in sheep and goats experimentally infected with H. contortus, those on the poor plane of nutrition exhibited higher egg counts which could not be explained by differences in faecal output. They therefore concluded that the differences reflected differences in worm egg production in the abomasum which possibly was due to either a large number of established worms or increased fecundity of female worms in animals on the low protein diet. Worm burdens were not assessed in animals on both rations. It would therefore seem that nutritional plane plays a more important role in modifying some of the pathophysiological changes in the host brought about by these parasites. Prominent among these changes are weight loss and growth retardation. From the observations of Gibson (1954b), Kates and Wilson (1955), Goldberg (1965), Hussain et al (1967), Downey et al (1967) on H. contortus, T. axei or O. circumcincta infections of sheep and calves, animals on a high plane of nutrition appeared more capable of withstanding the growth inhibition caused by the parasites. Those on the poor nutritional plane suffered the greatest retardation in growth rate, experienced more weight loss and also had up to 50% reduction in efficiency of food utilisation compared to those on the higher plane though the same diet did not cause any retardation in weight in studies where pair-fed controls were used. Reductions in red blood cell indices such as PCV, Hb and RBC counts have also been observed in poorly fed animals relative to those on a higher plane, although such reductions did not denote actual anaemias (Kates and Wilson, 1955; Gibson, 1954a; Downey et al, 1972). Downey et al (1972) working with O. circumcincta infected sheep also recorded elevation of serum pepsinogen levels in two groups on different nutritional levels during the first to fifth weeks of infection but subsequently higher/

higher values were only encountered in those on the low protein diet. Hyperglobulinaemia and hypoalbuminaemia occurred five weeks after infection in both groups but was more pronounced in those on the lower plane while infection also increased the weight of the abomasal mucosa with those on the poor plane being most affected.

Diets of the same energy content but from different sources were shown not to affect susceptibility of calves to O. ostertagi infections (Borgsteede et al, 1978). However diets of the same energy content but prepared differently have been observed to affect the biotic potential and pathophysiological effects of H. contortus, O. ostertagi and T. axei. Vetter et al (1963), Theur et al (1965),

Poeschel and Todd (1969) and Scroggs (1968) examined the effects of natural versus semi-purified synthetic diets on the pathogenesis of these parasites and found that the parasites established themselves in greater numbers and grew faster with no inhibition in those fed the semi-purified diets while weight gains and efficiency of food utilisation were better with the natural diet. Egg production by the worms were better with the natural diets and the conclusion was that the degree of inhibition of the parasites increased as the crude plant ingredient of the diet decreased and also that a factor(s) was present in the natural diet which was required by the parasites for egg production though such factor(s) were not isolated. Pelleting of the diet also led to decreased egg counts and worm recovery at necropsy (Vetter et al, 1963) and less severe drop in PCV (Kates and Wilson, 1955) than when the same diet was fed in loose form.

A deficiency of Vitamin A and Ca was found to increase the susceptibility of goats to Haemonchus infections (Kumar and Deo, 1970) while the addition of trace minerals to the diet greatly increased the resistance to H. contortus in sheep (Shumard et al, 1956). The role of/

of Cobalt supplementation in the diet in the pathogenesis of abomasal parasites was studied by Downey (1965, 1966a,b). With lambs infected with H. contortus, a low level of Cobalt in the diet was found to be detrimental to the worms when he observed that lambs given a Cobalt supplement had a higher egg count and more severe clinical signs of disease than those without. Similar conclusions were arrived at with T. axei infections, while in the case of O. circumcincta those on Cobalt deficient diets showed higher egg counts and more severe clinical signs and weight losses than those on cobalt supplemented diet. No reasons were given for these different responses.

2 - Small intestinal parasites.

The studies of Gordon (1948, 1950, 1964) and Stewart and Gordon (1953) provided much of the early observation on the effect of nutrition on the pathogenesis of small intestinal parasites. Sheep naturally infected with a mixed population of trichostrongyles and subsequently brought into pens were observed to lose their worm burdens at a rate inversely related to the nutritional plane. Those on the poor plane (47%SE and 7%CP) also resisted the development of a superimposed H. contortus infection less successfully than those on the high plane (50% SE and 18%CP)(Gordon, 1948). The 1950 work was along similar lines except that additional evidence was presented to show that Trichostrongyles spp. were more rapidly lost by the better fed sheep. In a later study Stewart and Gordon (1953) observed that in sheep naturally infected with T. colubriformis and subsequently penned and placed on two different diets, one providing 53%SE and 20%CP, the other 54%SE and 6%CP, more of those on the poorer plane of nutrition developed infections when challenged/

challenged with infective larvae. When the experiment was repeated with previously worm free sheep experiencing a first infection, there was little difference in the degree of infection established in the two groups. From these studies, poor nutrition was concluded to adversely affect the ability of the host to resist infections and that previous exposure to infection exerted more influence on resistance than the nutritional plane. Gordon (1964) showed that the duration as well as the quantity of feed offered the host affected susceptibility to T. colubriformis infection. He employed two diets which were qualitatively similar (56%SE and 10%CP) but quantitatively different - one group receiving half the quantity of the other. There was no difference in the establishment of the infection in the two groups following infection with 50,000 larvae of T. colubriformis and a feeding period of 7 weeks but when the experiment was prolonged for 23 weeks those on the half-ration appeared more susceptible to reinfection though resistance to the effects of the infection was not greatly different between the two groups, as shown by similar mortality patterns. Even this difference diminished with time as shown by the results of a second reinfection after another 120 days whereby those on the half-ration were only slightly more susceptible than those on the full ration. The only significant observation was the daily feed intake which declined with time in animals on full ration while those on half-ration maintained their appetites. The plane of nutrition was concluded to affect the weight gains and food intake of parasitised animals. Though later work has shown this to be true (Goldberg, 1965; Klosterman et al, 1973), these early studies failed to utilise pair-fed controls, hence their observations could also be explained by the degree of loss of appetite. Nutritional plane was consequently observed to have no effect on worm burdens or parasite egg production in small intestinal parasitisms (Lara et al, 1977) but rather affect the resistance of/

of the host to the effects of an established infection. Feeding of supplements in the diet has also been shown to have similar effects on the pathogenesis of gastrointestinal parasites irrespective of the pasture type (Ciorda et al, 1962). Extra supplements in the diet caused early maturity and less mortality from gastrointestinal parasites in lambs (Sharma and Kidwai, 1971).

3 - Large intestinal parasites.

Studies on the influence of nutrition on parasitism at this site have been carried out with Oesophagostomum columbianum which causes the condition called 'pimply gut' in sheep. The protein quality of the diet was found to inversely affect susceptibility to infection at this site (Bawden, 1969) even though parameters used for assessing susceptibility such as worm egg counts or worm recovery at necropsy are questionable. However, Herbert, Lean and Nickson (1969) incriminated a specific dietary deficiency of carbohydrates in the poor egg production and fecundity of the parasite in pigs. The anaerobic condition created in the colon through the incomplete digestion of the potato and bran was concluded to aid parasite survival and development.

Changes in blood characteristics of parasitised animals was found to be influenced by the nutritional plane (Bawden, 1969b) in sheep infected with 1,500 larvae of O. columbianum and placed on two qualitatively different nutritional levels. Significant reductions in Hb, PCV and number of circulating erythrocytes were recorded only in those on low protein diet while these indices were not changed in those on the high plane. There was no effect of nutritional plane on MCV, MCH and MCHC, i.e. the anaemia was normocytic and normochromic and was blamed on the severe haemorrhage caused by the parasites. The low protein sheep harboured more worms at 56 days post-infection and this coupled/

coupled with the deficient diet led to the observed haematological disturbances.

Infection affected weight gains and in some cases led to actual weight losses (Bawden, 1969a,b) but those on the poor nutritional level were most affected. The loss of weight resulted from decreased food intake and efficiency of food utilisation. Similar depressions in efficiency have been reported by Horak and Clark (1966) for O. columbianum infections of adequately fed sheep and by Bremner (1961) in O. radiatum infection of calves.

The plane of nutrition was found not to affect the establishment of O. columbianum infections (Bawden, 1969a,b; Dobson and Bawden, 1974) but there was an inconclusive evidence that there was a delayed re-emergence of the 4th stage larvae into the lumen in those on the poor plane of nutrition confirming earlier observations made by Veglia (1923) who attributed the phenomenon solely to the poor nutritional plane of the animals. Once in the lumen, the rate of passage of the worms to their predilection sites was found to be influenced by the rate of passage of ingesta through the gut and this rate of passage was found to be slower in animals on a poor plane of nutrition (Bawden, 1970). He then concluded that there was an enhanced survival ability of the worms in these poorly fed animals which could increase their susceptibility to the infection and possibly explain the earlier egg production and increased fecundity of the female worms (Dobson and Bawden, 1974).

Sheep on the high protein diet were thus concluded to have mounted a more efficient immunological response which enabled them to withstand the parasites'pathogenic effects. The immune mechanisms were postulated as operating at the level of exsheathment of the 3rd stage/

stage larvae and was attended by marked eosinophilic response and the formation of microscopic nodules (pimply gut). Various parameters were determined to show the more superior immunological response of the better fed sheep such as enhanced macrophage-lymphocyte cells hyperplasia; high mast cells, mucin secreting cells, eosinophils and globule leucocyte counts; and more encapsulated larvae showing arrested development (Dobson and Bawden, 1974). These parameters have earlier been pointed out by Dobson (1972) as important sequelae to the hypersensitivity reactions induced by the parasite. Similarly Dash (1970) was of the opinion that there were two histotrophic phases in the life cycle of the parasite which increased the number of antigenic stimulations and thereby governed the extent of the tissue reaction to the parasite. Bawden (1969a) speculated that O. columbianum frequently spent more time in the small intestine of animals on the poor plane of nutrition and this led Dobson and Bawden (1974) to surmise that the parasite probably failed to undergo the second histotrophic migration in poorly fed animals since caseous nodules which usually resulted from host reactions to the second migration were relatively fewer. Such animals would therefore be denied any enhanced immunological responsiveness that the second tissue invasion would have stimulated. Also Dobson (1967) drew attention to the inhibitory property of mucin against parasitic nematodes and its protective action against gut contents, hence the failure of sheep on low protein diet to show increased mucin cell responses (Dobson and Bawden, 1974) denied them an important immune response. A deficiency of thymic function was blamed for the poor lymphocyte and antibody production of the poorly fed sheep and coupled with malfunctions of the innate immunity of the gut involving decreased/

decreased peristalsis and failure of mucin cell response led to their increased susceptibility to the effects of the infection.

4 - Liver parasites.

Poor quality diets especially the protein and vitamin A content have been shown to increase the prevalence of clinical F.hepatica infections (Reid et al, 1970), lead to marked falls in weight gains (Elmagdoub and Badr, 1966) and to affect the natural resistance of sheep to the effects of F. gigantica infections (Deo et al, 1967). These studies gave no information on the effect of nutrition on the parasite itself and more importantly no pair-fed comparisons were made. These deficiencies were remedied by the studies of Berry and Dargie (1976, 1978) and Dargie, Berry and Parkins (1979). Berry and Dargie (1976) assessed the influence of nutrition on the pathogenesis of F. hepatica infections in 9 month old Blackface sheep using indirect indices of clinical manifestations of disease. Infection was established with a single large dose of 1,000 metacercariae and the dietary planes employed provided 13% CP for the high plane and 6% CP for the low plane. Nutritional plane had no effect on establishment of the infection as judged by fluke burdens at necropsy. Neither was the development of the worms affected by nutrition. Dimitrov et al (1978) did, however, observe fewer mature flukes and less development of flukes in sheep on poor nutritional plane and infected with F. hepatica. In the studies of Berry and Dargie (1976), there was an earlier development of symptoms and mortalities in the poorly fed sheep which were ascribed to the small size and limited metabolic reserves of these animals, thereby rendering them physiologically disadvantaged in counteracting the parasites' pathogenic effects. The superior ability of the better fed sheep was consequently observed not to be due to any innate ability to resist or limit/

limit infection, for when they were transferred to a low protein diet there was a rapid deterioration in body conditions. Diet also influenced the nature and timing of the haematological responses shown by the animals. Anaemia was evident in the poorly fed group during fluke migration and was characterised by a depletion of red cell haemoglobin and normochromia while the anaemia seen in the better fed group coincided with the onset of patency of infection in the bile ducts and was macrocytic with haemoglobin levels being depleted only terminally. Since macrocytosis usually is indicative of an enhanced bone marrow response, i.e. reticulocytosis, the better fed sheep were concluded to be synthesising red cells at an accelerated rate while the poorer response of those on low protein diet was attributed to a combination of iron and protein deficiency. A more severe and early weight loss, more pronounced hyperglobulinaemia and hypoalbuminaemia were also recorded in the poorer fed sheep and these changes were also directly related to the fluke burdens. No adverse weight changes occurred in the better fed sheep with fluke burdens of 200 or less due possibly to the regaining of appetite. Inappetence accounted mainly for the weight loss and hypoalbuminaemia but had no effect on the development of the anaemia. Carcase quality was also speculated to be affected due to the presence of oedema which was more pronounced in the poorer fed sheep.

Berry and Dargie (1978) assessed the influence of nutrition on the pathogenesis of liver fluke infections from a more direct measurement of the parasites' activity using ^{125}I-albumin and ^{51}Cr labelled plasma proteins to study the albumin turnover and enteric protein leak in the same animals used for the previous study. The hypoalbuminaemia which occurred during the biliary phase of the disease was/

shown to be influenced by the albumin pools of the body which, in turn, depended on such factors as nutritional plane, appetite and fluke burden. More albumin was catabolised by sheep with low fluke burdens and in those with the same level of infection, those on the higher plane catabolised more albumin, became least hypoalbuminaemic, survived longest and also synthesised more albumin. Chronically infected sheep also synthesised more albumin than pair-fed controls. Digestibility and nitrogen balance studies were also carried out (Dargie, Berry and Parkins, 1979). Intakes of metabolizable energy and CP digestibilities were higher in infected animals given hay and a pelleted supplement compared to those given hay alone due to the higher protein content of their diets. These differences were not significant when pair-fed comparisons were made. However, infected animals were in a negative N balance due to increased urinary losses and this was more pronounced in the poorer fed sheep, though again when compared with pair-fed controls the difference from those on supplementary feed was not very significant. They therefore concluded that differences between infected and control animals could be explained largely by the differences in food intake and protein catabolism, both factors being exaggerated with poorness of the diet offered the host.

5 - Schistosome infections.

Most studies on the influence of nutrition on the pathogenesis of schistosome infections have been carried out in laboratory animals and dietary deficiencies of proteins and vitamins were found to exert the greatest influence on the pathogenesis and course of infections. Early experiments examined the effect of malnutrition on either the parasite or the host and little attention was focussed on any possible interaction/

interaction between the host and parasite due to nutritional factors. Thus Vitamin C deficient diet in guinea-pigs infected with S. mansoni led to defective egg production or slower rate of destruction of the worms compared to controls on normal diet (Krakower et al, 1944) while De Witt (1957a,b) and de Meillon and Paterson (1958) recorded more worms which were stunted and immature in mice fed diets deficient either in proteins, selenium or vitamins. A more comprehensive picture of the influence of nutrition on both the host and the parasite emerged from the works of Bhattacharyya (1965), Coutinho-Abath et al (1962) Zuckerman and Macdonald (1964) and Knuft and Warren (1969). Portal cirrhosis was reported in the rat but not in the mice on a 4% protein deficient diet (Bhattacharyya, 1965). This difference was attributed to the fact that the rat was considered an abnormal host, albeit a poor one for the parasite (Warren and Peters, 1967). Coutinho-Abath et al (1962) could observe no histopathological differences between control and infected mice on either 8% or 60% protein diets while a high carbohydrate diet was observed to produce less portal cirrhosis than normal diets by Zuckerman and Macdonald (1964). Knuft and Warren (1969) failed to find any difference in cercarial penetration between control and infected mice on various levels of protein (12%, 8%, 4%) and calorie deficiency (25% and 50%) and they concluded that nutrition exerted no influence on the establishment of infections but there was a marked decrease in the number of eggs produced per worm pair in the liver. Inappetence was only evident in mice on 4% protein deficient diet but reductions in body weight gains, liver weight gains, spleen weight expressed on body seight basis, all fell with the degree of dietary deficiency and coincidental with the inception of oviposition. Total serum protein was unaffected by plane of nutrition but a fall in serum albumin/

albumin with a concomittant rise in gammaglobulins occurred, the extent being proportional to the degree of dietary protein or calorie deficiency. The haematocrit was affected solely by the protein deficient diets. Liver cell size diminished in animals on 4% protein diet and the numbers of granulomas and epitheloid cells contained therein decreased with nutritional plane except in animals on the 12% protein level which was considered adequate nutritionally. Nutrition was therefore concluded to have either an antagonistic effect on schistosome infections (decreased manifestation of overt disease through reductions in egg output) or a synergistic effect especially with severe protein deficiency (more marked pathophysiological effects) or to exert a balancing effect, especially under moderate protein deficiency (12%) whereby the harmful effects were offset by a negative effect on the parasites. Severe calorie deficiency was concluded to affect the parasite more than the host, whereas protein malnutrition exerted severe effects on both host and parasite (Knuft and Warren, 1969).

CONCLUSIONS:

Parasites affect the nutritional physiology of their hosts through reductions in food intake, decreased efficiency of use of metabolisable energy and through alterations in protein metabolism. From all the host-parasite systems reviewed, there was no definitive proof that protein digestibility was reduced. However, marked alterations in post-absorptive N metabolism occurred and even this was linked indirectly with the loss of whole blood or plasma and the attendant shift in catabolism and synthesis of proteins within and between the various body pools. Poor productivity ultimately supervened.

Nutritional level was found not to prevent the establishment of an infection but a high plane of nutrition invariably mitigated the effects/

effects of an infection and the adequately fed hosts developed immunity sooner which also persisted longer than in the poorly fed host. The only exception was in haemonchosis where more losses were observed when nutrition was excellent but environmental conditions were conducive to massive infestations.

The importance of trypanosomiasis is widely recognised but there appears to be a paucity of information on the effects of the parasite on the nutritional physiology of the host and the role of nutrition on the pathogenesis of infections. Nevertheless, the findings of Fiennes (1970) and Losos and Ikede (1972) showed that nutritionally stressed animals suffered most from the effects of the disease while Bevan (1936) cited by Murray and Urquhart (1977) reported that the tolerance of vaccinated cattle broke down under the stress of malnutrition.

REFERENCES.

Ackert, J.E. and Beach, T.D. (1933)
Transactions of the American Microbiology Society, 52, 51-58.

Andrews, J.S. (1939)
Journal of Agricultural Research, 57, 349 - 362.

Andrews, J.S., Kuffman, W. and Davis, R.E. (1944)
American Journal of Veterinary Research, 5, 22-29.

Armour, J., Jarrett, W.F.H. and Jennings, F.W. (1966)
American Journal of Veterinary Research, 27, 1267-1278.

Barger, I.A. (1973)
Australian Journal of Experimental Agriculture and Animal Husbandry, 13, 42-47.

Barger, I.A., Southcott, W.H. and Williams, V.J. (1973)
Australian Journal of Experimental Agriculture and Animal Husbandry, 13, 351-359.

Barker, I.K. (1973)
International Journal of Parasitology, 3, 743-757.

Bawden, R.J. (1969a)
Australian Journal of Agricultural Research, 20, 589.

Bawden, R.J. (1969b)
Australian Journal of Agricultural Research, 20, 601-606.

Bawden, R.J. (1969c)
Australian Journal of Agricultural Research, 20, 1151-1159.

Bawden, R.J. (1970)
British Journal of Nutrition, 24, 291-296.

Ben-Ghedalia, D., Tagari, H. and Bondi, A. (1976)
British Journal of Nutrition, 36, 211-217.

Berry, C.I., Dargie, J.D. and Preston, J.M. (1973)
Journal of Comparative Pathology, 83, 559-568.

Berry, C.I. and Dargie, J.D. (1976)
Veterinary Parasitology, 2, 317-332.

Berry, C.I. and Dargie, J.D. (1978)
Veterinary Parasitology, 4, 327-339.

Bhattacharyya, K.K. (1965)
Journal of Pathology and Bacteriology, 89, 13-21.

Borgsteede, F.H.M. and Hendricks, J. (1978)
In: Nutrition Abstracts (1979), 49(B).

Bremner, K.C. (1961)
Australian Journal of Agricultural Research, 12, 498-512.

Bremner, K.C. (1969)
Experimental Parasitology, 25, 382-394.

Bremner, K.C. (1970)
Experimental Parasitology, 27, 236-245.

Brundson, R.V. (1964)
New Zealand Veterinary Journal, 12, 105-107.

Brundson, R.V. (1964)
New Zealand Veterinary Journal, 10, 108-111.

Bueno, L., Dorchies, P. and Ruckebusch, Y. (1975) cited by
Dargie, J.D. (1980)
Digestive Physiology and Metabolism in Ruminants
(U. Ruckebusch and P. Thivend, eds.), 349-371.
Proceedings of the 5th International Symposium on
Ruminant Physiology, MTP Press Ltd., England.

Canale, A., Sarra, C., Valente, M.E., Dotta, U. and Balbo, T. (1973)
Annali della Faculta di Medicina Veterinaria di Torino,
20 (Suppl.), 72.

Chandler, A.C. (1953)
Journal of the Egyptian Medical Association, 36, 533 - 552.

Ciordia, H., Bizzelli, W.E., Baird, D.M., McCampbell, H.C.,
Vegors, H.H. and Sell, O.E. (1962)
American Journal of Veterinary Research, 23, 1001-1006.

Ciordia, H., Bizzelli, W.E., Baird, D.M., McCampbell, H.C.,
White, P.E. (1964)
American Journal of Veterinary Research, 25, 1473-1478.

Cohen, S. and Sadun, E. (1976)
Immunology of Parasitic Infections (Oxford Blackwell
Scientific Publications).

Coop, R.L., Angus, K.W. and Mapes, C.J. (1973)
International Journal of Parasitology, 3, 349-361.

Coop, R.L. and Angus, K.W. (1975)
Parasitology, 20, 1-9.

Coop, R.L., Sykes, A.R. and Angus, K.W. (1976)
Research in Veterinary Science, 21, 253-258.

Coop, R.L., Sykes, A.R. and Angus, K.W. (1977)
Research in Veterinary Science, 23, 76-83.

Coop, R.L., Angus, K.W. and Sykes, A.R. (1979)
Research in Veterinary Science, 26, 363-371.

Coop, R.L., Sykes, A.R. and Angus, K.W. (in press)
Veterinary Parasitology.

Coutinho-Abath, E., Filho, M. and Barbosa, J.M. (1962).
Rev. Inst. Med. trop. S. Paolo, 4, 311-322.

Cubarjan, F.A. (1965)
Nutrition Abstract, 35.

Dargie, J.D. (1973)
In: Helminth Diseases of Cattle, Sheep and Horses in Europe,
(eds. G.M. Urquhart and J. Armour), pp. 63-71.
The University Press, Glasgow, Robert Maclehose Co. Ltd.

Dargie, J.D., Maclean, J.M. and Preston, J.M. (1973)
Journal of Comparative Pathology, 83, 543-557.

Dargie, J.D. (1975)
In: Pathogenic Processes in Parasitic Infections, pp. 1-26.
(eds. A.E.R. Taylor and R. Muller).
13th Symposium of the British Society of Parasitology
(Oxford Blackwell Scientific Publications).

Dargie, J.D., Berry, C.I. and Parkins, J.J. (1979)
Research in Veterinary Science, 26, 289-295.

Dargie, J.D. and Berry,C.I. (1979)
International Journal of Parasitology, 9, 17-25.

Dargie, J.D. (1980)
In: Digestive Physiology and Metabolism in Ruminants, 349-371.
(eds. Y. Ruckebusch and P. Thivend).
Proceedings of the 5th International Symposium on Ruminant
Physiology, MTP Press Ltd., Lancaster, England.

Dash, K.D. (1970) cited by Dobson, C. and Bawden, R.J. (1974)
Parasitology, 69, 239-255.

de Meillon, B. and Paterson, S. (1958)
South African Medical Journal, 32, 1086-1088.

Deo, P.G., Tandon, K.C., Kumar, V. and Srivastava, H.D. (1967)
Indian Journal of Veterinary Science and Animal Husbandry,
37, 351-359.

De Witt, W.B. (1957a)
Journal of Parasitology, 43, 119-128.

De Witt, W.B. (1957b)
Journal of Parasitology, 43, 129-135.

Dimitrov, G., K'Ncheva, K. and Georgiev, B. (1978)
In: Nutrition Abstract, 50(7), 1980.

Dineen, J.K. and Adams, D.B. (1971)
Immunology, 20, 109-113.

Dobson, C. (1967)
Australian Veterinary Journal, 43, 291-296.

Dobson, C. (1972)
In: Immunity to Parasitic Infections (ed. E.J.L. Soulsby), 191-222, Academic Press, N. York.

Dobson, C. (1967)
Parasitology, 57, 201-219.

Dobson, C. and Bawden, R.J. (1974)
Parasitology, 69, 239-255.

Downey, N.E. (1965)
British Veterinary Journal, 121, 362 - 370.

Downey, N.E. (1966a)
British Veterinary Journal, 122, 201 - 208.

Downey, N.E. (1966b)
British Veterinary Journal, 122, 316 - 324.

Downey, N.E., Connolly, J F. and O'Shea, J. (1972)
Irish Journal of Agricultural Research, 11, 11-29.

Drurie, P.H. (1962)
Australian Journal of Agricultural Research, 13, 767-777.

Duwell, D., Sambeth, W. and Bassallier, W. (1972)
Parasitologica, 14, 35.

Edwards, C.M., al Saigh, M.N.R., Williams, G.L.I. and Chamberlain, A.G. (1976)
Veterinary Record, 98, 372.

Elmagdoub, A. and Badr, M.F. (1968)
In: Nutrition Abstract (1968) 38.

Fiennes, R.T.W. (1970)
In: The African Trypanosomiases (ed. H.W. Mulligan and W.H. Potts), George Allen & Unwin Ltd., London.

Fitzsimmons, W.H., Harness, E. and Sellwood, S.A. (1968)
Research in Veterinary Science, 9, 237.

Fourie, P.J. (1931)
17th Rept. Divn. Vet. Sci. Anim. Ind. S. Afr., 495-572.

Franklin, M.C., Gordon, H.McL. and MacGregor, C.H. (1946)
Journal of the Council of Scientific and Industrial Research, 19, 46-60.

Fraser, A.H.H. and Robertson, D. (1933)
Empire Journal of Experimental Agriculture, 1, 17-21.

Fraser, A.M.M., Thomson, W., Robertson, D. and George, W. (1938)
Empire Journal of Experimental Agriculture, 6, 316 - 322.

Geiman, Q.M. (1958)
Vitamins and Hormones, 16, 1-33.

Gibson, T.E. (1954a)
Journal of Comparative Pathology, 64, 127-140.

Gibson, T.E. (1954b)
Journal of Comparative Pathology, 64, 360-370.

Gibson, T.E. (1955)
Journal of Comparative Pathology, 65, 317-324.

Gibson, T.E. (1963)
Proceedings of the Nutrition Society, 22, 15-20.

Goldberg, A. (1965)
Journal of Parasitology, 51, 948-953.

Gordon, H. McI. (1948)
Australian Veterinary Journal, 24, 17-45.

Gordon, H. McI. (1958)
Proceedings of the Australian Society of Animal Production, 2, 59-63.

Gordon, H.McI. (1964)
Australian Veterinary Journal, 40, 55-61.

Grovum, W.L. and Philips, G.D. (1978)
British Journal of Nutrition, 40, 323-336.

Herd, R.P. (1971)
International Journal of Parasitology, 1, 251-263.

Herbert, I.V., Lean, I.J. and Nickson, E.W. (1969)
Veterinary Record, 84, 569 - 570.

Holder, J.M. (1964)
Australian Journal of Agricultural Research, 15, 408-416.

Holmes, P.H. and Maclean, J.M. (1971)
Research in Veterinary Science, 12, 265-271.

Horak, I.G. and Clark, R. (1964)
Onderstepoort Journal of Veterinary Research, 31, 163-176.

Horak, I.G. and Clark, R. (1966)
Onderstepoort Journal of Veterinary Research, 33, 139-160.

Hussein, M.Z., Durrani, M.Z. and Akrams, M. (1969)
In: Nutrition Abstract Review, 39.

Hunter, G.C. (1953)
Nutrition Abstract Review, 23, 705.

Jennings, F.W., Armour, J., Lawson, D.D. and Roberts, R. (1966)
American Journal of Veterinary Research, 27, 1249-1257.

Jackson, G.M. (1925)
The effects of Inanition and Malnutrition upon growth and structure.
Philadelphia Blackiston.

Kates, K.C. and Wilson, G.I. (1955)
Journal of Parasitology, 41, Suppl. 43.

Kloosterman, A., Hendricks, J., Borgsteede, F.H.M. and Brunk, R. van den (1973)
Tydschrift voor Diergenes Kunde, 98, 55-65.

Knuft, R.F. and Warren, K.S. (1969)
Journal of Infectious Diseases, 120, 560-575.

Krakover, C., Hoffman, W.A. and Antmayer, J.H. (1944)
Journal of Infectious Diseases, 74, 178-183.

Kumar, V. and Deo, P.G. (1970)
In: Nutrition Abstract 42(1), 1972.

Lara, S.I.M., Costa, H.M. de A., Costa, J.U. (1974)
In: Nutrition Abstract, 46, 1976.

Leland, Jr., S.E., Drudge, J.H., Wyant, Z.N. (1960)
American Journal of Veterinary Research, 21, 458.

Losos, G.J. and Ikede, B.O. (1972)
Veterinary Pathology (Suppl. ad), 9, 1 - 71.

Murray, M. and Rushton, B. (1975)
In: A.E.A. Taylor and R. Muller (eds.), Pathogenic Processes in Parasitic Infections, pp. 27-41.
Oxford Blackwell Scientific Publications.

Murray, M. and Urquhart, G.M. (1977)
In: Immunity to Blood Parasites of Animals and Man (eds. L.H. Miller, J.A. Pino and J.J. McKelvey, Jr.,) and Advances in Experimental Medicine and Biology, Vol.93, Plenum Press, New York and London, pp. 209-241.

Parkins, J.J., Holmes, P.H., Bremner, K.C. (1973)
Research in Veterinary Science, 14, 21-28.

Peters, E. and Weingartner, E. (1971)
Deutsche Tierarztliche Wochenschrift, 78, 535-537.

Peoschel, G.P. and Todd, A.C. (1969)
American Journal of Veterinary Research, 30, 1223-1228

Preston, J.M. and Allonby, E.W. (1978)
Veterinary Record, 103, 509-512.

Preston, J.M., Dargie, J.D. and Maclean, J.M. (1973)
Journal of Comparative Pathology, 83, 401-415.

Preston, J.M. and Dargie, J.D. (1974)
Journal of Comparative Pathology, 84, 73-81.

Pritchard, R.K., Hennessey, D.R. and Griffiths, D.A. (1974)
Research in Veterinary Science, 17, 182-187.

Reid, J.F.S., Armour, J., Urquhart, G.M. and Jennings, F.W. (1966)
Veterinary Record, 86, 242.

Reveron, A.E. and Topps, J.H. (1970)
Outlook in Agriculture, 6, 131-136.

Reveron, A.E., Topps, J.H., Macdonald, D.C. and Pratt, G. (1974)
Research in Veterinary Science, 16, 299-309.

Reveron, A.E., Topps, J.H. and Gelman, A.L. (1974)
Research in Veterinary Science, 16, 310-319.

Roseby, F.B. (1970)
Australian Veterinary Journal, 46, 361-365.

Roseby, F.B. (1973)
Australian Journal of Agricultural Research, 947-953.

Roseby, F.B. (1977)
Australian Journal of Agricultural Research, 25, 155-164.

Roseby, F.B. and Leng, R.A. (1974)
Australian Journal of Agricultural Research, 25, 363 - 367.

Schwartz, M.J. (1964)
American Journal of Digestive Diseases, 9, 128.

Scroggs, M.G. (1968)
Dissertation Abstract (B), 29, 5B, in Nutrition Abstract, 1969, 39, 706.

Sharma, K.M. and Kidwai, W.A. (1971)
Indian Veterinary Journal, 48, 349 - 355.

Shumard, R.F., Emerick, R.J., Bemrick, W.E., Herrick, C.A., Pope, A.L. and Philips, P.H. (1956)
American Journal of Veterinary Research, 252-255.

Shumard, R.F., Bolin, D.W., Eveleth, D.F. (1957)
American Journal of Veterinary Research, 330-337.

Silverman, P.H., Mansfield, M.E. and Scott, H.L. (1970)
American Journal of Veterinary Research, 31, 841-857.

Sinclair, K.B. (1967)
Helminth Abstract, 36, 115-134.

Sinclair, K.B. (1975)
Research in Veterinary Science, 19, 296-303.

Southcott, W.H., Heath, D.D. and Langlands, J.P. (1967)
Journal of the British Grassland Society, 22, 117-120.

Spedding, C.R.W. (1954)
Journal of Comparative Pathology, 64, 13.

Steel, J.W. (1972)
Proceedings of Australian Society of Animal Production, 9, 402-407.

Steel, J.W. (1974)
Proceedings of the Australian Society of Animal Production, 10, 139-147.

Stewart, D.F. and Gordon, H.McI. (1953)
Australian Journal of Agricultural Research, 4, 340 -348.

Sykes, A.R., Coop, R.L. and Angus, K.W. (1975)
Journal of Comparative Pathology, 85, 549-559.

Sykes, A.R. and Coop, R.L. (1976)
Journal of Agricultural Science, 86, 507-515.

Sykes, A.R. and Coop, R.L. (1977)
Journal of Agricultural Science, 88, 671-677.

Sykes, A.R. (1978)
Veterinary Record, TDC Article, 32-34.

Sykes, A.R., Coop, R.L. and Angus, K. . (1979)
Research in Veterinary Science, 26, 372-377.

Sykes, A.R., Coop, R.L. and Rushton, B. (1980)
Research in Veterinary Science, 28, 63-70.

Symons, L.E.A. and Jones, W.O. (1970)
Experimental Parasitology, 27, 496.

Symons, L.E.A., Jones, W.O. and Steel, J.W. (1974)
Experimental Parasitology, 35, 492-502.

Symons, L.E.A. and Jones, W.O. (1975)
Australian Journal of Agricultural Research, 26, 1063 - 1072.

Symons, L.E.A. and Jones, W.O. (1978)
Experimental Parasitology, 44, 7-13.

Taylor, E.L. (1943)
Veterinary Record, 55, 117-119.

Theur, R.C., Vetter, R.L., Hoekstra, W.G., Pope, A.L., and Todd, A.E. (1965)
American Journal of Veterinary Research, 26, 123-130.

Titchen, D.A. and Anderson, N. (1977)
Australian Veterinary Journal, 53, 369-373.

Urquhart, G.M. and Armour, J. (1973)
In: Helminth Diseases of Cattle, Sheep and Horses in Europe.
The University Press, Glasgow, Robert Maclehose Co. Ltd.

Veglia, F. (1923)
9th and 10th Reports, Director of Veterinary Educational
Research, Onderstepoort, p. 811.

Vegors, H.H., Baird, D.M., Sell, O.E. and Stewart, T.B. (1956)
Journal of Animal Science, 15, 1199-1206.

Vetter, R.L., Hoesktra, W.G., Todd, A.C., Pope, A.L. (1953)
American Journal of Veterinary Research, 24, 439-445.

Walsh, J.H. (1975)
Annual Review of Physiology, 81, 104.

Warren, K.S. and Peters, P.A. (1967)
Annals of Tropical Medicine and Parasitology, 61, 294-301.

Warren, K.S. and Peters, P.A. (1967)
American Journal of Tropical Medicine, 16, 718-722.

Wells, P.D. (1963)
Experimental Parasitology, 14, 15-22.

Zuckermann, A.J. and Macdonald, L. (1964)
British Journal of Experimental Pathology, 45, 589-594.

CHAPTER VI

THE CONTROL OF HAEMONCHOSIS AND TRYPANOSOMIASIS WITH PARTICULAR REFERENCE TO POSSIBLE VACCINATION

THE CONTROL OF HAEMONCHOSIS AND TRYPANOSOMIASIS WITH PARTICULAR REFERENCE TO POSSIBLE VACCINATION

A. Haemonchosis:

Gordon (1973) defined control of gastrointestinal parasites as the measures aimed at restraining the biotic potential of the parasites at a level compatible with the biologic requirements of economic husbandry. He also pointed out various ecological and epidemiological features of haemonchosis relevant to any control programme, e.g. the difficulty in predicting and diagnosing outbreaks, the post-parturient rise in worm burdens and hypobiosis during unfavourable weather, the high biotic potential and capacity for outbreaks, the additive effects of malnutrition, destruction of the free living stages, high susceptibility of the parasites to anthelmintics, violent population changes due to self-cure and the development of immunity by the host.

Much reliance has been placed in the past on agricultural practices such as rotational grazing and the use of anthelmintics in the control of haemonchosis (Blood and Henderson, 1968). Modern preventive control programmes employ the integrated approach and are based on well-defined epidemiological and ecological principles directed at limiting contact between the host and parasite (Brundson, 1980). An adequate knowledge of the life history of the parasite, the different sources of pasture contamination and their relationship, availability of infective larvae on pastures, the build-up and decline of infections and the timing of events are basic to this modern approach. Such integrated control can be achieved by a combination of three essential practices:- grazing management, judicious use of anthelmintics and dependence on acquisition of immunity by the host (Blood, Henderson and Radostits, 1979; Brundson, 1980; Morley and Donald, 1980). The control should be carried out on a herd basis

(Blood,/

(Blood, Henderson and Radostits, 1979) and since it forms an integral part of the whole farming enterprise, should be area specific or farm specific in its design taking the local environmental and climatic conditions into consideration (Brundson, 1980; Morley and Donald, 1980).

The control of haemonchosis is reviewed along established practices along with the possibility of exploiting the immune response to the disease to achieve control through vaccination.

I - ESTABLISHED CONTROL PRACTICES.

(a) Improvement in nutritional status: This has been recommended as a method of general application in the control of gastrointestinal nematode parasites (Blood, Henderson and Radostits, 1979) but according to Brundson (1980), improved nutritional status at best afforded protection against moderate infections and was of limited value in heavy infections.

(b) Rotational grazing: This was advocated principally on the assumption that an alternation of grazing on suitably rested pastures would break the cycle of reinfection by exposing the eggs and free living larvae to adverse environmental conditions. Other advantages of the system included a more efficient pasture use and higher levels of productivity as reported by Ciordia et al (1964) and McKinney (1974), though these advantages were at the expense of pasture contamination since worm burdens were actually higher in rotationally grazed animals. The most widely practised method was weekly alternations on pastures rested for 6-8 weeks and though a few reports have claimed advantages based on this schedule (Spedding, 1955; Lindahl et al, 1963) the majority of workers have not (Roe, Southcott and Turner, 1959; Levine

and/

and Clark, 1961; Zimmermann, 1965; Gibson & Everett, 1968; Goldberg and Lucker, 1969; Levine et al, 1975; Michel, 1976). These different results are probably correlated with climatic differences between regions where studies were carried out as the persistence and survival of the free living stages of Haemonchus spp. is dependent on climatic factors (Blitz & Gibbs, 1972; Gordon, 1973). Such persistence and survival have been shown to be much longer than previously thought (Michel, 1969; Southcott et al, 1976; Allonby, 1974; Donald et al, 1978) hence rotations based on a 6-8 weeks rested pasture may not be effective in controlling haemonchosis. Observations from different ecological zones has led to the discontinuity of this method in controlling the disease (Donald, 1969; Morley and Donald, 1980; Brundson, 1980).

(c) Improved husbandry and management practices: Various husbandry and management practices have been recommended for the control of gastrointestinal parasites which in most cases are either of limited or questionable value. Parnell (1962) recommended that, where feasible, lambing should be changed away from seasons favourable for survival of the free living stages. While this recommendation may be feasible in areas with distinct breeding seasons, its effectiveness is doubtful in most tropical areas where the sheep breed all the year round. Blood, Henderson and Radostits (1979) listed some routine barn management procedures such as avoidance of overcrowding, dung removal, provision of adequate level of nutrition, raising of water and feed troughs as part of the methods of general application in controlling gastrointestinal parasitism but also pointed out that where judiciously followed, these measures only lessen the hazard and

do/

do not achieve total control. Increase in stocking density has also not been successful in controlling worm burdens of Haemonchus (Michel, 1969; Southcott et al, 1970) though such increases have been reported to be effective in other host-parasite systems, e.g. ostertagiasis (Ciordia et al, 1971).

There are reports of control attempts through alternation of grazing by hosts of the same or different species. Two or more species graze a pasture alternatively, the changeover occurring when the pasture is helminthologically safe for the most susceptible group. The cattle/sheep system appear most common. Since cross-infection of H. contortus can occur between sheep and young calves (Southcott and Barger, 1975), it is best to graze sheep with yearling or older cattle though it is doubtful if such cattle can clean pastures for sheep (Morley and Donald, 1980; Armour, personal communication). Lambs have been grazed on pastures recently vacated by ewes because the ewes were once thought resistant and thus acted like vacuum cleaners (Taylor, 1961). As pointed out by Connon (1968), ewes can also be a source of infection for lambs due to post-parturient or lactation relaxation of resistance. Consequently, Gordon (1973) suggested that adult non-breeding ewes would be more effective as vacuum cleaners. The leader-follower system whereby calves are grazed ahead of cows has also been reported to successfully control parasitisms (Leaver, 1970).

A more common manipulation of grazing management in some tropical countries is zero grazing whereby livestock, especially cattle, are housed continuously and fed either preserved or cut grass./

grass. Ideally this method should greatly reduce, if not eliminate, gastrointestinal helminthiasis but unhygienic husbandry conditions coupled with the utilisation of their manure as fertilisers ensures continuity of infections on pastures. Should such animals eventually return to pasture, serious cases of helminthiasis occur due to their low immune status.

(d) Chemotherapeutic control: This is the most widely practised control measure in most of the tropics and sub-tropics where haemonchosis is endemic. Anthelmintics can either be used strategically when based on the epidemiological patterns of outbreaks in which case treatment is carried out at the same time in the management programme or tactically when based on an ad hoc criterion to abort outbreaks due to abnormal climatic or nutritional conditions(Gordon, 1971). In the control of haemonchosis a combination of strategic and tactical dosing or strategic dosing alone has been recommended (Gordon, 1973; Blood, Henderson and Radostits, 1979).

Many anthelmintics abound in the market and the choice of any is dictated by the ease of administration, cost, spectrum of activity and whether resistant strains have developed from its use. Among the earliest widely used drugs was phenothiazine which was applied continuously (Kutler and Marble, 1962) but has had to be discarded in most areas because the level fed failed to protect against heavy infections and, more importantly, strains of Haemonchus resistant to its use were reported (Drudge et al, 1957; Levine and Garrigus, 1962). More recent anthelmintics such as levamisole, morantel tartrate and thiabendazole are all effective while the newer benzimidazoles (ferber, oxfer and albendazole) are additionally said to be effective against hypobiotic larvae. The major setback to their/

their use appears to be the development of strains of H. contortus which are resistant to the benzimidazole anthelmintics. Such strains have been reported from U.S.A. (Drudge et al, 1964), Brazil (Colglazier et al, 1970), both against thiabendazole, and from Australia (Kelly et al, 1977) against related benzimidazoles. Perhaps as practised in the control of trypanosomiasis, drugs of different chemical groups should be used alternatively between Haemonchus seasons to overcome the problem of drug resistant strains.

(e) Integrated approach: This combines the judicious use of anthelmintics based on reliable epidemiological data with the provision through management of safe pastures to avoid reinfection of animals and has been applied mainly in the temperate regions and to a lesser extent in the sub-tropics to control mixed populations of trichostrongyles which included Haemonchus. The safe pastures can be provided through elimination of grazing on a pasture for a considerable time ranging from 4-5 months in autumn/winter to 3-4 months in summer or through alternation of grazing of cows and calves where heavy Haemonchus infections are present (Brundson, 1980; Morley and Donald, 1980) or by sowing new grass ley after 2 or 3 years of cash crops.

Spring born lambs are exposed to infection from their dams and from overwintered larvae. Using the integrated approach, the ewes are dosed before and after lambing and the lambs moved to safe pastures in early or mid-summer (Leaning et al, 1970; Donelly et al, 1972; Thomas and Boag, 1973; Brundson, 1974) though such movement was found unnecessary if the ewes lambed on safe pastures (Boag and Thomas, 1973). For post-weaned lambs who are further exposed to autoinfection, the drenching of the lambs followed by movement to safe pastures was found effective by Donald et al, cited by Donald (1974),/

(1974), but where such safe pastures cannot be provided, further treatments of the lambs in autumn and early winter were found necessary (Donald and Waller, 1973). Brundson (1976) and Vlassof (1973) also recommended the drench and move system for controlling gastrointestinal parasites in lambs in New Zealand.

Spring born calves, on the other hand, are exposed to infection only from overwintered larvae and Michel and Lancaster (1970) in England recommended a system of control based on movement of calves to safe pastures in mid-July accompanied by anthelmintic treatment (Weybridge System), while in New Zealand where conditions favourable for larval outbreaks extend through summer, autumn and early winter, various measures have been practised, e.g. drench and move (Michel, 1969a; Brundson, 1972) and two drenches, one at the beginning of summer and a second in autumn, each accompanied by a move to safe pastures (Brundson, 1980). The successful application of the integrated control method rests on reliable epidemiological and ecological information so that adaptations to different environmental conditions can be possible. In farming situations in areas of endemic haemonchosis, such information is usually lacking and control of haemonchosis is carried out through the use of anthelmintics. Such treatments are done at the beginning of the rains, middle of rains, end of the rains and once during the middle of the dry season for preventive purposes or the whole herd is treated when haemonchosis is diagnosed on routine faecal examinations (personal observation). The effectiveness of this regime is difficult to evaluate but, in the light of new knowledge regarding the persistence and survival of Haemonchus on pastures (Allonby, 1974), such treatments need to/

need to be more closely spaced and it should be remembered that faecal egg counts do not give an accurate picture of worm burdens nor the pathogenic effects of such burdens.

II - THE APPLICATION OF IMMUNITY IN THE CONTROL OF HAEMONCHOSIS THROUGH POSSIBLE VACCINATION.

Immune responses have been demonstrated in haemonchosis as early as 1950 by Stewart in his studies on the self-cure phenomenon. Such responses appeared to be of limited potency as they did not guarantee freedom from reinfection (Mulligan, 1976). There have been various attempts at vaccination. (Table I).

(a) Use of larval antigens: Dead worms were unsuccessfully used as antigens in vaccination trials by Mayhew (1949) and Stewart (1950) which led to the recognition that the living worms or their metabolic products contained the antigenic fractions. Normal larvae was next used by Luisenko (1956) as antigens and though he demonstrated resistance to a challenge dose of up to 150,000 larvae, the ages of the sheep were not given and the results were complicated by prior administration of antigens prepared from adult Haemonchus (Luisenko, 1956, cited by Christie et al, 1964). Subsequently two doses of 10,000 X-irradiated larvae given at an interval of 28 days were used as vaccines by Jarrett et al (1959) in sheep over 7 months of age and a solid immunity against a challenge dose of 10,000-50,000 normal larvae given almost four months after the last premunising dose was observed. The degree of protection was assessed by comparing resultant worm burdens in the vaccinated and control sheep. Since this report there have been various vaccination attempts using either X-irradiated larvae/

TABLE 1

Reported Experiments on Immunisation against H. contortus in sheep.

Author	Age of sheep (mths)	Immunising Dose: Size	Immunising Dose: Larvae	Interval before challenge (days)	Challenge Dose: Size	Challenge Dose: Larvae	Outcome of Vaccination
Lullens (1956)	?	Up to 150,000	live + antigens	?	-	-	100% protection
Jarrett et al(1959)a.	9	1 x 10,000	irradiated + normal	117	8,000	normal	87% - 100% protection in Blackface sheep
b.	9	1 x 10,000	irradiated + normal	117	8,000	normal	
Jarrett et al(1961)a.	8	2 x 10,000	irradiated	29	10,000	normal	Solid protection as above.
b.	8	2 x 10,000	irradiated	29	50,000	normal	
Milligan et al (1961)	7	2 x 10,000	irradiated	35	10,000	normal	Inconsistent protection in Merino sheep.
Manton et al(1962) a.	24	2 x 1,500 or 30 x 100 at 2-day intervals	normal	30	5,000	normal	No protection in Dorset sheep.
b.	10-12	2 x 4,500 or 30 x 300 every 2 days	normal	30	15,000	normal	100% protection in Dorset sheep
Christie et al (1964)	7½	14 x 10,000	normal	18	66 x 10,000	normal	75% protection in 3/4 Blackface sheep.
Urquhart et al (1962)	<6	1 x 10,000	normal	60-90	15,000-150,000	normal	Strong immunity confirmed earlier work (2).
		1 x 10,000	irradiated	60-90	15,000-150,000	normal	
Urquhart et al(1966)a.	3	1 x 10,000 or 2 x 10,000	irradiated	30	20,000	normal	No protection in Blackface sheep.
b. (i)	<3	2 x 10,000	irradiated	30	10,000	normal	No protection.
(ii)	5½	2 x 10,000	± adjuvant & Fasciola antigen	30	10,000	normal	Some degree of protection but poor compared to lambs aged 9 mths at vaccination.
Silverman (1965) and Scott et al (1971)	4-9	50,000-70,000 Anthelmintic removal of worms	normal + parenteral metabolic antigens	140	70,000	normal	Inconsistent protection.
Christie and Brambell (1966)	2½	2 x 25,000 + anthelmintics	normal	10	2x25,000	normal	Some measure of acquired resistance in Blackface sheep reported.
Bitakaramire (1966)	10-14	2 x 10,000	irradiated	30	50,000	normal	84% - 100% protection in Blackface sheep.
Dineen and co-workers (1966, 1967, 1969)	3-6	6 x 3,000 ± anthelmintics	normal	84	3,000	normal	No interference with immunity if vaccine worms persisted for a long time before their removal.
Lopez and Urquhart (1967)	0-24	2 x 10,000	irradiated	30	10,000	normal	No protection in Merinos at any age if reared from birth on infected pastures - immunity only in those 24 mths. old reared indoors.
Tetzlaff and Todd (1973) Manford et al (1974) Kennedy & Todd (1975)	3-6	2,000-8,000	normal ± antigens ± anthelmintic	30	25,000	normal	Initial infection caused developmental arrest in challenge inoculum attributed to presence of adult worms from initial infection. Role of immune responses not determined.
Wilson & Samson (1974)	2½	10,000	exsheathed	21	10,000	exsheathed	No protection
	2½-5	3 x 10,000 (3 week intervals)	exsheathed	63	15,000	exsheathed	Enhanced immunity in those aged 2½ mths.
Neilson (1975)	3	antigens	metabolic antigens parenterally + adjuvant	10	3,000	normal	No protection in Blackface sheep
Benitez-Usher et al (1977)	2½	2 x 10,000	irradiated	28	10,000	normal	No protection.
	8½	2 x 10,000	irradiated	28	10,000	normal	96.7% protection. Anthelmintics interfered with immunity.
Ross, Duncan and Halliday (1978)	2	2 x 10,000	irradiated	28	10,000	normal	No protection in Blackface sheep.
	2	2 x 10,000+TF	irradiated + TF*	28	10,000	normal	34% protection in Blackface sheep.
	5	2 x 10,000	irradiated	28	10,000	normal	40% protection in Blackface sheep.
	5	TF	TF alone	28	10,000	normal	45% protection in Blackface sheep.
Christie et al(1978)	10½-21	5 x 10,000for 25-26 weeks	normal	6	4,000,000	normal	High level of resistance following anthelmintic removal of challenge infection.
Duncan, Smith and Dargie (1978)	2	2 x 10,000	irradiated	21	10,000	normal	No protection in Blackface sheep.
	12	2 x 10,000	irradiated	21	10,000	normal	Solid protection (>90%)
Smith and Christie (1979)	7	2 x 10,000 + anthelmintics	irradiated	28	10,000	normal	High level of immunity. Confirmed earlier report (No. 18) that anthelmintics do not affect resistance if vaccine worms are allowed a long period of persistence before removal.
Smith & Angus (1980)	14	2 x 10,000	irradiated	28	10,000	normal	High level of protection.
	2	2 x 10,000	irradiated	28	10,000	normal	No protection.
	2	2 x 10,000	irradiated + antigen	28	10,000	normal	No protection.
	2	4 x 10,000	irradiated	28	10,000	normal	50% protection.

* Transfer factor.

larvae with or without adjuvant, normal larvae, exsheathed larvae or metabolic products of living worms as vaccines (Table I) and all have had only limited success due to other important factors. Firstly, the influence of age on the development of acquired resistance to the natural infection (Manton et al, 1962) also operated in experimental infections. For example, Blackface sheep and other breeds under three months of age were not protected by the same vaccination regime which conferred solid immunity in those over seven months (Jarrett et al, 1961; Mulligan et al, 1961; Urquhart et al, 1962, 1966a,b; Christie et al, 1964a,b; Benitez-Usher et al, 1977; Christie et al, 1978; Duncan, Smith and Dargie, 1978). Secondly, immunisation of merino sheep using X-irradiated larvae yielded inconsistent results. Thus vaccination of previously worm free merinos at 7 months gave inconsistent immunity while at 24 months a consistently high degree of resistance to subsequent challenge was observed (Mulligan et al, 1961). Lopez and Urquhart (1967) concluded that in addition to immunological immaturity, naturally acquired early infections led to a permanent impairment of immune responsiveness in later life giving rise to the rather prolonged unresponsive period. The possibility also exists that some of the variable results obtained following vaccination attempts in Merino sheep might have been related to the haemoglobin type of the sheep, the Hb type having been shown to influence host responses to H. contortus (Radhakrishnan et al, 1972; Allonby and Urquhart, 1973). The immunological immaturity view was also shared by Manton et al (1962), Urquhart et al (1966a,b) and Urquhart (1968, 1977). However, the work of Christie and Brambell (1966), Benitez-Usher et al (1977) and Smith and Angus (1980) has demonstrated that at least in Blackface sheep some measure of acquired resistance to haemonchosis can be demonstrated in young lambs implying that these lambs were not immunologically immature. Christie and

Brambell/

Brambell (1966) and Benitez-Usher et al (1977) successfully immunised 2-4 months old Blackface sheep primed either with two heavy doses of 25,000 normal larave or six monthly doses of 10,000 normal larvae followed by anthelmintic removal of the worms before challenge. This same regime had earlier been reported to confer no immunity in Dorset lambs (Urquhart et al, 1962; Manton et al, 1962) and in retrospect Urquhart (1980) was of the opinion that a different result might have been observed if priming had been done at an earlier age with less number of larvae at more closely spaced intervals to mimic natural infections. However some measure of acquired resistance was also demonstrated in $2\frac{1}{2}$ months old lambs in New Mexico by Wilson and Samson (1974) using three weekly doses of 10,000 exsheathed larvae and a long immunising period though the breeds of the sheep were not stated. More recently Smith and Angus (1980) detected antibody responses (mainly circulating and mucosal IgG) against H. contortus in young Suffolk-Greyface lambs aged $1\frac{1}{2}$ months given parenteral inoculations of larval antigens and adjuvant. Though these reports indicate a probable immune responsiveness in young lambs their value in practical control measures remain undetermined.

The failure to immunise young sheep has until recently also been blamed on their failure to elaborate abomasal mucus IgA and IgG and serum IgG antibodies, these antibodies having been found in successfully vaccinated adult sheep (Smith, 1977a,b; Urquhart, 1977; Smith and Christie, 1978, 1979) and not in young lambs (Duncan, Smith and Dargie, 1978). However Smith and Angus (1980) failed to corroborate/

corroborate the findings of Duncan et al (1978) when they showed that these antibodies were not depressed in young lambs and they thus concluded that, since these antibodies either in adult or young lambs were detected in the presence of numerous worms, they could not be solely involved in resistance to haemonchosis as was previously postulated but rather in combination with some unknown factors which are defective in the young lambs. These factors have not been identified.

There have been reports demonstrating some measure of acquired resistance following vaccinations in which immune responses have not been definitely determined. The works of Tetzlaff and Todd (1973), Manford et al (1974), Kennedy and Todd (1975) showed that lambs aged $3\frac{1}{2}$-6 months vaccinated with up to 25,000 normal larvae with or without antigen were able to resist a challenge infection which they attributed to the presence of adult worms from the initial infection which caused developmental arrest in the challenge inoculum, i.e. a direct age-group interaction between different populations of Haemonchus. More recently the possibility of transfer factor treatment (TF) conferring immunity to supposedly immune incompetent lambs has been investigated (Ross, Duncan and Halliday, 1978). The transfer factor is a dialysable low molecular weight derivative of leucocyte lysates which has been demonstrated to transfer delayed hypersensitivity and cell-mediated immunity in man (Lawrence, 1949, 1954), and lambs of 2-3 months infected with Trichostrongylus axei (Ross and Halliday, 1978, 1979). In the experiments of Ross, Duncan and Halliday (1978) the TF treatment produced a 34% reduction in the challenge infection in four months old/

old lambs and a 45% reducation in seven months old lambs similar to the level conferred by irradiated larval treatments (40%). It was therefore concluded that TF activity in this infection operated independently of immune competence. Experiments with TF treatments are still in the laboratory phase and it remains to be seen if it can achieve successful vaccination in areas of endemic haemonchosis.

(b) Genetic control: There are indications from field observations that breed differences in resistance to haemonchosis exist operating at the level of worm establishment or survival and which therefore may be beneficial in terms of production (Jilek and Bradley, 1969; Preston and Allonby, 1979a). Using clinical, parasitological or radioisotopic criteria, various workers have also supported the field observations experimentally in both primary and secondary infections (Radhakrishnan et al, 1972; Bradley et al, 1973; Altaif and Dargie, 1978a,b; Preston and Allonby, 1978). Individual variability in worm establishment within breeds have also been noticed which led to the search for probable genotypes associated with resistance of which the haemoglobin type of the animal was consequently identified. Using similar parameters as earlier mentioned, most reports suggested that haemoglobin type A (HbA) sheep were more resistant than either HbAB or HbB types (Evans and Blunt, 1961; Evans, Blunt and Southcott, 1963; Evans and Whitlock, 1964; Allonby and Urquhart, 1976; Preston and Allonby, 1979a; Altaif and Dargie, 1976, 1978a) although there are also reports where a definite relationship was either not conclusively demonstrated (Jilek and Bradley, 1969; Radhakrishnan et al, 1972; Bradley et al, 1973) or non-existent, especially in heavy infections (Le Jambre, 1978; Rifkin and Dobson, 1979; Altaif and Dargie, 1978a). These differences between and within breeds in response to H. contortus infections has further complicated immunisation studies and, according

to Mulligan (1976) possibly also goven the degree of unresponsiveness of lambs. The differences can, however, be exploited in controlling haemonchosis by breeding through selection for resistant traits if these traits can be shown conclusively to have a genetic linkage. In this regard, Whitlock and Marsden (1958) successfully created resistant populations of sheep grazing on H. contortus infected pastures through selection but the genetic basis of the selection was not determined as the resistance factor and was not enhanced by either cross-breeding or inbreeding. Recently Rifkin and Dobson (1979) identified another genetically controlled marker on lymphocytes which responded to larval and adult H. contortus antigens by undergoing blast transformation. This trait was found to be heritable and also positively correlated with resistance to subsequent primary, secondary or trickle infections of H. contortus in lambs not older than five months.

From these observations, there appears to be the possibility of controlling haemonchosis by breeding through selection of resistant strains of animals based on either the breed, haemoglobin type or the lymphocyte factor. However, there are no records of successful commercial control achieved by this method in areas of endemic haemonchosis. Various obstacles still remain to be surmounted. For example, there are indications that the haemoglobin marker appears quite fragile being broken by exposure to heavy challenge (Altaif and Dargie, 1978a) or low nutritional plane (Preston and Allonby, 1978). Secondly, extrapolating most of these observations obtained in temperate conditions to tropical situations where haemonchosis is endemic requires careful consideration of environmental factors such as high ambient temperatures, solar radiation, humidity,/

humidity, irregular food supply; which on their own limit production independently of parasitisms and hence possibly affect resistant genotypes. Thirdly, information presently available on the genetic markers identified appear limited as not much is known about the heritability, the interaction between genotypes and environmental conditions and, more importantly, the economics of such genetic control compared to other methods. Lastly, there are suggestions from the work of Rifkin and Dobson (1979) that animals resistant to H. contortus are not necessarily the most productive and that man has through the ages selected the most productive animals at the expense of resistance to this parasite.

(c) Anthelmintics and the immune response: The role of anthelmintics in aiding resistance to haemonchosis has not gained universal acceptance. Dineen and Wagland (1966) working with heavy priming infections which caused immunological exhaustion in young lambs showed that anthelmintic removal of the worms at eight days before challenge did not interfere with subsequent development of immunity. This was supported by the findings of Christie et al (1964a), Christie and Brambell (1966), Christie et al (1978). However when the priming dose was not so heavy, there was interference with development of immunity if the worms were removed before challenge (Wagland and Dineen, 1967). In later studies Donald, Dineen and Adams (1969) again showed that if the vaccine worms were allowed a long period of persistence before removal, there was no interference with the development of immunity. These conflicting reports are rendered more difficult to evaluate since in two of their experiments the lambs were immunologically immature at the start of the experiment. The works of Benitez-Usher et al (1977), Altaif and Dargie (1970a,b) demonstrated that anthelmintic removal of vaccine worms/

worms in nine months old lambs at three weeks in between two immunisation schedules led to a breakdown of vaccination due to cessation of antigenic stimulation and consequent waning of immunity. However Smith and Christie (1979) concluded that administration of anthelmintics one week before challenge did not affect the degree of protection to subsequent challenge implying that once protection had been afforded by the vaccine worms (possibly between weeks 3 to 7 of the immunising course), their subsequent presence was not required to sustain the protective effects. The importance of these observations in controlling haemonchosis practically has not been demonstrated and the possibility of boosting immune responses through the use of drugs, as occurs in trypanosomiasis, has not been achieved since the precise role of anthelmintics in aiding immune responsiveness in sheep remains unconfirmed.

(d) Exploitation of the 'self-cure' phenomenon: This can only be exploited to achieve control if shown conclusively to have a genetic basis whereby breeding through selection of animals showing high propensity for this trait can be embarked upon. Much has been documented about the 'self-cure' phenomenon such as its nature and timing (Stoll, 1929; Stewart, 1953; Allonby and Urquhart, 1973), how it accounted for the parasitological differences at grass between and within breeds (Allonby and Urquhart, 1976; Preston and Allonby, 1979b) and in animals undergoing experimental reinfections (Altaif and Dargie, 1978a). However the mechanism still remains controversial. Some workers considered the phenomenon to be immunological, especially when experimentally produced, and hence also a probable genetic basis (Stewart, 1950, 1953; Dargie and Allonby, 1975; Altaif and Dargie, 1978b), though they also conceded that the protection it afforded appeared short-lived or temporary as it did not guarantee freedom from reinfection./

reinfection. Others have not found an immunological basis and postulated that the natural occurrence of the phenomenon was associated with the ingestion of freshly growing grass and was independent of the presence of H. contortus (Gordon, 1967; Allonby and Urquhart, 1973). In the light of these different observations, the 'self-cure' phenomenon apart from controlling haemonchosis under natural conditions, has not been exploited commercially to achieve control.

Conclusion:

None of the manifestations of immune response to haemonchosis appear to have vaccine potential, especially in the most susceptible age group where it has hitherto been impossible to elicit immune responses on the field. Perhaps, as suggested by Mulligan (1976), the immune response to this parasite is not potent enough. The only possibilities offering any hope involve further investigation of transfer factor treatments and the possibility of exploiting resistance genotypes or breeds by selection for breeding improvements. In the meantime, much reliance will still be placed on other methods of control such as the use of the integrated approach where feasible which has been shown to be effective when rigorously applied. Should a vaccine breakthrough eventually occur, there are still bound to be practical problems in its use in endemic areas for the immune prophylaxis need to be highly effective, as anaemia and loss of condition can still occur with moderate worm burdens if nutrition is inadequate (Allonby and Urquhart, 1975; Preston and Allonby, 1978). Much reliance will continue to be placed on heavy anthelmintic therapy in controlling the disease in most areas of endemic haemonchosis in the tropics and sub-tropics.

B. Trypanosomiasis:/

B. Trypanosomiasis:

The control of bovine trypanosomiasis can be achieved through either suppressing the pathogenic effects of the parasites by the use of drugs or by eradicating their arthropod vectors (Maclennan, 1970). Ormerod (1976) and Holmes (1980) listed the problems associated with either method. With the former the frequency of drug treatment and the necessity for carefully monitoring such treatments in areas of high tsetse challenge, often to uneconomic proportions, the advent of drug resistant strains of trypanosomes, and the absence of new drugs has marred its effectiveness. With tsetse control, the major limiting factors include the high cost of undertaking and maintaining such programmes, failure to prevent reinfestation of reclaimed land and the facilitation of land destruction and soil erosion by some of the control measures.

Tsetse Control. Eradication of tsetse flies has led to successful control of bovine trypanosomiasis and, according to Finelle (1980), appears to be the only approach to the elimination of the disease, although Ormerod (1979) pointed out that trypanosomes often reverted to mechanical means of transmission in the absence of tsetse flies. Measures aimed at eradicating the flies can either be direct when the flies themselves are destroyed by hand catching, traps or insecticides, or indirect when the habitat and food supply of the flies are destroyed through game eviction, bush clearing or sterilised males which in effect reduce the net reproductive rate of the flies.

(a) Direct approach:

(i) Hand catching - Heavy manpower outlay and isolation of area to be treated are essential requirements of this method of control and where successes have been achieved, e.g. the eradication of Glossina palpalis/

palpalis from the island of Principe by Austen and Hugh (1922) cited by Glasgow (1970), these requirements were met. Despite the obvious advantages of specificity and non-alteration of the environment, this sytem has been discarded because of the advent of better and less expensive control measures (Glasgow, 1970; Leach, 1973).

(ii) Traps and attractants - Traps of various designs have been used to control Glossina spps. without success and later for distribution studies also with doubtful success (Potts, 1970). Leach (1973) suggested that traps may find a return to favour if new and effective attractants are developed either in distribution studies or as an adjunct to eradication when tsetse population has been brought to a low level by other methods.

(iii) Parasites and predators - Nash (1970) reviewed the diverse species of parasites and predators of Glossina spp. which possibly control populations of Glossina under natural conditions but so far these have been of no practical application.

(iv) Repellants - Laboratory trials with natural pyrethrum conferred protection for 1-2 days against tsetse bite but length of protection was considered uneconomical (Galun, 1975, cited by Galun et al, 1980). Recent research has led to the development of a technique of micro-encapsulation of natural pyrethrum which has lengthened the protective period to about 10 days (Galun et al, 1980) and, though still not tested practically in the field, these workers hoped that this might replace the use of chemoprophylactic drugs especially in slaughter cattle.

(v) Insecticidal control - Two uses of insecticides were defined by Burnett (1970) in the control of trypanosomiasis, viz:- (a) those which act by virtue of residual deposits killing insects contracting them over days or months and also permit more discriminative application to vegetation food traps and pupation sites, and (b) those without

detectable/

detectable residual effect needing repeated application from aircrafts, smoke generators or ground-based fogging machines. For successful insecticidal control, the environment must remain favourable for tsetse flies and once control has been achieved, the reclaimed land should be isolated and made free from reinfestation by either urbanisation or food cultivation (Burnett, 1970). The most firmly established method of control in terms of cost and damage to the environment is the residual application to resting sites (Burnett, 1970; Hadaway, 1972; Leach, 1973) and organochlorine compounds like D.D.T., B.H.C. dieldrin and endosulfan are the most widely used inseciticides for this purpose. Endosolfan has been used for sequential aerosol applications in various parts of Africa (Hocking et al, 1966; Park et al, 1972; Finelle, 1973b; Kendrick and Alsop, 1974) while dieldrin is preferred for residual applications using either ground-spraying equipment, rotary atomizers or helicopters (Davis, 1971; Spielberger and Abdurrahim, 1972, cited by Hadaway, 1977; Finelle, 1973b; Spielberger and Na'isa, 1975). The major disadvantage appears to be the destruction of non-target species such as birds, fishes and reptiles (Koeman et al, 1976, cited by Lee, 1979) due to the high dosage rates of residual applications. However the fear of development of resistance to the currently used insecticides by tsetses, the growing concern for environmental protection and the restrictions on the use of D.D.T. and dieldrin has intensified the search for safer and more economical insecticides (Hadaway, 1977).

B. Indirect control:

(i) Release of sterile males - Male Glossina can be successfully sterilised by either cross-mating different species (Vanderplank, 1944) or use of mutant genes or high energy radiation (Potts, 1958; Dean and Wortham, 1969) but successful control of tsetse flies on the field has been/

been limited. The only reported work was the 90% reduction of the indigenous G. morsitans population of the 200 km^2 Mkaja ranch in Tanzania (Dame and Williamson, 1979; Dame et al, 1980) where tsetse population had already been brought to a low level by insecticides and bush clearing before the release of the sterile males. According to Leach (1973), the practical difficulties notwithstanding, resistant tsetses may develop and non-target species may also be affected.

(ii) Vegetation clearing - This is perhaps the oldest and most effective method of tsetse control (Ford et al, 1970). Methods of clearing have varied from the total or ruthless destruction of all vegetation facilitated nowadays by bulldozers and arboricides but which precipitated land destruction (Ormerod, 1976) to more recent modifications such as selective or discriminative clearing (Ford et al, 1970) based on the knowledge that not all vegetation was essential to the flies. Finelle (1973a) pointed out that nowadays these ecologically damaging measures are only employed to create a tsetse barrier so that the confined areas can be attacked by insecticides.

(iii) Game eviction - Leach (1973) claimed that destruction of a large variety of game animals, which are the principal food source of tsetse flies especially the morsitans group, has cleared the flies from many areas while Ormerod (1976) cited that the great rinderpest epizootic of 1895 which greatly reduced the population of game animals also achieved successful control of the flies. The eviction has been rendered more selective based on the finding that not all species are favoured by the flies but the major constraint to its use is the conflict with environmental preservators and the fact that reinvasion by flies follows recovery of the game animal population.

Control/

Control in the Mammalian Host:

In the absence of any practicable vaccine, control in the mammalian host has relied solely on the use of trypanocidal drugs and there are many reports of successful control of bovine trypanosomiasis achieved by their use (Bevan, 1928, 1936; Smith, 1959; Shaw, 1960; Scott and Pegram, 1974; Bourn and Scott, 1978) with the attendant problems as earlier mentioned (Holmes, 1980). The therapeutic control has been achieved mainly by two groups of drugs, Diminazene aceturate (Berenil) which has high activity, low toxicity, rapid excretion but limited prophylactic property and the phenanthridium drugs - Homidium Chloride and Isomethamidium chloride (Samorin) both drugs having considerable prophylactic properties, especially the latter. Strains resistant to one drug often show cross-resistance to other drugs because the drugs are related chemically. Because of this cross-resistance, Whiteside (1962) developed the concept of using Sanative pairs of drugs which did not cause mutual cross-resistance such as Berenil and Homidium or Berenil and Samorin. These pairs are used alternatively in an area until resistance to one is observed whereby a switch is made to the other. Therapeutic control require a high level of veterinary supervision and careful monitoring which, where existing, can lead to widespread economic advantages due to successful control and even enhancement of immunity *vide infra* (Wilson *et al*, 1975a,b; Bourn and Scott, 1978). In traditional systems of husbandry such has not been the case and drug prophylaxis has been impracticable since there is no guarantee that cattle under such prophylactic treatment regimes, will be retreated within the protective period. Massive therapeutic treatments are therefore embarked upon in/

in controlling the disease which often precipitates drug resistance (Jones-Davis, 1967a,b, 1968; Maclennan and Jones-Davis, 1967; Maclennan and Na'isa, 1970).

Vaccination:

A pronounced immune response follows infection by trypanosomes as evidenced by the high levels of circulating immunoglobulins (mainly IgM), marked expansion of the mononuclear phagocytic system and the demonstration of predominantly opsonising antibodies (Lumsden and Herbert, 1967; Takayanagi et al, 1974; MacAskill et al, 1980) by various in vitro tests. Theoretically it should be possible to protect animals against trypanosomiasis by vaccination and limited success has been reported though no commercial vaccine is as yet available. The immune responses can also be boosted by trypanocidal drugs, while the tolerance of some breeds which have been speculated to have a genetic basis can also be exploited to achieve control. These different aspects are examined.

(a) Vaccination with trypanosomal antigens - Successful vaccination of laboratory rodents against defined variants of the genus Trypanozoon has been reported using such regimes as infection followed by treatment, inoculation of trypanosomal extracts or infection with irradiated trypanosomes (Seed, 1963; Duxbury et al, 1972b; Duxbury and Sadun, 1969; Wellde et al, 1973) for T. rhodesiense; (Dodin and Fromentin, 1962; Seed and Gam, 1966; Seed, 1972) against T. gambiense; (Dodin and Fromentin, 1962; Miller, 1965; Soltys, 1967; Herbert and Lumsden, 1968; Cross, 1975) against T. brucei; (Dodin and Fromentin, 1962; Johnson et al, 1963) against T. congolense.

Experimental/

Experimental studies were frequently unsatisfactory due to use of small numbers of animals, poor controls and failure to define the exact nature of the challenge and vaccine antigens (Murray and Urquhart, 1977). Furthermore, any successes were only of limited practical value as cross-protection in most cases did not extend to other variants. In cattle vaccination trials, similar problems were also encountered, i.e. successful vaccination only occurred when the same variant was used for vaccination and challenge. Bevan (1928) was the first to establish that tolerance or vaccination of cattle could be achieved by inoculating a laboratory passaged strain of T. congolense followed by treatment with a trypanocidal drug though later work showed that the tolerance could be broken by exposure to T. vivax infections, malnutrition or piroplasmosis (Bevan, 1936). The claim that dead T. brucei, T. congolense or T. vivax conferred full immunity in cattle by Schilling (1953) was not substantiated by Hornby (1941). However, Stephen (1966) in Nigeria showed that calves infected at monthly intervals with wild-caught tsetse flies for up to two years developed acquired non-sterile immunity to T. vivax and T. congolense needing only occasional drug treatments while untreated controls generally died within 3 months. Similar successes were reported for T. brucei vaccinations using infection and treatment by Cunningham (1966), for T. vivax and T. congolense in cattle in Uganda by Wilson (1971) and for T. congolense infections in sheep by Uilenberg (1974) cited by Holmes (1980). Results from experiments using X-irradiated T. congolense as vaccines in cattle were not as successful as those reported for mice (Duxbury et al, 1972) which was attributed to antigenic variation having occurred in the vaccine or challenge inocula./

inocula. However, a lasting immunity was observed when X-irradiated T. rhodesiense was used as the antigens, while T. brucei also gave a limited degree of immunity (Wellde et al, 1973; Duxbury et al, 1973).

From these observations in laboratory and domestic animals, immunisation against individual defined antigenic type is normally highly successful but cross-protection does not extend to other antigenic types. This antigenic variation exhibited by both bloodstream and metacyclic trypanosomes has been the major constraint to the development of a trypanosome vaccine and investigations into its nature have revealed the enormous barrier to be surmounted and vaccination against trypanosomiasis at best remains a research objective. Firstly, the variant antigen was revealed as a glycoprotein which constituted the major part of the surface coat and was made up of a single polypeptide chain (Vickerman, 1969); successive variants were structurally dissimilar entities with different amino-acid sequences (Cross, 1975), and the phenomenon occurred spontaneously in a random fashion independent of host immune responses (Jenni, 1977). Secondly, the number of variants appearing during the course of an infection appear unlimited up to 101 variable antigenic types (VATs) having been isolated from one clone of T. equiperdum (Capbern et al, 1977, cited by Holmes, 1980) though limited numbers have been associated with the three relevant species T. vivax, T. congolense and T. brucei (Wilson and Cunningham, 1972; Jones and Clarkson, 1974; Clarkson, 1976). Thirdly, opinion appears divided as to whether these three relevant trypanosomes always reverted to a very limited number of antigenic types after passage through tsetse flies or in the early phases of infection in their mammalian hosts. Recent observations by Van Meirvenne et al (1977) & Hudson et al (1980) would seem to support the earlier observations by Gray (1965), Clarkson and Awan (1969), Wilson and Cunningham (1971) that in fact they do but

Van Meirvenne/

Van Meirvenne et al (1975), Le Ray et al (1977) and Barry et al (1979) failed to support this conclusion when clone-derived metacyclic populations of T. brucei were observed to give rise to antibody production to several variants in the hosts. However, if the number of antigenic types is limited, a cocktail vaccine based on such VATs may be effective. Limited work along these lines has come from the attempted protection of Zebu cattle against T. congolense in East Africa using a multi-stabilate vaccine of 11 different stabilates of defined antigenic types (Scott et al, 1978) based on an earlier finding by Herbert and Lumsden (1968) that mice can be protected against four stabilates concurrently following challenge by any one of the four original stabilates. The immunising regime consisted of priming with two doses of the 11 stabilates either as live organisms or dead vaccines, followed by chemotherapy and challenge 40 days later with 9 of the original stabilates. All the vaccinated and control cattle eventually died but there was evidence from the increased survival time of the group given the live vaccines that a measure of protection had been induced. Various reasons were given for the observed failure, such as lack of precise timing of chemotherapy to allow slowly replicating VATs to confer their own immunity, competition between stabilates and too massive a challenge dose (10^8 - 10^9 organisms). Thus so far field vaccination against bovine trypanosomiasis still remains completely impractical.

(b) Immunoprophylaxis - Cattle and sheep kept under conditions of natural challenge have been observed to produce a significant degree of immunity which can be boosted by trypanocidal drugs. Urquhart (1977) considered this approach as offering a better and more practicable method of controlling trypanosomiasis through exploiting the host immune responses rather than attempting to produce a trypanosome vaccine/

vaccine. The idea originated from the observations of Bevan (1928, 1936) that cattle clinically recovered from trypanosomiasis by drug treatment remained in good health despite reinfection as evidenced by positive blood smears and the term 'tolerance' was used to describe this phenomenon. Similar observations were made by Whiteside (1962) and Cunningham (1966) whereby after a variable number of natural infections and treatment regimes of cattle in an endemic zone, the interval between drug treatments and parasitaemias increased, which was attributed to the development of a non-sterile immune state. Such immunities also broke down when infection rate was high. The successful control of trypanosomiasis through exploitation of this ability of cattle to develop an immune response to trypanosome challenge assisted by chemotherapy has been reported from East Africa (Wilson et al, 1975a,b; 1976; Bourn and Scott, 1978). The non-sterile immunity was achieved by exposure of cattle to natural infection and treating them when their PCV fell below 20% or when they were overtly sick while the effectiveness of the immunity was assessed on the basis of frequncy of drug therapy, development and duration of parasitaemia, ability to maintain normal blood levels in the face of infections, calving rate in breeding stock and growth rate in beef herds. In a high tsetse challenge zone, a breeding herd was kept successfully for 2 years with eight Berenil treatments and an average of 35 days between parasitaemias and reduced susceptibility evidenced by increased number of calves born, lessened calf mortalities and abortions (Wilson et al, 1975a). In an area of medium challenge, steers which originally required Berenil treatment every 50-60 days only required such treatments after 130 days by the ninth treatment by which time the parasitaemic interval had also increased to 30.9 days from 11.7 days. Some steers which/

which received no treatment during the last six months of the experiment survived and continued to grow at the same rate as those receiving treatments which showed that the immunity was not drug dependent (Wilson et al, 1975b). In a second group of steers treated on an average every 26 days when parasites appeared in the blood, there was no development of immunity and when the drug treatment was withdrawn, severe signs of illness and mortalities resulted. In this group better results and the development of immunity were observed when Samorin was used for treatments. Bourn and Scott (1978) reported the successful keeping of work oxen in an area of endemic trypanosomiasis in Eithiopia (Angar-Gutin Settlement Scheme) based on infection and treatment but, more importantly, on the ability of the animals to develop a non-sterile immunity to the disease. These successes, according to Holmes (1980), were determined largely by the level of careful management, good veterinary supervision and the judicious use of drugs, measures which cannot be guaranteed under traditional husbandry practices but nevertheless emphasise the point that susceptible herds can be kept successfully in areas of low or medium tsetse challange through immunoprophylaxis.

(c) Trypanotolerance - Certain breeds of cattle, especially the West African dwarf breeds such as the Muturu and N'dama, certain breeds of sheep and goats and game animals are more resistant to infection with trypanosomes than others when challenged for the first time or during reinfections with the same or similar numbers of organisms, suggesting that trypanotolerance has a genetic basis (Desowitz, 1959; Stephen, 1966; Fiennes, 1970; Roberts and Gray, 1973a,b; Murray et al, 1979a,b; Griffin and Allonby, 1979). Recent evidence from tolerant mice (Whitelaw et al, 1980; Jennings et al, 1978; Morrison et al, 1978) would seem to suggest that trypanotolerance has an immunological basis. Breeding for tolerance, according to Finelle (1980) should increase

the/

the number of tolerant animals and their degree of tolerance, but it has not been shown conclusively that trypanotolerance can be inherited, i.e. enhanced by selective breeding within breeds or whether cross-breeding with productive breeds can enhance productivity. From evidence presently available, trypanotolerance does not confer protection to the effects of the disease (Murray et al, 1979a,b) but rather enables such animals to limit the first wave of parasitaemia, i.e. a superior resistance to infection, and consequently limit the severity of the anaemia. Practical constraints to successful control of trypanosomiasis through exploitation of the innate resistance of these breeds include the cost and limited stock of breeding animals available (Holmes, 1980), the long term nature before full realisation of breeding objectives and the necessity for good nutrition, careful veterinary supervision, good husbandry without which breeding cannot be successful. These measures can also achieve successful control in the more productive Zebus (Urquhart, 1977).

Prospects for Vaccination:

At present the only practical exploitation of the host's immune responses in controlling trypanosomiasis has been through acquired or genetic trypanotolerance. The problems of antigenic variation notwithstanding, the search for possible vaccines against trypanosomiasis continues and various options are being considered (Murray et al, 1979c; Holmes, 1980) and optimism appears based on the long recognised fact that cattle under natural conditions of challenge can control parasitaemias, and on recent scientific advances in other fields:-

(i) Further exploitation of the multi-stabilate vaccine approach: The requirements for success along these lines have been earlier enumerated (Murray et al, 1979).

(ii)/

(ii) There is the possibility that different VATs of the same species or between different species have cross-reacting determinants (Barbet and McGuire, 1978) and it has been suggested that, if the immunogenicity of such determinants can be increased, vaccination may be achieved.

(iii) The large scale production of antigens hitherto a problem has now been made possible by the works of Hirumi et al (1977, 1978a,b, 1980) who also identified and cloned together antigenically more stable mamallian bloodstream forms with the same VAT repertoire as metacyclic forms. Thus it may be possible to define the VAT complement of metacyclic populations and, hopefully, produce a vaccine against that VAT repertoire.

(iv) Recent advances in molecular and genetic engineering in other fields such as recombinant DNA technology, use of specific messenger RNA and specific target tissues to produce immunising proteins, transfer of specific genes from one genome to another, production of trypanosome antigens in bacteria, as well as weak spots in the life cycle of the parasite, are being watched for possible vaccine potentials.

(v) A search for possible antagonists capable of inhibiting antigenic variation continues but so far without success.

(vi) Possible use of immunostimulants to induce non-specific immunity against trypanosomes. Limited success has been reported in a pilot trial by Murray and Morrison (1979) using the immunostimulants Bordetella pertusis, Corynebacterium parvum and Bacillus-Calmette Guerin (B.C.G.) in T. congolense infected mice but Holmes (1980) was unable to produce similar results with another stabilate of T. congolense.

(vii) In vivo attenuation of the parasites to boost non-sterile immunity is also undergoing laboratory investigation (Holmes, 1980) but there is the/

the fear that such attenuated parasites may revert to more pathogenic forms under field conditions.

Conclusion.

An integrated approach, as suggested by Finelle (1980), probably offers the best scope for control. Using this approach, chemotherapy can be used to eliminate existing trypanosome infections, thereby avoiding mechanical transmission and tsetse control achieved by insecticidal application followed by release of sterile males to obtain total tsetse eradication. Alternatively, in areas where effective control cannot be achieved due to economic reasons and location in marginal tsetse areas, breeding of trypanotolerant animals can be embarked upon. Either method should be integrated with rural development taking into consideration all the problems likely to be encountered and a programme designed accordingly.

The joint FAO/WHO programme for the control of African trypanosomiasis and related development in Africa is an example of such an integrated approach (Finelle, 1980). In the meantime, much reliance will continue to be placed on sporadic chemotherapy and limited tsetse eradication in areas of endemic trypanosomiasis.

REFERENCES.

Allonby, E.W. (1974)
Ph.D. Thesis, University of Glasgow.

Allonby, E.W. and Urquhart, G.M. (1973)
Parasitology, 66, 43-53.

Allonby, E.W. and Urquhart, G.M. (1975)
Veterinary Parasitology, 1, 129-143.

Allonby, E.W. and Urquhart, G.M. (1976)
Research in Veterinary Science, 20, 212-214.

Altaif, K.I. and Dargie, J.D. (1976)
I.A.E.A., Vienna, 449-462.

Altaif, K.I. and Dargie, J.D. (1978a)
Parasitology, 77, 161-175.

Altaif, K.I. and Dargie, J.D. (1978b)
Parasitology, 77, 177-187.

Anderson, N. (1973)
Australian Journal of Agricultural Research, 24, 559-611.

Austen, E.E. and Hegh, E. (1922) cited by Glasgow, J.P. (1970)
'The African Trypanosomiases'(ed. H.W. Mulligan),
Allen & Unwin Ltd., London, 456-458.

Barbet, A.F. and McGuire, T.C. (1978)
Proceedings of the National Academy of Science, 75, 1989-1993.

Barger, I.A. and Southcott, W.H. (1975)
International Journal of Parasitology, 5, 39-44.

Barry, J.D., Hadjuk, S.L., Vickermann, K. and Le Ray, D. (1979)
Transactions of the Royal Society of tropical Medicine
and Hygiene, 73, 205-208.

Benitez-Usher, C., Armour, J., Duncan, J.L., Urquhart, G.M. and
Gettinby, G. (1977)
Veterinary Parasitology, 3, 327-342.

Bevan, Ll.E.W. (1928)
Transactions of the Royal Society of tropical Medicine and
Hygiene, 22, 147-156.

Bevan, Ll.E.W. (1936)
Transactions of the Royal Society of tropical Medicine and
Hygiene, 30, 199-207.

Bitakaramire, P.K. (1966)
Parasitology, 56, 619-622.

Blitz, N.M. and Gibbs, H.C. (1972)
International Journal of Parasitology, 2, 5-12.

Blood, D.C. and Henderson, J.A. (1968)
Veterinary Medicine, Bailliere, Tindall and Casell (3rd Edition) London, 585-628.

Blood, D.C., Henderson, J.A. and Radostits, O.M. (1979)
Veterinary Medicine, Bailliere, Tindall (London) 750-791.

Boag, B. and Thomas, R.J. (1973)
Research in Veterinary Science, 14, 11-20.

Bourn, D. and Scott, M. (1978)
Tropical Animal Health & Production, 10, 191-203.

Bradley, R.E., Radhakrishnan, C.V., Patel-Kulkarni, V.G. and Loggins, P.E. (1973)
American Journal of Veterinary Research, 34, 729-735.

Brundson, R.V. (1972)
New Zealand Veterinary Journal, 20, 214-220.

Brundson, R.V. (1974)
New Zealand Journal of Experimental Agriculture, 2, 223-230.

Brundson, R.V. (1976) cited by Brundson, R.V. (1980)
Veterinary Parasitology, 6, 185-215.

Brundson, R.V. (1980)
Veterinary Parasitology, 6, 185-215.

Burnett, G.F. (1970)
In 'The African Trypanosomiases' (ed. H.W. Mulligan), Allen and Unwin Ltd., 464-489, 490-519.

Capbern, A., Giroud, C., Baltz, T. and Mattern, P. (1977) cited by Holmes, P.H. (1980)
Vaccines against Parasites (ed. A.E.R. Taylor and R. Muller), Symposia of the British Society for Parasitology, 18, 75, Blackwell Scientific Publications, Oxford.

Ciordia, H., Bizzell, W.E., Baird, D.M., McCampbell, H.C. and White, C.E. (1964)
American Journal of Veterinary Research, 25, 1473-1478.

Ciordia, H., Neville, W.E., Baird, D.M. and McCampbell, H.C. (1971)
American Journal of Veterinary Research, 32, 1353-1358.

Clarkson, M.J. (1976)
Veterinary Parasitology, 2, 9-29.

Clarkson, M.J. and Awan, M.A.O. (1969)
Annals of Tropical Medicine and Parasitology, 63, 515-527.

Colglazier, M.L., Enzie, F.D. and Lehmann, R.P. (1970)
Journal of Parasitology, 56, 768-772.

Connan, R.M. (1968)
World Review of Animal Production, 4, 55-58.

Cross, G.A.M. (1975)
Parasitology, 71, 393-417.

Cross, G.A.M. (1978)
Proceedings of the Royal Society, London B, 202, 55-72.

Cunningham, M.P. (1966)
East African Medical Journal, 43, 394-397.

Dame, D.A. and Williamson, D.L. (1979)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 73, 133.

Dame, D.A., Williamson, D.L., Cobb, P.E., Gates, D.B., Warner, P.V., Mtuya, A.G. and Baumgartner, H. (1980)
I.A.E.A./F.A.O., 267-280.

Dar, F.K. (1972)
Tropical Animal Health and Production, 4, 237-244.

Dargie, J.D. and Allonby, E.W. (1975)
International Journal for Parasitology, 5, 147-157.

Davis, H. (1971)
Journal of Applied Ecology, 8, 563-578.

Dean, G.J.W. and Wortham, S.M. (1969)
Bulletin of Entomological Research, 58, 505-514.

Christie, M.G., Brambell, M.R. and Charleston, W.A.G. (1964a)
Research in Veterinary Science, 5, 202-212.

Christie, M.G., Brambell, M.R. and Charleston, W.A.G. (1964b)
Journal of Comparative Pathology, 74, 427-434.

Christie, M.G. and Brambell, M.R. (1966)
Journal of Comparative Pathology, 76, 207-216.

Christie, M.G., Hart, R., Angus, R.N., Devoy, J. and Patterson, J.E. (1978)
Journal of Comparative Pathology, 88, 157-165.

Desowitz, R.S. (1959)
Annals of Tropical Medicine and Parasitology, 53, 293-313.

Dineen, J.K. and Wagland, B.M. (1966)
Parasitology, 56, 665-677.

Dodin, A. and Fromentin, H. (1962)
Bulletin of Society of Pathologists, exot., 55, 128-133.

Donald, A.D. (1969)
Vict. Veterinary Proceedings, 27, 34-36.

Donald, A.D. (1974)
Proceedings of Australian Society of Animal Production, 10, 148-155.

Donald, A.D., Dineen, J.K. and Adams, D.B. (1969)
Parasitology, 59, 497-503.

Donald, A.D., Morley, F.H.W., Waller, P.J., Axelsen, A. and Donelly, J.R. (1978)
Australian Journal of Agricultural Research, 29, 189-204.

Donald, A.D. and Waller, P.J. (1973)
International Journal of Parasitology, 3, 219-233.

Donelly, J.R., McKinney, G.T. and Morley, F.H.W. (1972)
Proceedings of the Australian Society of Animal Production, 9, 392-395.

Drudge, J.H., Azanto, J. and Wyant, A.M. (1957)
American Journal of Veterinary Research, 18, 317-329.

Drudge, J.H., Leland, S.E.Jr., Wyant, Z.N. (1959)
American Journal of Veterinary Research, 20, 670-676.

Drudge, J.H., Azanto, J., Wyant, A.M. and Elam, G. (1964)
Americal Journal of Veterinary Research, 25, 1512 - 1518.

Duncan, J.L., Smith, W.D. and Dargie, J.D. (1978)
Veterinary Parasitology, 4, 21-27.

Duxbury, R.E. and Sadun, E.H. (1969)
Journal of Parasitology, 55, 859 - 865.

Duxbury, R.E., Anderson, J.S., Wellde, B.T., Sadun, E.H. and Muriithi, I.E. (1972a)
Experimental Parasitology, 32, 527 - 533.

Duxbury, R.E., Sadun, E.H., Anderson, J.S., Wellde, B.T., Muriithi, E.H. and Warui, G.M. (1973)
I.A.E.A., Vienna, 179.

Duxbury, R.E., Sadun, E.H. and Anderson, J.S. (1972b)
Americal Journal of Tropical Medicine and Hygiene, 21, 885-888.

Duxbury, R.E., Sadun, E.H., Schoenbechler, M.J. and Stroupe, D.A. (1974)
Experimental Parasitology, 36, 70 - 76.

Evans, J.V. and Blunt, M.H. (1961)
Australian Journal of Agricultural Research, 14, 100-108.

Evans, J.V., Blunt, M.H., and Southcott, W.H. (1963)
Australian Journal of Agricultural Research, 14, 549-558.

Evans, J.V. and Whitlock, J.H. (1964)
Science, 145, 1318 - 1323.

Fiennes, R.N.T.W. (1970)
In 'The African Trypanosomiases' (ed. H.W. Mulligan), George Allen & Unwin Ltd., (London), 729.

Finelle, P. (1973a)
World Animal Review, 7, 1-6.

Finelle, P. (1973b)
World Animal Review, 8, 24-27.

Finelle, P. (1980)
I.A.E.A./F.A.O., Vienna, 3-32.

Ford, J., Nash, T.A.M. and Welch, J.R. (1970)
In 'The African Trypanosomiases' (ed. W.H. Mulligan),
Allen and Unwin Ltd. (London), 543-571.

Ford, J. and Blaser, E. (1971)
Acta. tropica, 28, 67-79.

Galun, R. (1975) cited by Galun, M.N., Ben-Eliahu, D.,
Ben-Tamar, D., Simkin, J. (1980)
I.A.E.A./F.A.O., Vienna, 207-317.

Galun, M.N., Ben-Eliahu, D., Ben-Tamar, D., Simkin, J. (1980)
I.A.E.A./F.A.O., Vienna, 207-317.

Gibson, T.E. and Everett, G. (1968)
British Veterinary Journal, 124, 287-298.

Glasgow, J.P. (1970)
In 'The African Trypanosomiases', (ed. H.W. Mulligan),
Allen and Unwin Ltd. (London), 456-458.

Goldberg, A. and Lucker, J.T. (1969)
American Journal of Veterinary Research, 30, 2137-2144.

Gordon, H.McI. (1967)
In 'Reaction of the host to parasitism' (ed. E.J.L. Soulsby),
Marburg/Lahn, Germany, pp. 174-190.

Gordon, H. McI. (1971)
Proceedings of World Veterinary Congress (19th), 3, 892-896.

Gordon, H.McI. (1973)
Advances in Veterinary Science and Comparative Medicine, 17,
395-431.

Gray, A.R. (1965)
Journal of General Microbiology, 41, 195-214.

Griffin, L. and Allonby, E.W. (1979)
Veterinary Parasitology, 5, 97-105.

Hadaway, A.B. (1972)
Bulletin of World Health Organisation, 46, 353-362.

Hadaway, A.B. (1977)
Transactions of the Royal Society of Tropical Medicine and
Hygiene, 71, 6-8.

Heath, D.F. and Park, P.O. (1953)
Nature (London), 172, 206.

Herbert, W.J. and Lumsden, W.H.R. (1968)
Journal of Medical Microbiology, 1, 23-32.

Hirumi, H., Doyle, J.J. and Hirumi, K. (1977)
Science, 196, 992-994.

Hirumi, H., Hirumi, K. and Doyle, J.J. (1978a)
4th International Congress on Parasitology, Sc. A.
(Warszawa, Poland). p.54.

Hirumi, H., Hirumi, K. and Doyle, J.J. (1978b)
In Vitro, 14, 379.

Hirumi, K., Hirumi, H., Doyle, J.J. and Cross, G.A.M. (1980)
Parasitology, 80, 371-382.

Hocking, K.S., Lee, C.W., Beesley, J.S.S.and Matsuchi, H.T. (1966)
Bulletin of Entymological Research, 56, 737-744.

Holmes, P.H. 1980)
In 'Vaccines against Parasites' (ed. A.E.R. Taylor and R. Muller),
Symposia of the British Society for Parasitology, 18, 75-105.

Hornby, H.E. (1941)
Transactions of the Royal Society of tropical Medicine and
Hygiene, 35, 165.

Hudson, K.M.,Taylor, A.E.R. and Elie, B.J. (1980)
Parasite Immunology, 2, 57-69.

Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., Mulligan, W.
and Sharp, N.C.C. (1959)
American Journal of Veterinary Research, 20, 527-531.

Ibid, (1961)
American Journal of Veterinary Research, 20, 186 - 188.

Jenni, L. (1977)
Acta. tropica, 34, 35-41.

Jennings, F.W., Whitelaw, D.D., Holmes, P.H. and Urquhart, G.M. (1978)
Research in Veterinary Science, 25, 399-400.

Jilek, A.F. and Bradley, R.E. (1969)
American Journal of Veterinary Research, 30, 1773-1778.

Johnson, P., Neal, R.A. and Gall, D. (1963)
Nature (Lond.), 200, 83.

Jones-Davis, W.J. (1967a)
Veterinary Record, 80, 531-532.

Jones-Davis, W.J. (1967b)
Veterinary Record, 81, 367-368.

Jones-Davis, W.J. (1968)
Bulletin of Epizootic Diseases in Africa, 16, 213-216.

Jones, T.W. and Clarkson, M.J. (1974)
Annals of Tropical Medicine and Parasitology, 68, 485-486.

Kelly, J.D., Hall, C.A., Whitlock, H.V., Thompson, H.G., Campbell, N.J. and Martin, I.C.A. (1977)
Research in Veterinary Science, 22, 161-168.

Kendrick, J.A. and Alsop, N. (1974)
Pest Articles and News Summaries, 20, 392-399.

Kennedy, T.J. and Todd, A.C. (1975)
American Journal of Veterinary Research, 36, 1195-1198.

Koch, H. (1914) cited by Glasgow, J.P. (1970)
In 'The African Trypanosomiases', (ed.H.W. Mulligan), Allen & Unwin Ltd., London.

Koeman, J.H., den Boer, W.M.J., Feith, A.F.,de longh, H.H., Spliethoff, P.C., Na'isa, B.K. and Spielberger, U. (1976) cited by Lee, C.W. (1939)
Transactions of the Royal Society for Tropical Medicine and Hygiene, 73, 137-138.

Kutler, K.L. and Marble, D.W. (1962)
Veterinary Medicine, 57, 39-45.

Leach, T.M. (1973)
Advances in Veterinary Science and Comparative Medicine, 17, 119-162.

Leaning, W.H.D., Cairns, G.C., McKenzie, J.K. and Hunter, W.R. (1970)
Proceedings of the New Zealand Society of Animal Production, 30, 52-64.

Leaver, J.D. (1970)
Journal of Agricultural Science, Camb., 75, 265-272.

Lawrence, H.S. (1949)
Proceedings of Society of Experimental Biological Medicine, 41, 516.

Lawrence, H.S. (1954)
Journal of Clinical Investigation, 35, 951-961.

Lee, C.W. (1979)
Transactions of the Royal Society for Tropical Medicine and Hygiene, 73, 137-138.

Le jambre, L.F. (1978)
In 'Epidemiology and Control of Gastrointestinal Parasites of Sheep in Australia' (eds. A.D. Donald, W.H. Southcott and J.K. Dineen), C.S.I.R.O., p. 157.

Le Ray, D., Barry, J.D., Easton, C. and Vickermann, K. (1977)
Annales de la Societe Belge de Medicine tropicale, 57, 369-381.

Levine, N.D. and Garrigus, V.S. (1962)
American Journal of Veterinary Research, 23, 489 - 493.

Levine, N.D. and Clark, D.T. (1961)
Veterinary Parasitology, 4, 89-97.

Levine, N.D., Clark, D.T., Bradley, R.E. and Kantor, S. (1975)
American Journal of Veterinary Research, 36, 1459-1 64.

Lindahl, I.L., Kates, K.C., Turner, J.H., Enzie, F.D. and Whitmore, G.E. (1963)
Journal of Parasitology, 49, 209-217.

Lopez, V. and Urquhart, G.M. (1967)
In 'The reaction of the host to parasitism', (ed. E.J.L. Soulsby), Elvert, Germany, 153-159.

Luisenko, A.A. (1956) cited by Christie, M.G., Brambell, M.R. and Charleston, W.A.G. (1964a)
Research in Veterinary Science, 5, 202-212.

Lumsden, W.H.R. and Herbert, W.J. (1967)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 61, 42.

MacAskill, J.A., Holmes, P.H., Whitelaw, D.D., McConnell, I., Jennings, F.W. and Urquhart, G.M. (1980)
Immunology, 40, 629-635.

Maclennan, K.J.R. (1970)
In 'The African Trypanosomiases', (ed. W.H. Mulligan), Allen & Unwin Ltd., London, 799-821.

Maclennan, K.J.R. and Na'isa, B.K. (1970)
Tropical Animal Health and Production, 2, 189-195.

Maclennan, K.J.R. and Jones-Davis, W.J. (1964)
Veterinary Record, 80, 389-390.

Manton, V.J.A., Peacock, R., Poynter, D., Silvermann, P.H. and Terry, R.J. (1962)
Research in Veterinary Science, 3, 308-314.

Mayhew, R.L. (1949)
Proceedings of Helminthological Society, Washington, 16, 19-23.

Manford, E.M., Czerol, N.H., Courter, M., Green, C. and Levine, N.D. (1974)
American Journal of Veterinary Research, 35, 1423-1428.

McKinney, G.T. (1974)
Australian Journal of Experimental Agriculture and Animal Husbandry, 14, 726-734.

Michel, J.F. (1969a)
Journal of Helminthology, 43, 111-133.

Michel, J.F. (1969b)
Advances in Parasitology, 7, 211-282.

Michel, J.F. and Lancaster, M.B. (1970)
Journal of Helminthology, 44, 107 - 113.

Michel, J.F. (1976)
Advances in Parasitology, 14, 355-397.

Miller, J.K. (1965)
Immunology, 9, 521.

Morley, F.H.W. (1978) cited by Morley, F.H.W. and Donald, A.D. (1980)
Veterinary Parasitology, 6, 105-134.

Morley, F.H.W. and Donald, A.D. (1980)
Veterinary Parasitology, 6, 105-134.

Morrison, W.I., Roelants, G.E., Mayor-Withey and Murray, M. (1978)
Clinical Experimental Immunology, 32, 25-40.

Mulligan, W., Gordon, H.McI., Stewart, D.F. and Wagland, B. (1961)
Australian Journal of Agricultural Research, 12, 1175-1187.

Mulligan, W. (1976)
IAEA/FAO, Vienna, 413-420.

Murray, M. and Urquhart, G.M. (1977)
In 'Immunity to Blood Parasites of Animals and Man',
(eds. Miller, L.H. and McKelvey, Jr., J.J.),
Advances in Experimental Medical Biology, 93, 209-241,
Plenum Press, New York and London.

Murray, M. and Morrison, W.I. (1979)
Parasitology, 79, 349 - 366.

Murray, P.K., Murray, M., Wallace, M., Morrison, W.I. and
McIntyre, W.I.M. (1979a)
In 'International Scientific Council for Trypanosomiasis
Research and Control', 15th Meeting, The Gambia, 1977,
Eleza Services, Nairobi.

Murray, P.K., Murray, M., Wallace, M., Morrison, W.I. and
McIntyre, W.I.M. (1979b)
In 'International Scientific Council for Trypanosomiasis
Research and Control', 15th Meeting, The Gambia, 1977,
Eleza Services, Nairobi.

Murray, M., Barry, J.D., Morrison, W.I., Williams, R.O.,
Hirumi, H. and Rovis, L. (1979c)
World Animal Review, 32, 9-13.

Nash, T.A.M. (1970)
In 'The African Trypanosomiases' (ed. H.W. Mulligan), Allen & Unwin Ltd., London, 521-532.

Neilson, J.T.M. (1975)
International Journal of Parasitology, 5, 427-430.

Ormerod, W.E. (1976)
Science, 191, 815-821.

Ormerod, W.E. (1979)
Pharmaceutical Therapeutics, 6, 1-40.

Park, P.O., Gledhill, J.A., Alsop, N. and Lee, C.W. (1972)
Bulletin of Entomological Research, 61, 373-384.

Parnell, I.W. (1962)
Journal of Helminthology, 36, 161-188.

Potts, W.H. (1958)
Annals of Tropical Medicine and Parasitology, 52, 484.

Potts, W.H. (1970)
In 'The African Trypanosomiases', (ed. H.W. Mulligan), Allen & Unwin, Ltd., London, 458-463.

Potts, W.H., Jones, R.M. and Cromwell, R.L. (1974)cited by Brundson, R.V. (1980)
Veterinary Parasitology, 6, 185-215.

Preston, J.M. and Allonby, E.W. (1978)
Veterinary Record, 103, 509 - 513.

Preston, J.M. and Allonby, E.W. (1979a)
Research in Veterinary Science, 26, 134-139.

Preston, J.M. and Allonby, E.W. (1979b)
Research in Veterinary Science, 26, 134-139.

Radhakrishnan, C.V., Bradley, R.E. and Loggins, P.E. (1972)
American Journal of Veterinary Research, 33, 817-823.

Rifkin, G.G. and Dobson, C. (1979)
Veterinary Parasitology, 5, 365-378.

Roberts, F.H.S. (1942)
Australian Veterinary Journal, 18, 19-27.

Roberts, F.H.S., O'Sullivan, A.J. and Rick, R.F. (1952)
Australian Journal of Agricultural Research, 3, 187-226.

Roberts, C.J. and Gray, A.R. (1973a)
Tropical Animal Health and Production, 5, 211-233.

Roberts, C.J. and Gray, A.R. (1973b)
Tropical Animal Health and Production, 5, 220-233.

Rocha, C.A., Campos, M.S., Pracoli, J.O., Costa, J.W. and Ribeiro, R. (1967) cited by Gordon, H. McI. (1973) Advances in Veterinary Science and Comparative Medicine, 17, 395-437.

Roe, R., Southcott, W.H. and Turner, H.N. (1959) Australian Journal of Agricultural Research, 10, 530-554.

Ross, J.G. and Halliday, W.G. (1978) Veterinary Record, 102, 240-241.

Ross, J.G. and Halliday, W G. (1979) Research in Veterinary Science, 26, 41-46.

Ross, J.G., Duncan, J.L. and Halliday, W.G. (1978) Research in Veterinary Science, 27, 258-259.

Ross, I.G., Chamberlain, W.E. and Turner, H.N. (1937) cited by Gibson, T.E. and Everett, G. (1968) British Veterinary Journal, 124, 287.

Ross, J.G., Armour, J., Hart, J. and Lee, R.A. (1959) Veterinary Record, 71, 751.

Schilling, C. (1953) Journal of Tropical Medicine and Hygiene, 38, 106-112.

Scott, H.L., Silvermann, R.H., Mansfield, M.E. and Levine, H.S. (1977) American Journal of Veterinary Research, 32, 249-262.

Scott, J.M. and Pegram, R.G. (1974) Tropical Animal Health and Production, 6, 215-221.

Scott, J.M., Holmes, P.H., Jennings, F.W. and Urquhart, G.M. (1978) Research in Veterinary Science, 25, 115-117.

Seed, J.R. (1963) Journal of Protozoology, 10, 380-389.

Seed, J.R. (1972) Experimental Parasitology, 31, 98 - 108.

Seed, J.R. and Gam, A.A. (1966) Journal of Parasitology, 52, 1134-1140.

Shaw, G.D. (1960) Symposium on Animal Trypanosomiases, Luanda, 1958. Report of the Committee on technical co-operation in Africa south of the Sahara, London, 45, 125-137.

Silvermann, P.H., Poynter, D. and Podger, K.R. (1962) Journal of Parasitology, 48, 562-571.

Silvermann, P.H. (1965) American Journal of Zoology, 5, 153-163.

Smith, I.M. (1959)
Journal of Comparative Pathology, 69, 105-115.

Smith, W.D. (1977a,b)
Research in Veterinary Science, 22, 128-129, 334-338.

Smith, W.D. and Angus, K.W. (1980)
Research in Veterinary Science, 29, 45-50.

Smith, W.D. and Christie, M.G. (1978)
International Journal of Parasitology, 8. 219 - 223.

Smith, W.D. and Christie, M.G. (1979)
Journal of Comparative Pathology, 89, 141-150.

Soltys, M.A. (1964)
Parasitology, 54, 585 - 591.

Soltys, M.A. (1967)
Canadian Journal of Microbiology, 13, 743 - 749.

Southcott, W.H., Langlands, J.P. and Heath, D.D. (1970)
Veterinary Parasitology, 6, 105-134.

Southcott, W.H. and Barger, I.A. (1975)
International Journal of Parasitology, 5, 45-48.

Southcott, W.H., Major, G.W. and Barger, I.A. (1976)
Australian Journal of Agricultural Research, 27, 277-286.

Spedding, C.R.W. (1955)
Proceedings of the British Society of Animal Production, 30-37.

Spielberger, U. and Abdurrahim, U. (1972) cited by Hadaway, A.B. (1977)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 71, 6-7.

Spielberger, U. and Na'isa, B.K. (1975) cited by Lee, C.W. (1979)
Transactions of the Royal Society of Tropical Medicine and Hygiene, 73, 137-138.

Stephen, L.E. (1966)
Annals of Tropical Medicine and Parasitology, 60, 230.

Stewart, D.F. (1950)
Australian Journal of Agricultural Research, 1, 301-321.

Stewart, D.F. (1953)
Australian Journal of Agricultural Research, 4, 100-117.

Stoll, N.R. (1929)
American Journal of Hygiene, 10, 384-418.

Takayanagi, T., Nakatake, Y. and Enreque , G.L. (1974)
Journal of Parasitology, 60, 336-339.

Taylor, E.L. (1961)
Outlook in Agriculture, 3, 139-144.

Tetley, J.L. (1953) cited by Gordon, H. McI. (1957)
Advances in Veterinary Science, 3, 287-351.

Tetzlaff, D. and Todd, A.C. (1973)
American Journal of Veterinary Research, 34, 1549-1554.

Thomas, R.J. and Boag, B. (1973)
Research in Veterinary Science, 15, 238-249.

Uilenberg, G. (1974) cited by Holmes, P.H. (1980)
In 'Vaccines against Parasites', (ed. A.E.R. Taylor and R. Muller), Symposia of the British Society for Parasitology, 18, 75-105.

Urquhart, G.M. (1962)
Proceedings of the International Congress on Microbiology, Stand. 1961, 517-519.

Urquhart, G.M. (1977)
In 'Immunity in Parasitic Diseases',
INSERM, 72, 263-274.

Urquhart, G.M. (1968)
I.A.E.A., Vienna, 35-41.

Urquhart, G.M., Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., Mulligan, W. and Sharp, N.C.C. (1966a)
American Journal of Veterinary Research, 27, 1641-1643.

Urquhart, G.M., Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M. and Mulligan, W. (1966b)
American Journal of Veterinary Research, 27, 1645-1648.

Urquhart, G.M. (1980)
Proceedings of the 8th International Congress of the World Association for the Advancement of Veterinary Parasitology, 1977, Veterinary Parasitology, 6, 217-239.

Vanderplank, F.L. (1944),
Nature (London), 154, 607-608.

Van Meirvenne, N., Janseens, P.G., Magnus, E., Lumsden, W.H.R. and Herbert, W.J. (1975)
Annals of the Societe Belge Medicine tropicale, 55, 25-30.

Van Meirvenne, N., Magnus, E. and Vervoort, T. (1977)
Annals of the Societe Belge Medicine tropicale, 57, 409-423.

Vickermann, K. (1969)
Journal of Cellular Science, 5, 163-193.

Vickermann, K. (1978)
Nature (London), 273, 613-617.

Vlassoff, A. (1973)
New Zealand Journal of Experimental Agriculture, 1, 293-301.

Wagland, B.M. and Dineen, J.K. (1967)
Parasitology, 57, 59-65.

Wellde, B.T., Duxbury, R.E., Sadun, E.H., Lanbehn, H.R., Lotzch, R., Deindl, G. and Warui, G. (1973)
Experimental Parasitology, 34, 62-68.

Wellde, B.T., Schoenbechler, M.J., Diggs, C.L., Langbehn, H.R. and Sadun, E.H. (1975)
Experimental Parasitology, 37, 125 - 129.

Whitelaw, D.D., MacAskill, J.A., Holmes, P.H., Jennings, F.W. and Urquhart, G.M. (1980)
Infectivity and Immunity, 27, 707-713.

Whiteside, E.F. (1962),
East African Agricultural and Forest Journal, 28, 67-73.

Whitlock, J.H. and Masden, H. (1958)
Cornell Veterinarian, 48, 134-135.

Williamson, J. (1970)
In 'The African Trypanosomiases', (ed. H.W. Mulligan), Allen & Unwin, London.

Williamson, J. (1976)
Tropical Diseases Bulletin, 73, 531-542.

Wilson, A.J. (1971)
Tropical Animal Health and Production, 3, 14-22.

Wilson, A.J. and Cunningham, M.P. (1971)
Tropical Animal Health and Production, 3, 133-139.

Wilson, A.J. and Cunningham, M.P. (1972)
Experimental Parasitology, 32, 165-173.

Wilson, A.J., Paris, J. and Dar, F.K. (1975a)
Tropical Animal Health and Production, 7, 63-71.

Wilson, A.J., Le Roux, T.D., Paris, J., Davidson, C.R. and Gray, A.R. (1975b)
Tropical Animal Health and Production, 7, 187-199.

Wilson, A.J., Paris, J., Luckins, A.G., Dar, F.K. and Gray, A.R. (1976)
Tropical Animal Health and Production, 8, 1-12.

Wilson, G.I. and Samson, K.S. (1974)
Research in Veterinary Science, 17, 390-394.

Zimmerman, W.J. (1965)
J.A.V.M.A., 147, 499-505.

CHAPTER VII

THE EFFECTS OF DIFFERENT LEVELS OF PROTEIN INTAKE ON THE PATHOPHYSIOLOGY OF ACUTE HAEMONCHOSIS AND THE SELF-CURE PHENOMENON

GENERAL INTRODUCTION

INTRODUCTION:

Helminth and protozoan parasites exert by far the greatest limiting factor on economically viable livestock production in most tropical and sub-tropical countries. Unfortunately in such areas a large number of humans suffer from chronic protein malnutrition and various efforts are being made to correct this deficiency. One approach has involved a shift from the traditional methods of husbandry to more intensive techniques. Paradoxically the problems posed by these parasites have assumed more importance under the new management systems. Attempts are also being made to exploit the genetic resistance of some indigenous breeds to boost productivity through cross breeding with the more productive exotic breeds but so far with limited success. The finding that irregularity of food supply, typical of situations in most endemic countries, can affect genetic markers of resistance independently of the presence of parasitism has further complicated this latter approach.

Of the helminth infections associated with poor productivity in livestock, especially sheep, in tropical countries, haemonchosis caused by either Haemonchus contortus or H. placei is generally regarded as the most important. Annual losses due to this parasite in sheep from various tropical countries are not widely available. One report from the semi-arid regions of Kenya puts such losses in the order of 3 million pounds (Allonby, 1974). The widespread prevalence of the disease in most tropical countries and in the warmer temperate regions of Southern Europe and elsewhere was highlighted in Chapter I.

The basic features of the epidemiology and pathogenesis of the disease were also reviewed in Chapter I. Anaemia, hypoproteinaemia and weight loss or reduced weight gains are the major consequences of infections.

The/

The mechanism of the anaemia has long been recognised as haemorrhagic (Fourie, 1931). This has been supported and further extended by the use of radioisotopic tracer techniques (Dargie, 1974; Dargie and Allonby, 1975). However the wide variation in the severity of the anaemia exhibited by infected animals suggests the involvement of other factors in the pathogenesis of the anaemia. Some of these factors, i.e. age, breed, haemoglobin type, have been identified and their influence on the disease process determined (Gordon, 1958; Urquhart et al, 1966a,b; Altaif and Dargie, 1978a, b; Preston and Allonby, 1978, 1979a,b) - Chapter I. More importantly, the nutritional status of the host affects susceptibility to infections but the exact relationship has not been easy to define. Most of the early observations on this aspect were reviewed in Chapter V. The important conclusions are:- (a) the adequately fed host is more resistant than the poorly fed host to most parasitic infections, i.e. nutritional status can affect the immune response of hosts to their parasites; (b) the infections may upset the nutritional physiology of the hosts thereby leading to poor productivity, performance and even death; and (c) the necessity to differentiate between resistance to the establishment of an infection and resistance to the effects of an established infection.

So far no firm conclusions can be drawn as to whether the establishment of H. contortus is affected by the nutritional plane of the host. Similarly, the contention that the nutritional plane of the host affects the severity and ultimate outcome of haemonchosis in common with other gastrointestinal parasites has been widely accepted but has not been verified in sufficient detail experimentally. Since protein level per se can impair erythropoiesis it is necessary to define if the differences in susceptibility observed in animals on different dietary protein regimes reflect differences in impairment of erythropoiesis or/

or a faster and more severe pathogenesis. This necessitates a study of the functional state of the erythron under the different dietary regimes, as was attempted in the present experiment.

The changes in plasma protein metabolism following single experimental infections were documented by Dargie (1974, 1975, 1980) using isotopic tracer techniques (Chapters I and IV). He concluded that the underlying changes in plasma protein metabolism in haemonchosis involved disturbances in the balance between catabolism, distribution and synthesis. The extent to which these changes are influenced by nitrogen intake was examined to a limited extent by Dargie (1974) in a comparison of ad libitum fed H. contortus infected and pair-fed control sheep. He showed that infected animals, although consuming more food than controls, failed to gain weight at the same rate as the controls. This he attributed to the hypercatabolism and re-channelling of essential amino acids away from tissues such as the muscle to organs involved in essential amino acid synthesis. He consequently suggested that sheep on a low protein diet would have lower rates of fractional and absolute albumin degradation. This assumption has been verified in other host parasite systems, e.g. fascioliasis (Berry and Dargie, 1978), but no actual measurements have been made nor diets affording different crude protein intakes used to test this assumption in haemonchosis. Hence it is worthwhile to examine this aspect as dietary deprivation forms an integral part of the epidemiology of the disease in endemic areas.

Loss of body weight or failure to gain weight thus occur in haemonchosis (Dargie, 1974). This could arise from either inappetence or other metabolic disturbances. Using a system of pair-feeding he showed that inappetence was of no major importance in this disease. Similarly, evidence for reduced digestibility of nitrogen contributing

to/

to the weight differences between infected and pair-fed controls has been inconclusive. This is because the reduced digestibilities observed were more or less accounted for by the passage of endogenous nitrogen into the gut and there was no indication that this nitrogen was not ultimately processed. He thus concluded that poor digestion or malabsorption was not associated with haemonchosis. The weight difference was attributed solely to the greatly accelerated rate of protein synthesis and the attendant shift in amino acid metabolism from tissues such as muscle into organs such as liver and bone marrow which are involved in synthesis of essential proteins. If this mechanism truly operates in haemonchosis, then the quality and quantity of the host diet will be of particular importance, since the more nutrients available from the diet the less the animal will have to release from its body reserves. A nutritional balance and digestibility study was consequently carried out to determine if nutritional plane affected the nitrogen balance or digestibility of various food constituents at a time when the sheep were harbouring patent infections of H. contortus.

A further aim of the work described in this thesis was to examine the influence of dietary protein intake on the pathophysiological changes associated with the self-cure phenomenon. Larval challenge in animals carrying patent infections of H. contortus has been shown to cause a dramatic fall in egg production and expulsion of the previously acquired infection due to a local hypersensitivity reaction in the abomasum initiated by the newly acquired larvae (Chapter I). There are indications that natural self-cure is not immunogenic in origin but rather caused by a possible anthelmintic factor in freshly growing grass (Allonby and Urquhart, 1973).

Information/

Information is currently available both on the functional changes in the worm which accompany expulsion, particularly as regards egg output and blood sucking activities, as well as on the patho-physiological changes in the host associated with the phenomenon (Dargie and Allonby, 1975; Dargie, 1974) - (Chapters I and III).

Despite this detailed information only in a few instances has the nutritional status of the host been linked with the manifestation of self-cure. Preston and Allonby (1978) concluded that animals on a high protein diet self-cured more effectively than those on the low protein diet indicative of a better immune response in the former. Effectiveness of self-cure was based solely on faecal egg counts (eggs/gram of faeces) and no worm recoveries were made nor any pathophysiological alterations monitored. Attempts were made to examine these aspects in the present experiment.

The influence of nutrition on the pathophysiology of acute haemonchosis and the self-cure phenomenon was examined by offering two groups of sheep diets of different crude protein content. The course of the infection in each group was followed using parasitological, haematological and radioisotopic parameters. Red cells labelled with ^{51}Cr were used to study the alterations in red cell kinetics following primary and challenge infections. Changes in the iron kinetics and hence erythroid marrow activity were monitored with the help of plasma labelled radioiron (^{59}Fe), while albumin trace labelled with ^{125}I was used to follow the changes in albumin metabolism in both parasitised and pair-fed control sheep. A nutritional balance and digestibility trial was also carried out to detect any adverse effect of protein intake on the nitrogen status and digestibility of various nutrients by the host. For ease of presentation the work is divided into sections, each embracing materials and methods and results, followed by a general discussion.

GENERAL MATERIALS AND METHODS.

I - Experimental sheep:

(a) Rearing and maintenance before experiments:

Twelve Scottish Blackface wethers aged 9-12 months were purchased commercially and drenched with a 2.5 per cent suspensions of benzimidazole (Panacur-Hoechst Pharmaceuticals) at the rate of 1 ml./kg. body weight to remove gastrointestinal worms. The treatment was repeated four weeks later. Their parasite-free status was checked by regular faecal examinations.

The animals were housed in covered pens with concrete floor and straw bedding. For two weeks after purchase they were fed on ad libitum hay and pelleted concentrate. Subsequently they were divided into two groups of six animals each, kept in two adjacent pens and introduced to the special compound diet. Group A received the high protein diet which consisted of sugar beet pulp, siftings, soya bean meal and mineral and vitamin mixture. Group B received the low protein diet made up of the same ingredients as the former but without the soya bean meal. These diets were fed twice daily for a further two weeks before the animals were moved to the metabolism cages.

(b) Maintenance of animals during experiments:

The sheep were confined in standard metabolism cages throughout the duration of the experiment having been moved to these cages a week before the commencement of the experiment. The special high or low protein diets were offered to the appropriate animals of each group. Each sheep was fitted with a faecal collection bag lined with a polythene bag which allowed complete and separate collections of faeces and urine. The urine passed through the grid floor of the cage and was collected in a bucket placed behind the cage. Each infected sheep was paired on a body weight and haemoglobin type basis to an uninfected control and thereafter/

thereafter the latter was offered same amount of food as that eaten by its partner the previous day. This pair-feeding lasted throughout the first part of the experiment and the first week of the second part, after which the animals were fed their respective diets to appetite. Six litres of water was offered to each sheep daily.

II - Experimental protocol:

The sheep were used in two separate but comparable experiments consisting of a primary infection and a challenge infection.

During the five weeks before the animals were moved into the metabolism cages the sheep were weighed weekly, usually in mid-morning, using an Avery-Spring balance pig weigher. Blood for haematological and biochemical analyses was also collected weekly usually before weighing. These parameters (weight, packed cell volume and haemoglobin type) enabled the division of the animals into the two groups A and B.

For the experimental period the sheep were confined in standard metabolism cages and fed their respective high or low protein diets. On day 1 of the experiment three sheep in each group were individually infected with 350 3rd stage larvae of H. contortus per kg. body weight. The other three served as worm-free controls and were allotted on a body weight, PCV and Hb type basis to the three infected sheep of each group. All the six animals in each group were injected with ^{51}Cr-labelled red cells and ^{59}Fe on the same day and later that day infected with H.contortus larvae. Injections of ^{125}I-labelled albumin were unavoidably delayed until three days later. Pair-feeding commenced a week earlier and was maintained throughout this part of the experiment. To ensure the rapid excretion of iodine from degraded albumin each sheep was dosed with 10 ml. of 0.75 per cent solution of potassium iodide beginning four days before the injection of labelled substances and continued throughout the duration of the experiment. Red cell, iron and albumin kinetics were measured during the following 40 days. The daily feed and water intakes, urine/

urine and faecal outputs were recorded. A 7-day nutritional balance study was carried out between days 31 and 36 which entailed special treatments of the daily urine and faecal collections as described in Section D.

For the challenge infection on day 42, each infected sheep of both groups was again reinfected with the same number of infective larvae (350/kg. body weight) in an attempt to induce self-cure. The various measurements were continued for a further 28 days except that pair-feeding was discontinued on day 49 and the animals fed their respective diets to appetite. Intakes were recorded. The animals were necropsied at the end of the experiment and worm burdens determined.

Blood samples for haematological and biochemical analyses were collected once weekly on Mondays before the morning feeding throughout the duration of the experiment. The animals were weighed after the bleeding. Blood for radioisotopic measurements were collected daily as described below. Faecal egg counts (total output) were determined once weekly following primary infection and thrice weekly after the challenge infection.

III - Injection of radioisotopes and preparation of samples:

Each sheep received approximately 500 µCi (^{125}I), 167 µCi (^{59}Fe) and 833 µCi (^{51}Cr) on each of two occasions. Injections of isotopically labelled substances were made into the jugular vein via a jugular catheter (Portex Plastics Ltd., Hythe, England) connected to a three-way tap. The catheter was flushed out with isotonic saline before being withdrawn. The syringes were weighed prior to and after injections for determination of amounts of isotope injected.

The/

The first blood sample was taken 10 minutes after injection from the opposite vein and other samples at 30 60, 90, 120 and 180 minutes. They were subsequently bled twice daily for the next three days and thereafter once daily for the duration of the experiment. 5 ml. of blood was withdrawn into heparinised tubes at each bleeding. Daily urinary and faecal outputs were collected and recorded.

1 ml. samples of whole blood and plasma were pipetted into counting tubes and made up to a volume of 10 ml. with 0.02 N NaOH. A 10 ml. aliquot of the daily urine output was taken for radioassay. Each 24-hour faecal collection was weighed, mixed thoroughly inside the polythene bag and random duplicate 10 g. samples taken and packed to a volume of 10 ml. in counting tubes.

1 ml. of each of the isotopically labelled preparations was weighed in a syringe and the contents emptied into a 100 ml. volumetric flask. The syringe was re-weighed. The contents of the flask were made up to the mark with 0.02 N NaOH. 1 ml. of this was drawn into counting tubes and made up to 10 ml. with 0.02 N NaOH. These standards were made up for each sheep except the ^{125}I standard. The standards served as corrections against decay, changes in the sensitivity of the counting equipment and for calculations of injected dose.

Count rates of the three isotopes in the samples were determined simultaneously as described in Section C.

IV - Statistical methods:

The statistical methods employed were those described by Snedecor (1956) and Bishop (1966). The exponential phase of the radioactivity disappearance curves were calculated by linear regression analysis/

analysis and all radioactivity curves were drawn by such regressions. A correlation coefficient $r > 0.95$ was accepted.

Deviations from mean values were expressed as standard errors (SE) of the means. The 't' values were considered significant at $p \leq 0.05$.

SECTION A

CLINICAL AND PARASITOLOGICAL OBSERVATIONS

SECTION A - CLINICAL AND PARASITOLOGICAL OBSERVATIONS.

A. MATERIALS AND METHODS.

I - Preparation and administration of infective larvae:

Infective third stage larvae of H. contortus were kindly supplied by the Wellcome Laboratories for Experimental Parasitology, University of Glasgow. These larvae were recovered from cultures of faeces from sheep with patent infections of H. contortus. The concentration of the larval suspension was determined by examining forty 0.025 ml. aliquots, with thorough mixing during sampling. Doses for each sheep (350/kg. body weight) were measured by pipetting the calculated volumes of the original larval suspension into narrow-necked universal bottles. An error of ± 10% was usually envisaged.

The larvae were administered to each animal by oral drenching with the universal bottles. Each inoculum was followed by a drench of water to ensure swallowing of infective dose.

II - Faecal egg counts:

Faecal egg determinations were performed using a modification of the McMaster technique as suggested by Whitlock (1948). Samples were taken per rectum or from the faecal bags, from which three grams were weighed and broken up in 42 ml. of water using a homogeniser. This was poured through a 100 mesh sieve to remove the coarse debris while the filtrate was collected, agitated and then used to fill a 15 ml. flat bottomed test tube. The tube was centrifuged at 2,000 r.p.m. for two minutes, the supernatant poured off and the sediment agitated with a whirlmixer. Saturated sodium chloride was then used to top up the tube to the previous level and the tube inverted six times so that the rising air bubbles could mix the solution. This was pipetted rapidly into both chambers of a McMaster slide; the slide examined under the microscope and the number of eggs counted in the two chambers multiplied by 50 to give the number of eggs per gram of faeces.

III/

III - Necropsy procedure and assessment of worm burdens:

All sheep to be necropsied were starved for 24 hours prior to slaughter. They were killed by rapid intravenous injection of Pentobarbitone sodium (Euthatal, May & Baker, Dagenham, Essex). A ventral midline incision was made and the alimentary tract removed from the body cavity. The abomasal/duodenal junction was quickly ligatured to prevent transfer of parasites from one site to another.

Estimations of worm burdens were performed according to the techniques of Ritchie et al (1966). The abomasal contents were washed gently under slow running water into a bucket and the contents made up to 2 litres. This was thoroughly mixed and two 200 ml. samples taken into jars. 10 x 4 ml. aliquots of the mixed suspension were counted in a lined petri dish after iodine staining and decolorisation with 5% sodium thiosulphate. The average count was multiplied by 50 (dilution factor) to give an estimate of the worm burden.

The abomasum was divided transversely at the pyloric-fundic region and the mucosa scrapped off and digested in a pepsin-HCl mixture for 6 hours at 42°C. The digested material was formalised, made up to 2 litres and duplicate 200 ml. aliquots again counted using a stereoscopic microscope (x 12 objective) as described for abomasal contents. The two counts were added to give the total worm burden. Worms were classified as adults or developing stages.

B. RESULTS.

I - Clinical observations:

The main clinical sign observed in both groups of infected sheep was pallor of the visible mucous membranes. Only in one animal on the low protein diet was there evidence of facial and submandibular oedema. All infected animals maintained good appetites throughout both primary and challenge infections and no weight losses were recorded. However,

infected/

infected animals on the high protein diet gained more weight than those on the low protein diet. This difference was more pronounced during the primary infection but was still noticeable following the challenge infection when all the sheep were fed to appetite. On an average infected sheep on low protein diet gained 8.5 kg. during the duration of the experiment, while those on the high protein gained 11.0 kg. Control sheep on the low protein diet gained 10 kg. during the duration of the experiment, while those on high protein gained 12.8 kg. Weight gains between infected and control sheep on either diet were not significantly different though noticeably higher in the latter (Fig. 1).

II - Parasitological data:

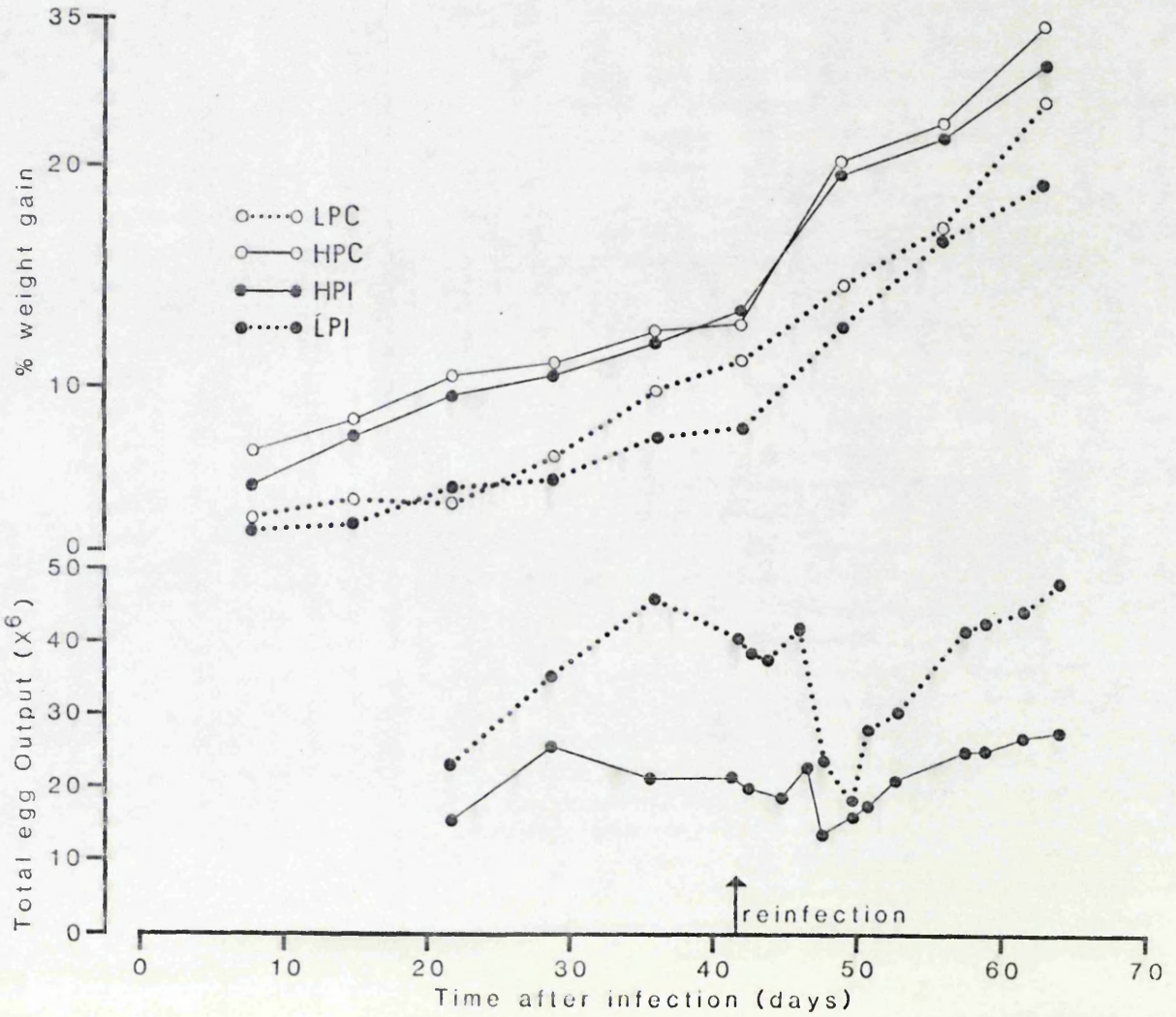
(a) Primary Infections: The mean faecal worm egg output of each group is depicted in Fig. I from which it can be observed that the general pattern was similar in both groups. Eggs were first detected during the third week after the administration of larvae and increased substantially over the next two weeks to reach a level which was more or less maintained until reinfection on day 42. Egg output was clearly higher in sheep on the low protein diet than those on the high protein diet. For example, mean egg output was 42 million per day by two weeks following patency in the former group and only 21 million/day in the latter. These differences were significant ($P < 0.05$). The faeces of the worm-free controls remained negative.

(b) Challenge Infections: Although some noticeable drop in egg output occurred within a week of reinfection in both dietary groups, the percentage drop was not significant. Over the next two weeks egg output returned to pre-challenge levels and increased steadily until the termination of the experiment.

(c)/

Fig. 1: Mean faecal egg counts and percentage body weight gains of sheep infected with H. contortus (•) and normal animals (o) maintained on low protein (LP -----) and high protein (HP ———) diets.

Fig. 1


35
20
10
0
% weight gain
LPC
HPC
HPI
LPI
50
40
30
20
10
0
Total egg Output (X6)
reinfection
0
10
20
30
40
50
60
70
Time after infection (days)

(c) Worm Burdens: The worm burdens of individual sheep determined at the end of the experiment are presented in Table II. No significant differences in worm burdens occurred between the two dietary groups - mean for the high protein group being 5,033 ± 605 and the low protein group 5,133 ± 586. A small proportion of immature adults (L5) was recovered from one sheep (R95) on the high protein diet. No significant alteration of the male:female ratio was noticed.

TABLE II.

The worm burdens of sheep maintained on high and low protein diets after primary and challenge infections with 350 larvae/kg. of *H. contortus*.

Diet	No. of sheep	Worm Burdens			
		Immature (L5)	Adults	Total	Male: Female.
Low Protein	Y75	-	5,800	5,800	1.32
	Y76	-	4,700	4,700	0.94
	Y81	-	4,900	4,900	0.69
Mean + SE				5,133 ± 586	0.98 ± 0.18
High Protein	R82	-	5,250	5,250	0.86
	R92	-	4,350	4,350	0.78
	R95	700	4,800	5,500	0.78
Mean + SE				5,033 ± 605	0.81 ± 0.03

SECTION B - HAEMATOLOGICAL AND BIOCHEMICAL OBSERVATIONS.

B. MATERIALS AND METHODS.

I - Collection and storage of samples:

All blood samples were taken from the jugular vein. Samples for radioisotopic and daily packed cell volume determinations were collected into evacuated 5 ml. glass tubes (Vacutainer, Becton and Dickinson and Co., New Jersey, U.S.A.).containing 100 international units of heparin as anti-coagulant. Those for the weekly haematological analysis were collected into 5 ml. Monovette tubes (Sarstedt, Monovette, Leicester, England) containing ethylene diamino tetracetic acid (EDTA) as anti-coagulant. For analyses involving serum, blood was collected into 10 ml. evacuated tubes, allowed to clot and left standing overnight at room temperature with the tubes inverted. The serum was transferred by means of a Pasteur pipette into plastic vials which were immediately stored at $-5^{\circ}C$.

II - Red cell count (RBC), Packed cell volume (PCV), Mean cell volume (MCV) and Haemoglobin concentration (Hb):

The total red cell counts (x $10^6/mm^3$), percentage PCV, mean cell volume (μ^3) and haemoglobin concentration (g.%) were determined using an electronic particle counter and Coulter haemoglobinometer respectively (Coulter Model ZF, Coulter Electronics Ltd., Harpenden, Herts., England). The PCVs of the daily blood samples were determined by the capillary microhaematocrit method. Capillary tubes containing the blood samples were sealed at one end by plasticine, centrifuged for 5 minutes in a microhaematocrit centrifuge (Hawksley & Sons Ltd., London, England) and the percentage PCV read from a scale on a Hawksley micro-haematocrit reader.

III/

III - Mean cell haemoglobin concentration (MCHC) and mean cell haemoglobin (MCH):

These indices were calculated as follows:-
globin (MCH

$$MCHC = \frac{Hb \times 100}{PCV}$$

$$MCH = \frac{Hb \times 10}{RBC}$$

IV - Haemoglobin typing:

Haemoglobin typing was carried out by electrophoresis of haemolysed red cells on cellulose acetate strips (Smithers, 1955).

V - Serum iron:

Serum iron was determined using a commercial test kit (Roche Diagnostics, RochеProducts Ltd., London). Serum obtained from clotted blood collected in iron-free tubes was first treated with an anionic detergent (Teepol in acetate buffer pH 5.8) to split the Fe^{3+} transferrin complex and thereafter with sodium dithionite to reduce free Fe^{3+} to Fe^{2+}. One drop of bathophenanthroline disulphate was added to give a reddish pink complex, the colour intensity of which was read on a spectrophotometer at 546 mμ. Serum iron concentration was calculated by reference to the colour intensity of a standard solution treated as above.

VI - Serum proteins:

Total serum proteins were estimated by a biuret colorimetric method (Weischselbaum, 1946) and albumin by the bromocresol green technique of Rodkey (1965). Serum globulins were calculated as the difference between total proteins and albumin concentrations.

VII - Serum pepsinogen:

Serum pepsinogen concentrations were estimated according to the methods of Edwards et al (1960).

B. RESULTS./

B. RESULTS.

Haematological and biochemical results from the infected sheep and their worm-free counterparts are illustrated in Figures 2 and 3. For clarity and ease of presentation the interrelationship of the various parameters determined and the features of the anaemia are described separately for the initial and challenge infections.

Haematological observations:

(a) Primary Infection:

The packed cell volume (PCV) of all infected sheep and their controls were comparable before the infection, although these were relatively higher in animals on the high protein diet. The most marked haematological disturbances occurred at about the same time in both groups, i.e. during the 2nd - 4th week after infection, when PCVs of all infected animals fell significantly. The fall in PCV was more pronounced in the low protein group (from 33% to 17% compared to 34%-22% in the high protein group). Sheep on either diet subsequently maintained their PCV at this low level until reinfection.

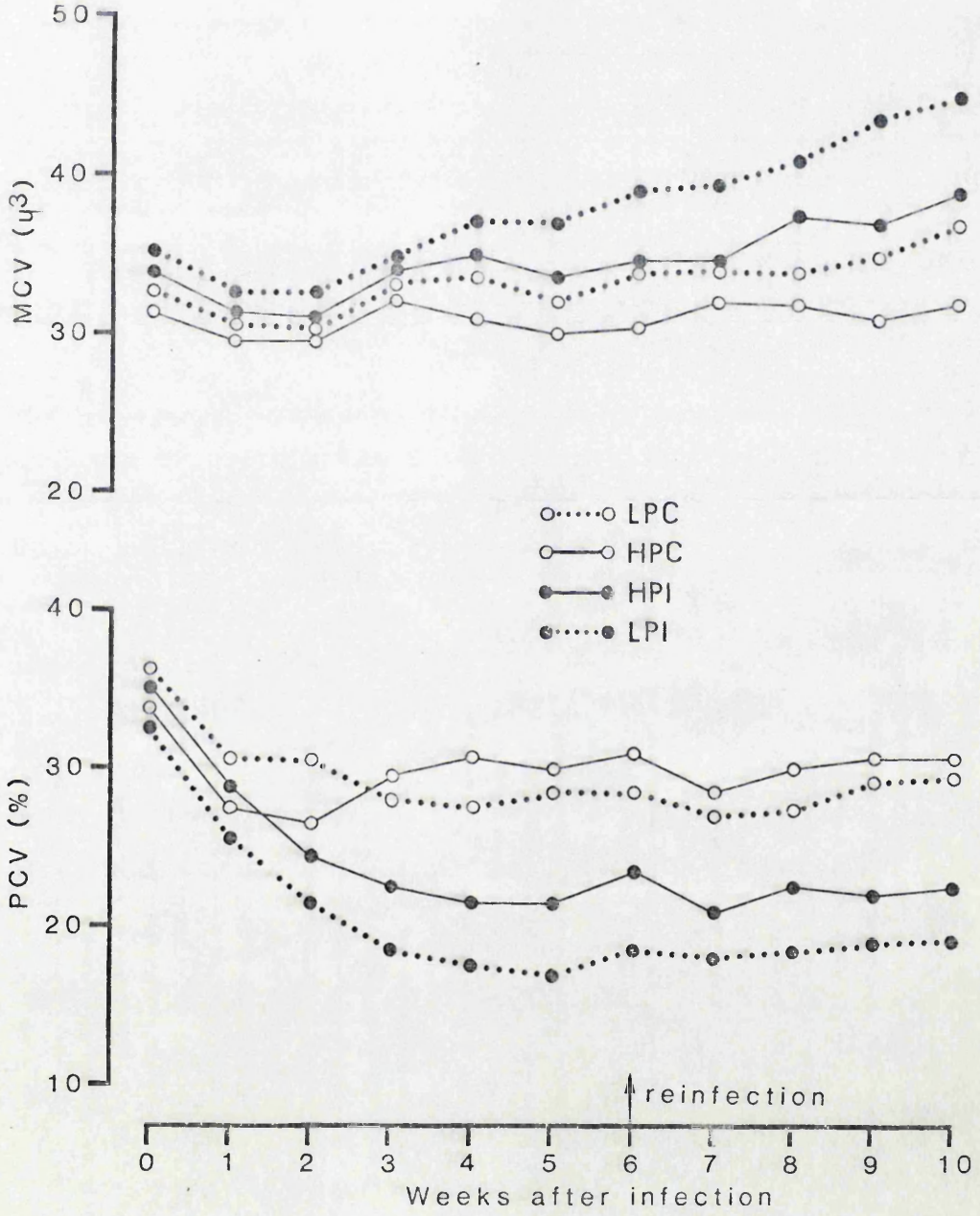
The trends observed with regard to total red cell counts and haemoglobin concentration was generally similar to those described for PCV but differences were observed which were reflected in alterations in erythrocyte size (MCV) in animals on the low protein diet. The MCV was steady during the early phase of the infection in both dietary groups but from about the 3rd week onwards macrocytosis became progressively dominant in the low protein group. The MCV of the high protein group was maintained within normal values during the primary infection. Mean values for the two groups of control sheep were similar throughout this period although those on low protein tended to have higher values. While MCVs tended to decline in the control sheep, the MCHC remained relatively constant.

(b) Secondary Infection:

Reinfection/

Fig. 2: Haematological indices of sheep infected with H. contortus (•) and normal animals (o) maintained on low protein (LP ----) and high protein (HP ——) diets.

Fig. 2


50
40
30
20
MCV (μ3)
LPC
HPC
HPI
LPI
40
30
20
10
PCV (%)
reinfection
0
1
2
3
4
5
6
7
8
9
10
Weeks after infection

Reinfection had no noticeable effect on the PCV of the sheep on either diet. The values were maintained more or less at pre-challenge values, the high protein group maintaining higher values (22%) than the low protein group (17%). Similar trends were observed in total red cell counts, Hb concentration and the main differences were again reflected in the MCV values. The macrocytosis observed in the low protein group before challenge was maintained, albeit at an elevated level, until the end of the experiment. Small increases in MCV were noted in animals on the high protein diet during the latter part of the challenge infection (last two weeks). There was no change in the haematological picture of the control sheep on either diet from that described for the primary infection period. MCHC values deviated little from control values in the infected sheep throughout the primary and challenge infections.

Biochemical observations:

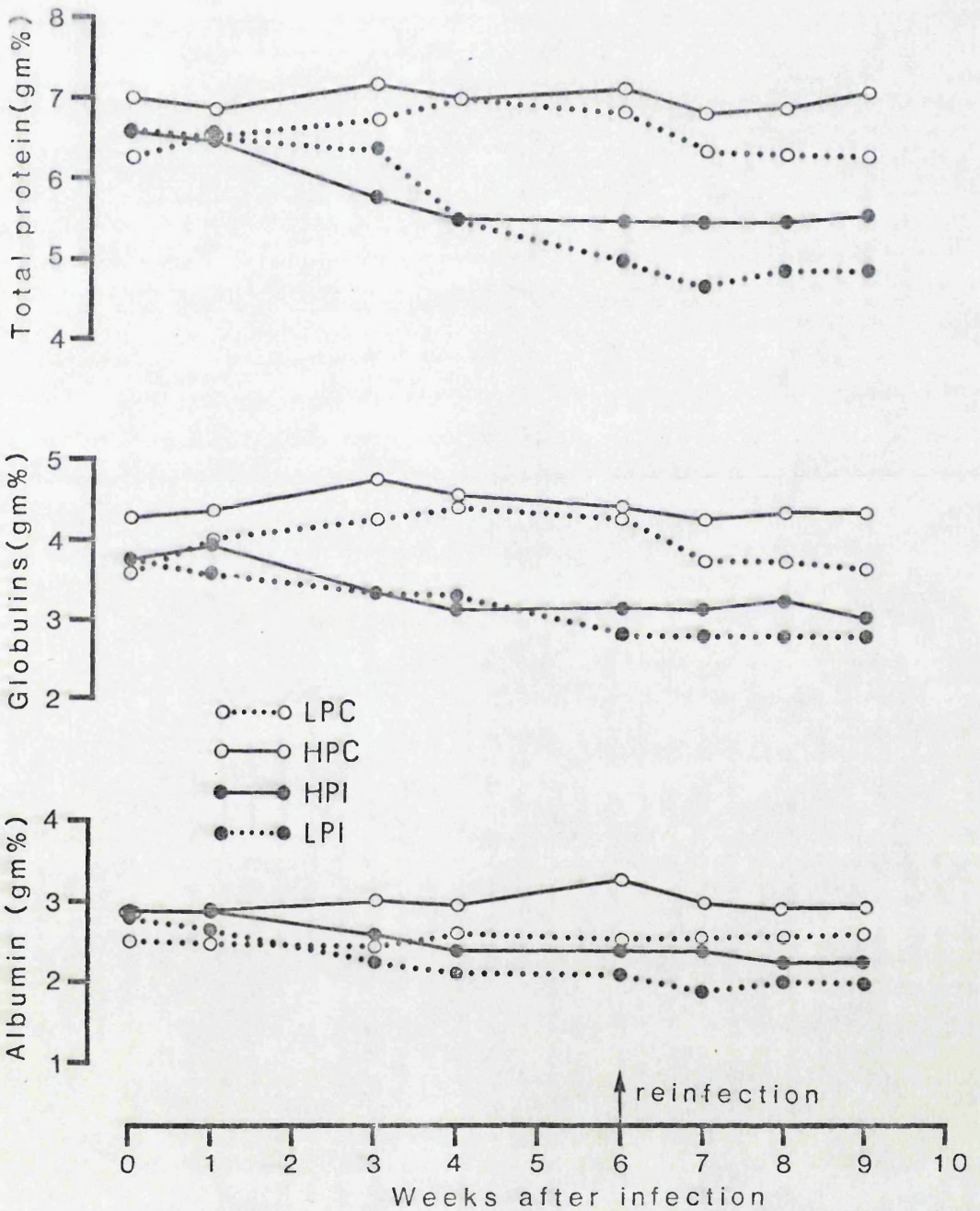
The mean results of the serum protein estimations are illustrated in Figure 3. Both groups of infected animal exhibited similar responses but of varying magnitude. Total serum proteins of the low protein group increased slightly in the week following infection but subsequently decreased to less than 5 g.% by reinfection on week 6. This level was maintained throughout the course of the challenge infection. Sheep given the high protein ration also developed hypoproteinaemia during the latter stages of the infection but this was less rapid and well maintained at 5.4g.% throughout the duration of the experiment. Control values were well maintained throughout but were consistently higher in the high protein group.

Serum albumin concentration followed a downward trend in all infected animals but was more pronounced in animals on the low protein ration.

Serum/

Fig. 3: Serum protein concentrations in sheep infected with *H. contortus* (•) and normal animals (o) maintained on low protein (LP ----) and high protein (HP ——) diets.

Fig. 3


Total protein(gm%)
8
7
6
5
4
Globulins(gm%)
5
4
3
2
LPC
HPC
HPI
LPI
Albumin (gm%)
4
3
2
1
reinfection
0
1
2
3
4
5
6
7
8
9
10
Weeks after infection

Serum globulin levels were reasonably well maintained throughout the infection in all infected animals at about 3g.% and there was little change in albumin:globulin ratios.

Serum iron concentrations were similar in all animals at the outset (161-175 µg%) and surprisingly changed little during the course of both the primary and challenge infections in both groups (Table III). However, those on the low protein diet experienced detectable falls in serum concentrations between the 3rd week of infection until reinfection (164 - 135 µg%) but the fall was not significant.

Serum pepsinogen levels were elevated throughout the course of the primary and challenge infections in both groups but particularly more so in animals on low protein diet. Differences between the two groups were not significant.

TABLE III MEAN IRON CONCENTRATION (μg%) AND SERUM PEPSINOGEN LEVELS (i.u.) IN *H. CONTORTUS* INFECTED AND CONTROL SHEEP ON HIGH AND LOW PROTEIN DIETS.

	Week No.							
	0	1	3	4	6	7	8	9
LOW PROTEIN:								
Serum Iron:								
Infected	161.7 ± 23	164.8 ± 21	145.6 ± 21	144.5 ± 11	135.1 ± 6	138.9 ± 23	129.6 ± 18	119.5 ± 19
Control	175.6 ± 16	148.3 ± 36	140.9 ± 30	157.6 ± 30	177.0 ± 24	167.0 ± 14	177.6 ± 9	181.6 ± 20
Serum Pepsinogen:								
Infected	300 ± 0.3	623 ± 1.2	778 ± 1.3	828 ± 1.0	813 ± 1.1	890 ± 0.9	800 ± 1.2	830 ± 0.8
Control	307 ± 0.01	320 ± 0.03	321 ± 0.4	303 ± 0.1	220 ± 0.5	143 ± 2.3	156 ± 3.6	260 ± 1.3
HIGH PROTEIN:								
Serum Iron:								
Infected	162.4 ± 23	169.8 ± 34	180.5 ± 27	194.3 ± 32	167.3 ± 24	163.0 ± 32	155.1 ± 27	164.5 ± 24
Control	160.9 ± 24	190.4 ± 27	177.4 ± 21	166.4 ± 25	178.3 ± 18	165.5 ± 5	181.6 ± 10	192.8 ± 6
Serum Pepsinogen:								
Infected	373 ± 0.02	610 ± 0.8	512 ± 1.2	723 ± 0.9	597 ± 1.3	656 ± 0.6	623 ± 2.1	601 ± 2.0
Control	321 ± 0.01	306 ± 0.01	373 ± 0.1	312 ± 0.1	310 ± 0.3	323 ± 1.0	159 ± 2.1	180 ± 2.4

SECTION C

RED CELL TURNOVER STUDIES

SECTION C - RED CELL TURNOVER STUDIES.

C. MATERIALS AND METHODS.

I - Labelling of red cells with ^{51}Cr:

Erythrocytes were labelled according to the method of Gray and Sterling (1950) as described in Chapter III. Approximately 10 ml. of $Na_2\ ^{51}CrO_4$ in isotonic saline obtained from the Radiochemical Centre, Amersham (specific activity 1mc/ml, chromium content 5.4 µg/ml) was divided among the twelve blood samples with gentle mixing, each sheep thus received approximately 833 µCi of ^{51}Cr.

II - Injection and sampling:

On each of the two occasions each sheep received approximately 833 µCi of ^{51}Cr which was injected intravenously using a jugular catheter. 5 ml. blood samples were taken from the opposite jugular vein at regular intervals and prepared for radioactivity measurements as described under General Materials and Methods.

III - Radioactivity measurements:

Count rates of the three isotopes in the blood, plasma, urine and faeces were determined simultaneously in an automatic well-type scintillation spectrometer (Packard Tri-carb Liquid Scintillation Spectrometer). The calculations of overlap factors were based on the relative count rates of standard solutions of these isotopes at each photopeak.

IV - Calculation and expression of results:

(1) Counts/ml/red blood cell: The radioactivity of each blood sample was corrected for background radioactivity and physical decay and expressed as counts/minute/ml of red cells using the PCV value of each sample, i.e.

$$\text{Counts/min/ml RBC} = \frac{\text{Counts/min/ml blood} \times 100}{\text{PCV}}$$

(2)/

(2) Apparent 'half-life' ($t_{\frac{1}{2}}$): This was calculated from the linear phase of the curve obtained from a semi-logarithmic plot of counts against time as the time taken for the red cell radioactivity to fall by 50%. The half-life was determined following each injection of radioisotope.

(3) Red cell volumes, Blood volumes: Red cell volumes and hence blood volumes were calculated by using the dilution principle. Since this calculation involves the packed cell volume, values obtained are not entirely quantitative as the venous haematocrit may slightly overestimate the mean body haematocrit.

(4) Gastrointestinal blood and red cell losses: Since ^{51}Cr remains firmly attached to red cells and is not reabsorbed from the intestine of man and animals (Owen et al, 1954; Ebaugh et al, 1958), it can be used quantitatively to estimate gastrointestinal blood and red cell losses and these are expressed as faecal 'clearances'. These 'clearance' values are obtained by dividing the total radioactivity in each 24-hour collection of faeces by the activity per ml of whole blood and of red cells respectively at the beginning of the collection period.

Similar clearance values can be worked out for the urine. In this case ^{51}Cr lost by elution from tagged cells will contribute substantially to the urine activity.

(5) Iron lost into the gut: Iron lost into the gut was estimated from the formula of Roche, Perez-Giminez and Levy (1957) on the assumption that there is no significant reabsorption of haemoglobin iron in ruminants.

$$\text{Iron lost (mg)} = \frac{\text{Hb(g/100ml)} \times 3.34 \times {}^{51}\text{Cr blood clearance}}{100}$$

C. RESULTS/

C. RESULTS.

In infected animals turnover calculations were no longer valid after the fourth week following the initial infection due to the blood counts having fallen below the level of practical significance (less than twice background). This problem was also encountered following the challenge infection at variable periods in each sheep. For ease of presentation of the results, mean results are given (Figs. 4 and 5).

(a) Blood volumes:

The results of the blood volume measurements determined at infection and repeated at reinfection (42 days later) are given in Table IV.

There were marked reductions in red cell volume of both infected groups compared with their pair-fed partners. At infection the high protein group had higher red cell volumes than their low protein counterparts but the differences were not significant when expressed on body weight basis and possibly reflect the superior weight gains of the former group.

When the measurements were repeated six weeks later, all infected animals had lower red cell volumes with little or no change in plasma volumes. In line with the PCV changes, animals on the low protein diet had slightly greater falls in red cell volume (31.8%) compared to their high protein partners (28.9%) but blood volume changes were more remarkable, the former suffering reductions of 17.3% and the latter 7.8% The fall in red cell volumes generally agreed closely with reductions in blood volume, implying that plasma volumes were little affected by the disease. Figures for the controls were generally steady in the low protein group but the high protein group had improved red cell volumes due possibly to their superior growth rate.

(b)/

TABLE IV MEAN BLOOD VOLUMES IN H. CONTORTUS INFECTED AND CONTROL SHEEP ON HIGH AND LOW PROTEIN DIETS FOLLOWING PRIMARY AND CHALLENGE INFECTIONS.

Diet	DAY 0			DAY 42			% decrease in	
	Red cell volume (ml/kg)	Plasma volume* (ml/kg)	Blood volume (ml/kg)	Red cell volume (ml/kg)	Plasma volume* (ml/kg)	Blood volume (ml/kg)	Blood volume	Red cell volume
Low Protein								
INFECTED	17.9 ± 0.8	50.7 ± 0.3	68.6 ± 0.8	12.5 ± 0.2	46.4 ± 3.9	58.9 ± 3.7	16.9	30.2
CONTROL	19.8 ± 1.9	48.9 ± 5.9	69.5 ± 4.5	17.7 ± 1.9	44.7 ± 5.6	62.5 ± 4.0	10.1	10.6
High Protein								
INFECTED	19.7 ± 0.1	54.3 ± 1.1	74.1 ± 1.1	14.1 ± 0.6	53.3 ± 0.4	67.4 ± 0.2	9.0	28.4
CONTROL	18.0 ± 4.4	42.9 ± 2.3	60.8 ± 2.0	20.6 ± 1.8	44.3 ± 4.5	64.8 ± 2.8	-	-

* Obtained from ^{125}I-albumin data (Section E).

(b) Red cell survival:

Red cell survival was estimated from the circulating half-life ($t_{\frac{1}{2}}$) of ^{51}Cr-labelled erythrocytes during two stages of the investigation - following primary infection and for four weeks after the challenge infection. The results are presented in Table V and Figs. 4 and 5.

Differences were noticed during both periods. Following initial infection, red cell survival was lower in both infected groups relative to the controls but more so in the low protein group. Mean red cell survival was 6 days in the low protein group and averaged 8 days in the high protein group. These differences were more marked following reinfection with the low protein group averaging 4.4 days and 7.4 days in the high protein group. Labelled cells thus survived longer in the circulation of the high protein group following both primary and challenge infections. Values for the control sheep were similar in both groups during the two stages of the investigation, i.e. 12 days.

(c) Gastrointestinal red cell losses:

Gastrointestinal red cell losses were estimated during the six weeks following the initial infection and for four weeks following the challenge infection by relating the daily faecal ^{51}Cr radioactivity to that present in the red cells. Fig.6 shows the average losses recorded and gives an insight into the pattern and extent of bleeding suffered by each group.

Significant haemorrhage into the gut was first detected in both groups following the second week of infection and increased progressively over the next three weeks to reach a peak which was generally maintained until reinfection. Losses were greater in animals on low protein which lost an average of 42 ml. of red cells between the 2nd and 5th weeks of infection compared to 27 ml. in the high protein group within the same period. Cumulative red cell losses into the gastrointestinal tract were considerably in excess of the fall in circulating red cell volume. For example,/

TABLE V MEAN ^{51}Cr RED CELL SURVIVAL IN *H. CONTORTUS* INFECTED AND CONTROL SHEEP MAINTAINED ON HIGH AND LOW PROTEIN DIETS.

	$t_{\frac{1}{2}}$ (days)	
	Primary Infection	Challenge Infection
Low Protein:		
Infected	6.0 ± 0.3	4.4 ± 0.4
Control	12.8 ± 1.3	11.5 ± 0.3
High Protein:		
Infected	8.0 ± 0.3	7.4 ± 0.5
Control	13.7 ± 0.7	12.3 ± 0.7

Fig. 4: ^{51}Cr-red cell survival in sheep following primary infection with H. contortus (•) and normal animals (o) maintained on low protein (LP ----) and high protein (HP ——) diets.

Fig. 4

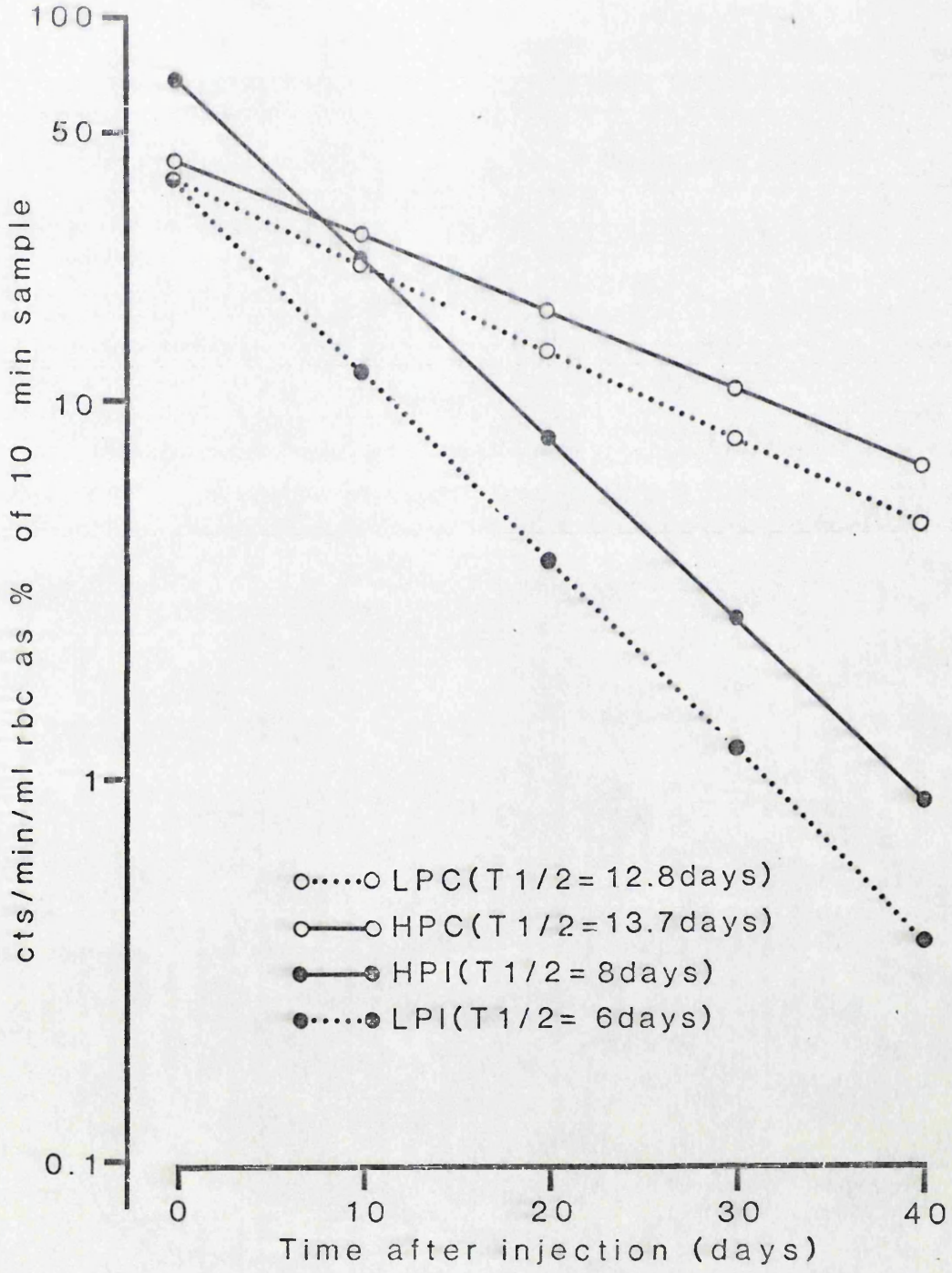

100
50
10
1
0.1
cts/min/ml rbc as % of 10 min sample
LPC(T1/2=12.8days)
HPC(T1/2=13.7days)
HPI(T1/2=8days)
LPI(T1/2= 6days)
0
10
20
30
40
Time after injection (days)

Fig. 5: ^{51}Cr-red cell survival in sheep following a challenge infection with H. contortus (•) and normal animals (o) maintained on low protein (LP ----) and high protein (HP ——) diets.

Fig.5

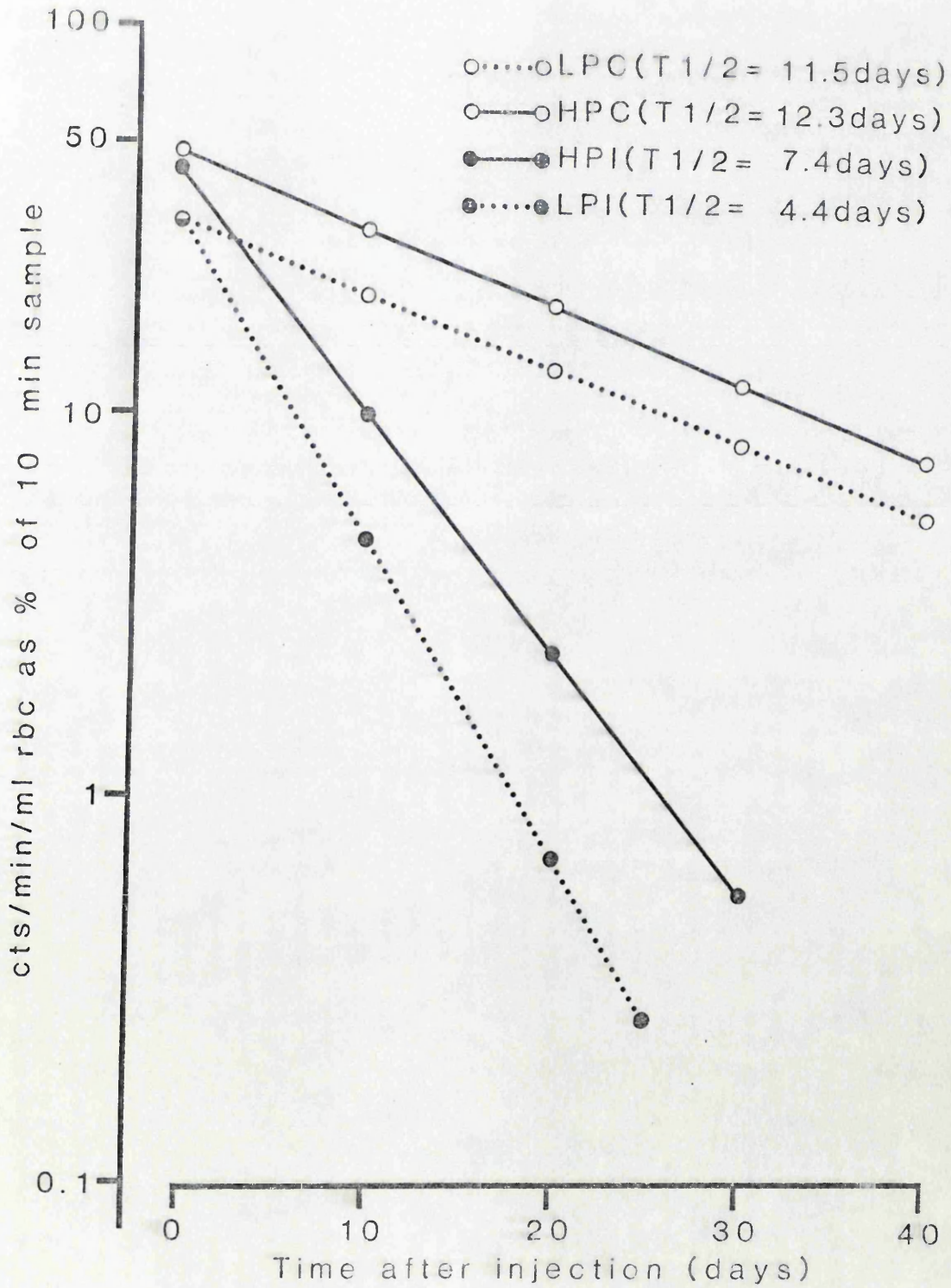
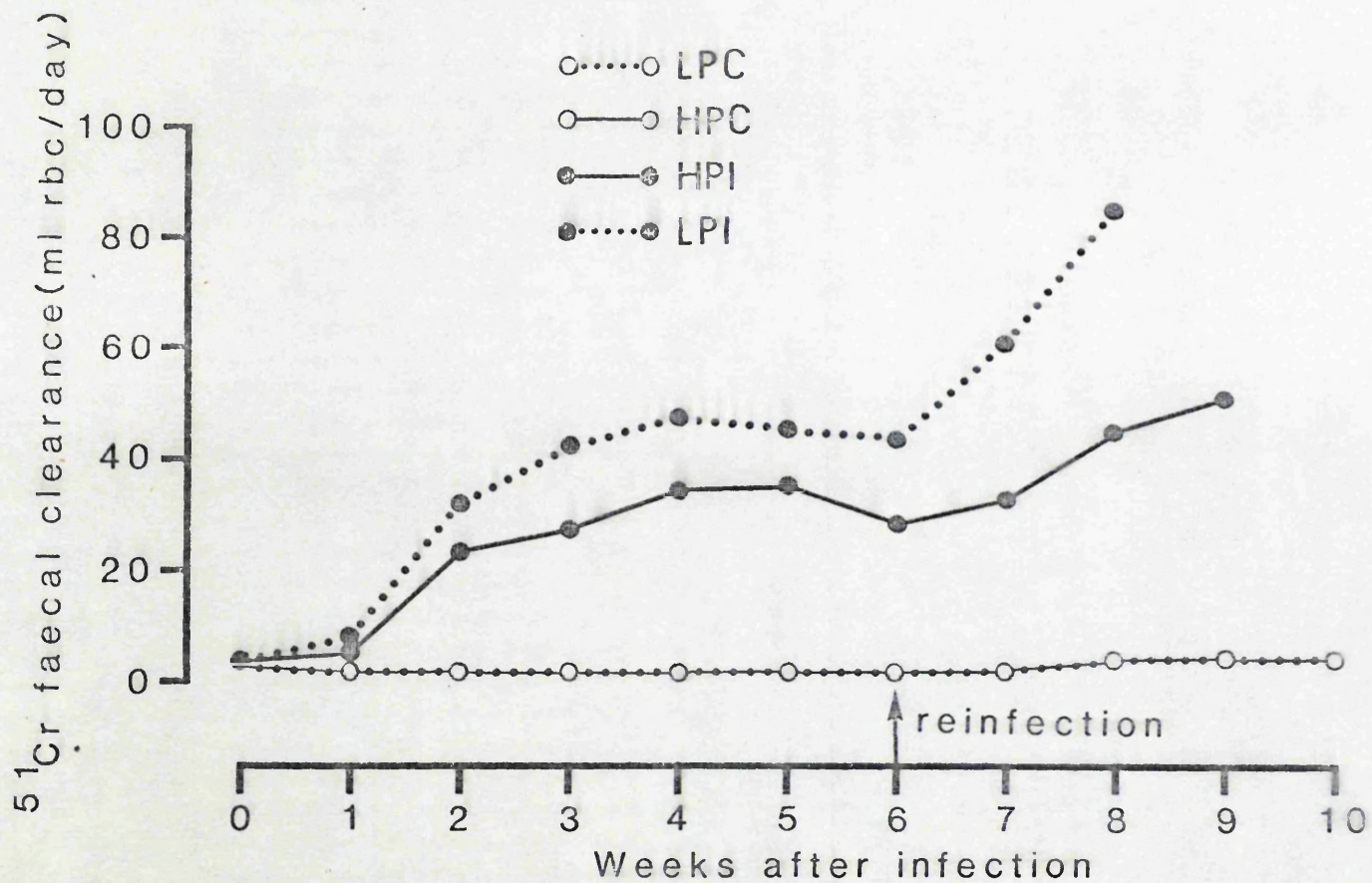

100
50
10
1
0.1
cts/min/ml rbc as % of 10 min sample
oLPC(T1/2= 11.5days)
oHPC(T1/2= 12.3days)
HPI(T1/2= 7.4days)
LPI(T1/2= 4.4days)
0
10
20
30
40
Time after injection (days)

Fig. 6: Gastrointestinal red cell losses in sheep infected with *H. contortus* (●) and normal animals (o) maintained on low protein (LP ----) and high protein (HP ——) diets.

Fig. 6


LPC
HPC
HPI
LPI
^{51}Cr faecal clearance(ml rbc/day)
100
80
60
40
20
0
reinfection
0
1
2
3
4
5
6
7
8
9
10
Weeks after infection

example, reductions in red cell volumes within the same period amounted to 5.5 ml/kg. for the low protein group and 5.6ml/kg. for the high protein group. This suggests indirectly that erythropoiesis must have been greatly accelerated in both groups since they were the more anaemic. Although group variations in blood loss were most pronounced following patency, significant losses were also observed during the pre-patent phase.

The general pattern of blood loss continued during the course of the challenge infection. Haemorrhage eventually became more severe than during the initial infection and was again more pronounced in the low protein group. There was an indication of a temporary suppression of the severity of abomasal bleeding following reinfection, especially in the high protein group. Clearance figures were not available in the week preceding infection in the low protein group, hence this relationship was not so obvious.

(d) Inter-relationships between haemorrhage, egg output and plane of nutrition:

The relationship between haemorrhage, egg output and plane of nutrition is aptly demonstrated in Figs.1,2 &6. Excessive haemorrhage generally preceded the appearance of eggs in the faeces but blood loss and egg output were closely correlated during the latter part of the initial infection. Animals on the low protein diet produced more eggs, had the lowest PCV and also suffered more blood loss than the high protein group. The same relationship generally was observed following patency of the challenge infection except that no significant changes in PCV were observed despite continuous and even more severe abomasal haemorrhage.

SECTION D

FERROKINETIC STUDIES

SECTION D - FERROKINETIC STUDIES

D. MATERIALS AND METHODS.

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

The retained plasma from all the twelve blood samples was pooled and mixed with ^{59}Ferric citrate (specific activity 15 μCi/μg., Radiochemical Centre, Amersham) for five minutes at room temperature in order to label transferrin. Each sheep received approximately 167 μCi of ^{59}Fe.

II - Injection, sampling and radioactivity measurements:

The injection, sampling and radioactivity measurements are as described under General Materials and Methods.

III - Calculation and Expression of results:

(a) Plasma iron turnover rate: The decrease in plasma activity curve with time usually follows a single exponential curve when plotted on a semi-logarithmic scale which on integration can be expressed as: $k = \frac{0.693}{t\frac{1}{2} \text{ (mins)}}$

where k represents the fraction of iron in the plasma removed per unit time, 0.693 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 calculated from the $t\frac{1}{2}$, the serum iron concentration and venous haematocrit at the beginning of the injection using the formula of Bothwell, Hurtado, Donohue and Finch (1957).

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

The total plasma iron turnover was calculated from this figure and the blood volume. The values obtained overestimate the true turnover because a certain proportion of the iron leaving the plasma is transported to the iron stores of the liver, spleen, gut, etc., and moreover approximately

25%/

25% of that going to the bone marrow becomes reversibly bound to a labile erythropoietic iron pool present at or within the membrane of developing red cells and is subsequently fed back to the plasma. These values therefore represent approximate indices of erythropoiesis but nevertheless still prove useful for comparative studies of the type undertaken.

(b) Red cell utilisation of ^{59}Fe: Red cell utilisation of iron was obtained from a plot of the activity/ml RBC against time calculated from the activity and haematocrit of each blood sample. The percentage red cell utilisation rate was estimated from the formula:

$$\text{utilisation \%} = \frac{100 \times \text{blood volume} \times {}^{59}\text{Fe activity/ml blood}}{\text{total injected activity}}$$

using the mean blood volume and maximal ^{59}Fe activity appearing in the red cells. The red cell iron turnover rate (incorporation rate) was obtained as a product of PITR and % utilisation expressed as mg/day and mg/kg/day.

(c) Gastrointestinal blood and red cell losses: These clearance values were obtained by dividing the daily total faecal activity by the corresponding activity per ml of whole blood or packed red cells respectively.

(d) Faecal losses of haemoglobin iron: With a knowledge that each g of haemoglobin contains 3.34mg. of iron, the amount of iron excreted in the faeces each day as a result of haemorrhage may be calculated from the ^{59}Fe whole blood 'clearance' and the blood haemoglobin concentration at that time using the formula of Roche, Perez-Giminez and Levy (1957)

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

The difference between this figure and the amount of iron lost in the faeces estimated from the ^{51}Cr faecal activity gives an estimate of haemoglobin iron reabsorbed.

D. RESULTS/

D. RESULTS.

(a) Plasma iron turnover and red cell incorporation:

The mean results of the ferrokinetic measurements made immediately at infection and again at six weeks are given in Table VI & Figs 7&8 No significant differences in ^{59}Fe plasma half-life, plasma iron turnover rate and red cell turnover rate were observed in both groups when the measurements were first performed. On repeating the measurements six weeks later, a number of striking differences were observed. The rate of ^{59}Fe plasma disappearance was greatly accelerated in both infected groups compared to the controls. Similar differences were also recorded in the plasma iron turnover rate, percentage incorporation of label by red cells and in the red cell turnover rate. These differences were highly significant. Although these values were higher in the low protein group, they were not significant when compared to the high protein group due possibly to the scatter and size of the groups. The major factor responsible for these changes appears to be the greatly accelerated plasma ^{59}Fe disappearance during the second measurement which was almost three times faster than at infection and about half as much again faster in the low protein group relative to the high protein group. There was no significant change in the controls on either diet during the two measurements.

(b) Faecal haemoglobin iron losses and reabsorption:

Iron which passes into the gut as a result of the excessive haemorrhage is eventually lost in the faeces if no significant reabsorption occurs. Such faecal losses may be estimated from the ^{59}Fe faecal clearance of blood and the haemoglobin concentration. The estimation was carried out weekly following infection and Figure 9 gives the pattern of such losses and possible reabsorption. Initially all iron/

iron which passed into the gut (estimated from the ^{51}Cr faecal clearance of blood) was lost from the body with no absorption occurring. Enteric iron loss was actually lower than that excreted in the faeces at this stage due to the additional passage of iron from senescent red cells and desquamated iron containing epithelial cells into the faeces. Losses from this route are, however, minimal in the face of excessive haemorrhage. As haemorrhage became more marked, faecal iron losses increased progressively in both groups in accordance with the magnitude of blood loss. Iron reabsorption appeared insignificant but there was an indication that limited reabsorption was occurring in the most anaemic sheep, i.e. those on the low protein diet during the latter part of the infection (from the 7th - 10th week).

TABLE VI MEAN RESULTS OF ^{59}Fe TURNOVER STUDIES IN *H. CONTORTUS* INFECTED AND NORMAL SHEEP ON HIGH OR LOW PROTEIN DIET

	^{59}Fe plasma $T_{\frac{1}{2}}$(hrs)	PITR (mg/day)	PITR (mg/kg/day)	Incorporation by RBC	RBC turnover (mg/day)	RBC turnover (mg/kg/day)
PRIMARY INFECTION. Low Protein:						
Infected	1.4 ± 0.1	71.0±6.5	1.9 ± 0.3	66.6 ± 2.4	47.2	1.3 ± 0.2
Control	1.9 ± 0.3	54.4±10.4	1.7 ± 0.4	64.6 ± 6.4	35.1	1.3 ± 0.3
't' test	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
High Protein:						
Infected	1.2 ± 0.1	59.3±9.8	1.7 ± 0.4	68.1 ± 5.7	40.4	1.0 ± 0.1
Control	1.5 ± 0.08	63.6±15.7	2.0 ± 0.7	62.3 ± 2.2	39.6	1.2 ± 0.4
't' test	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CHALLENGE INFECTION. Low Protein:						
Infected	0.62 ± 0.09	242.6±4.8	6.0 ± 0.9	91.3 ± 5.2	221.5	5.6 ± 1.1
Control	1.7 ± 0.13	73.0±4.2	2.0 ± 0.2	62.0 ± 2.2	45.3	1.4 ± 0.06
't' test	P<0.01	P<0.1	P<0.02	P<0.01		P<0.02
High Protein:						
Infected	0.8 ± 0.2	168.5±19.3	4.4 ± 0.7	92.3 ± 10.4	155.5	4.0 ± 0.3
Control	1.7 ± 0.2	73.5±6.7	1.9 ± 0.2	69.3 ± 4.3	50.9	1.2 ± 0.1
't' test	P<0.01	P<0.01	P<0.05	P<0.01		P<0.001

Fig. 7: ^{59}Fe-plasma iron disappearance in sheep following primary infection with H. contortus (•) and normal animals (o) maintained on low protein (LP ----) and high protein (HP ——) diets.

Fig.7

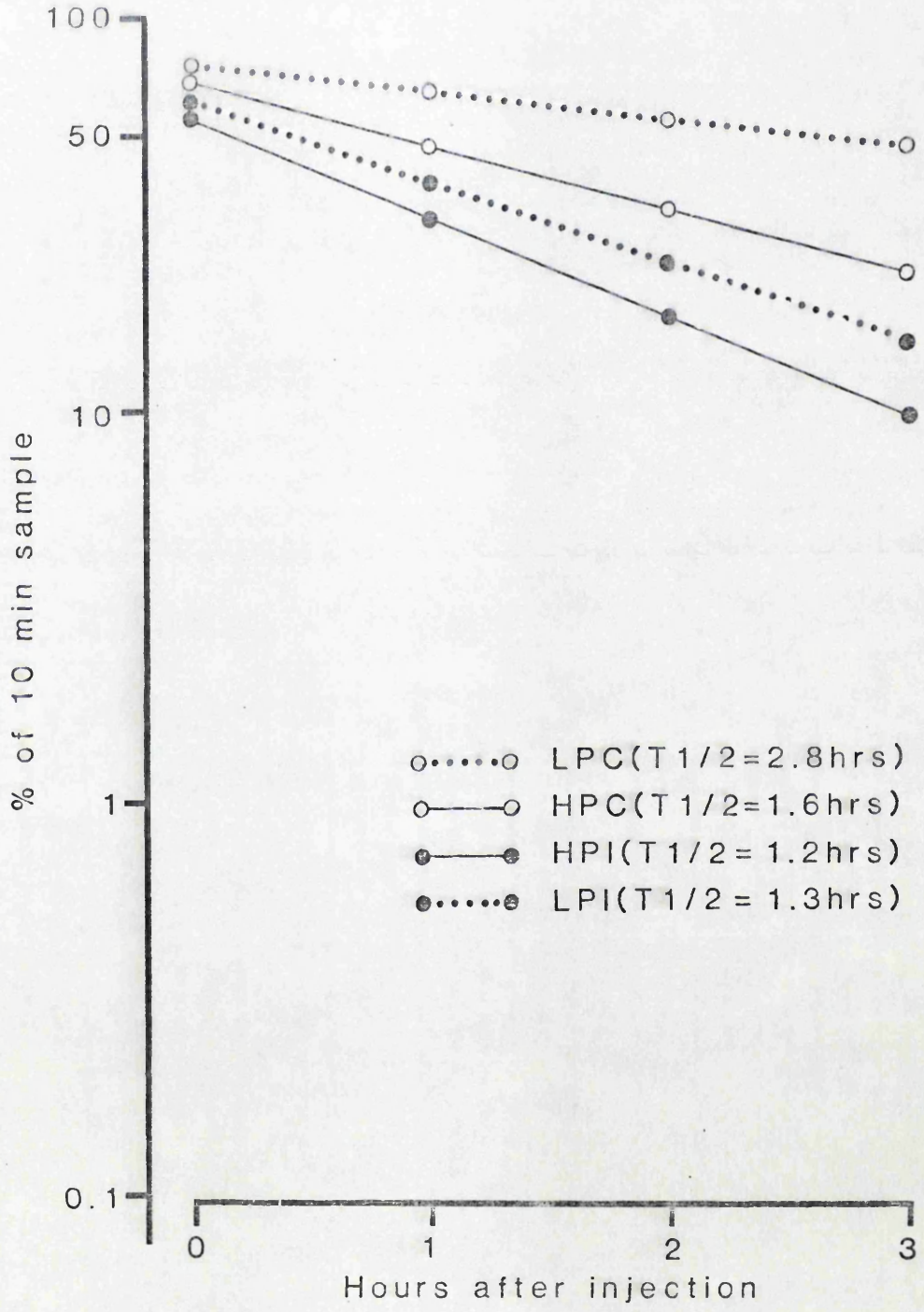

100
50
10
1
0.1
% of 10 min sample
LPC(T1/2=2.8hrs)
HPC(T1/2=1.6hrs)
HPI(T1/2=1.2hrs)
LPI(T1/2=1.3hrs)
0
1
2
3
Hours after injection

Fig. 8: ^{59}Fe-plasma iron disappearance in sheep following a challenge infection with H. contortus (●) and normal animals (o) maintained on low protein (LP ----) and high protein (HP ——) diets.

Fig.8

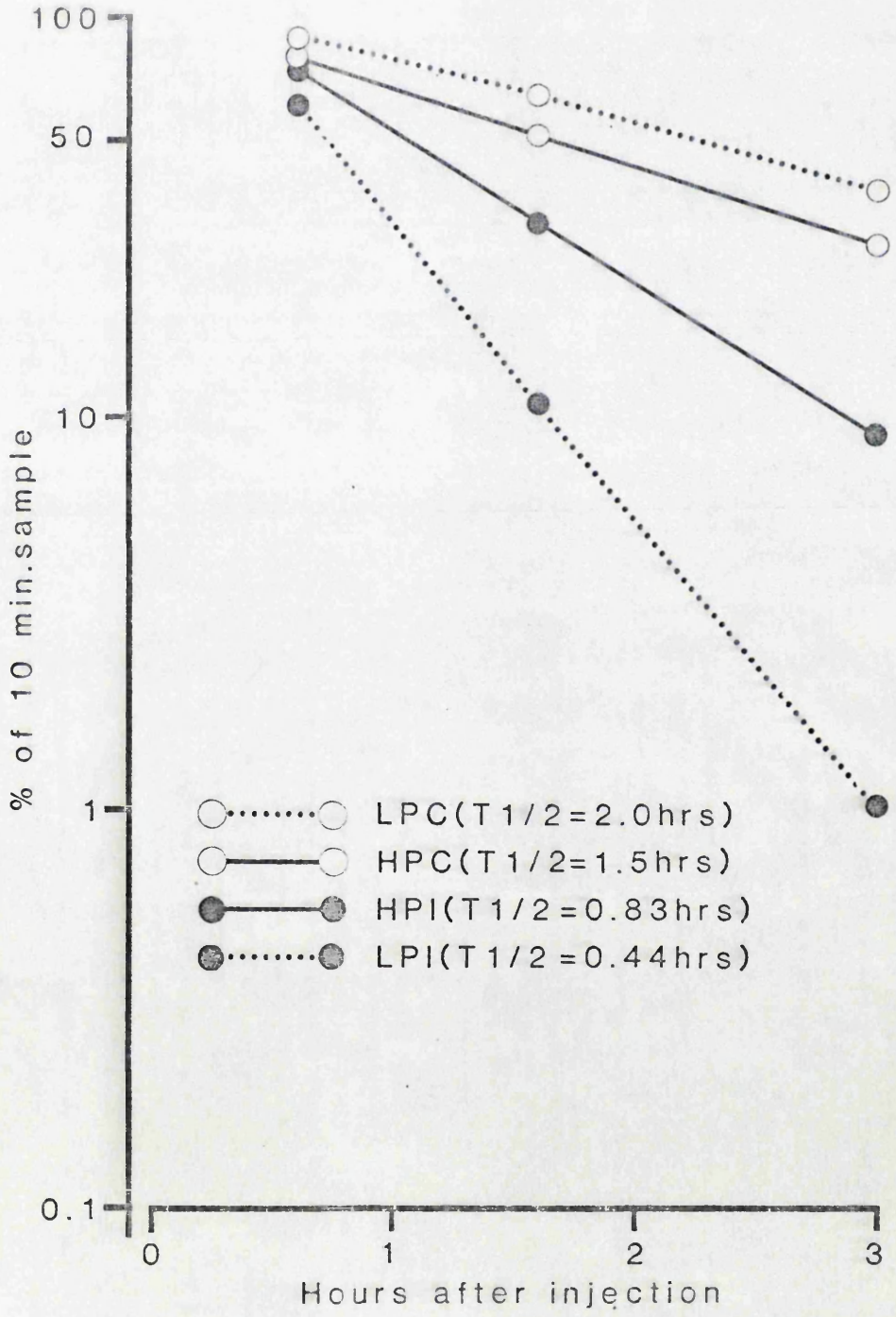
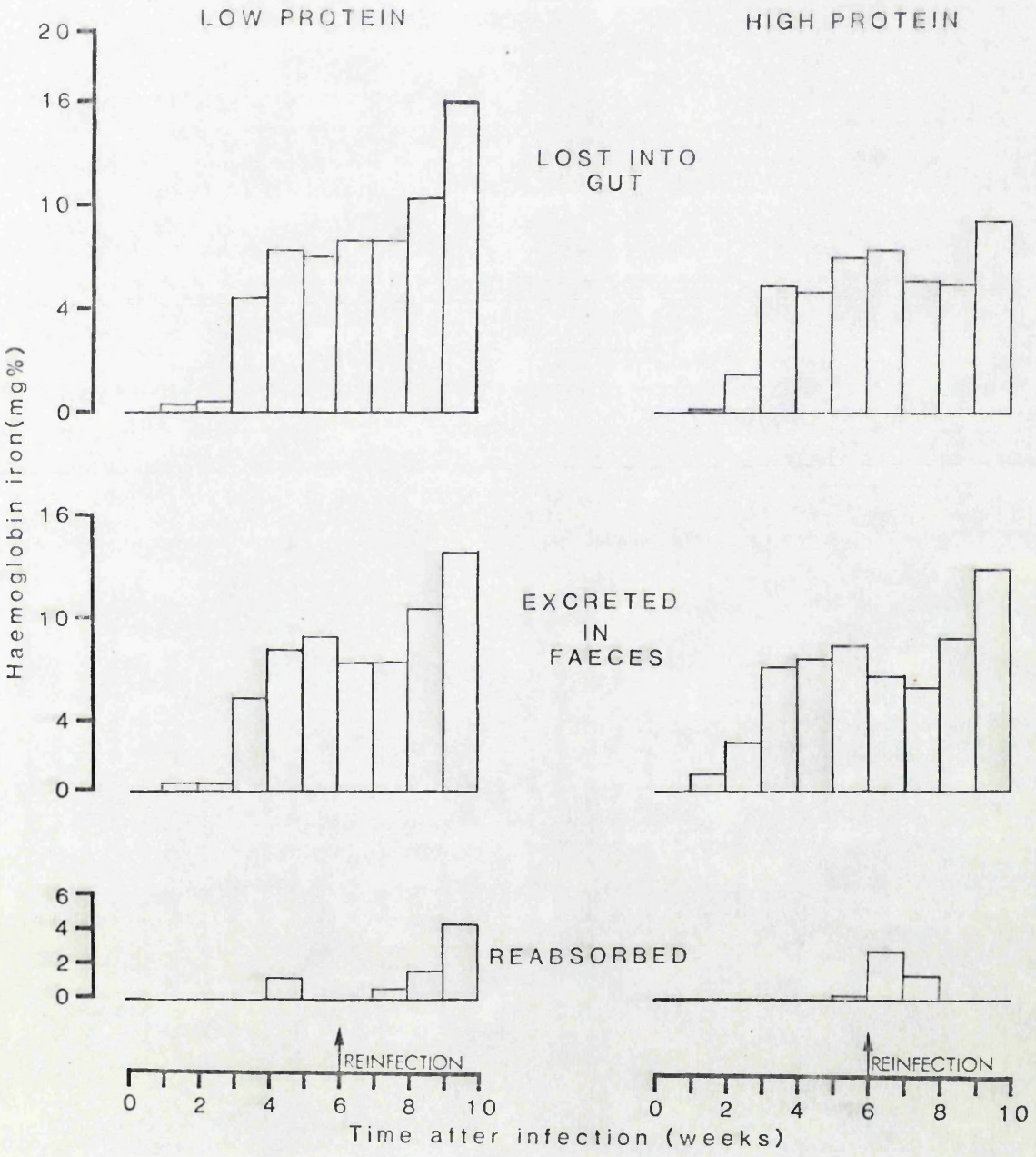

100
50
10
1
0.1
% of 10 min.sample
LPC(T1/2=2.0hrs)
HPC(T1/2=1.5hrs)
HPI(T1/2=0.83hrs)
LPI(T1/2=0.44hrs)
0
1
2
3
Hours after injection

Fig. 9: Gastrointestinal iron loss and reabsorption in sheep infected with H. contortus maintained on high and low protein diets.

Fig.9


LOW PROTEIN
HIGH PROTEIN
LOST INTO GUT
EXCRETED IN FAECES
REABSORBED
Haemoglobin iron(mg%)
20
16
10
4
0
16
10
4
0
6
4
2
0
REINFECTION
REINFECTION
0 2 4 6 8 10
0 2 4 6 8 10
Time after infection (weeks)

SECTION E

ALBUMIN TURNOVER STUDIES

SECTION E - ALBUMIN TURNOVER STUDIES.

E. MATERIALS AND METHODS.

I - Labelling of albumin with ^{125}I:

Commercial sheep albumin was trace labelled with radioiodine by the iodine monochloride method of McFarlane (1958).

Materials:

(a) Albumin: Sheep albumin was obtained commercially (Cohn Fraction, V. Pentex Incorp., Kankee, Illinois, U.S.A.).

(b) Stock iodine monochloride solution: The stock iodine monochloride solution was prepared by dissolving 5.0gm of potassium iodide and 3.25gm of potassium iodate in 37.5 ml of distilled water. 37.5 ml of concentrated HCl and 5 ml of carbon tetrachloride were added to give a faint pink colour. This solution was diluted 1:350 to give a solution containing 0.42 gm of iodine/ml as iodine monochloride which was used for the labelling procedure.

(c) Glycine buffers (A and B): Two glycine buffers were prepared. Buffer A (pH 8.5) was prepared by adding 9 ml molar glycine in $^{N}/4$ NaCl solution to 1 ml NaOH. This was used to convert iodine monochloride to the hypoiodite.

Buffer B (pH 9.0) was prepared by adding 8 ml molar glycine in $^{N}/4$ NaCl solution to 2 ml NaOH. This provided the alkaline medium necessary for the reaction to occur.

II - Procedure:

15 ml of buffer A was added to 6 ml of the diluted stock iodine solution. The radioactive iodine (approximately 10 mCi) was added to this solution and immediately transferred to the buffered protein solution (30 ml of 2% sheep albumin + 15 ml of buffer B). The solution was poured into a dialysis sac. Carrier protein was added to bring the specific activity/

activity of the preparation to below 5 μC/mg protein. The labelled protein was dialysed for 48 hours against two changes of 20 litres of isotonic saline and then dispensed for injection. Assuming 100% incorporation, labelled albumin prepared in this way contains 0.9 atoms/molecule. In practice, incorporation is often in the order of 50%-60%. Each sheep consequently received approximately 500 μCi of ^{125}I.

III - Injection and sampling:

Injection and sampling were as described in General Materials and Methods.

IV - Calculations and expression of results:

Calculations and expression of results are as described in Chapter IV. The fractional catabolic rate (k) was calculated by the method of Campbell et al (1956) based on the excreted activity in urine and faeces. Total body albumin pool was estimated by the extrapolation method.

E. RESULTS.

(a) Albumin pool sizes:

Albumin pool sizes and distribution were measured at infection and again at reinfection six weeks later, whilst catabolic rates were measured in the period following each injection of ^{125}I-labelled albumin. The results are illustrated in Table VII and Figures 10 and 11.

When the measurements were first made at the time of infection no significant differences were observed in total body pool between the infected and control sheep of each protein group. However, the extravascular pools were noticeably higher in the controls and more so in those on high protein diet. On repeating the measurements six weeks later, the infected sheep on each diet suffered reductions in the sizes of all/

TABLE VII DISTRIBUTION AND CATABOLISM OF ^{125}I-LABELLED ALBUMIN IN ACUTE HAEMONCHOSIS OF SHEEP ON HIGH AND LOW PROTEIN DIETS.

			Albumin distribution					Albumin degradation		
			Serum Albumin (g%)	CA (g/kg)	EA (g/kg)	TA (g/kg)	EA/CA	Half-life (hrs.)	F(CA)	Faecal Clearance of plasma (ml./day)
Mean Infected	LP	1	2.5	1.5	1.6	3.1	1.1	283	0.13	4.7
		2	2.0	0.9	0.8	1.7	0.9	160	0.22	10.8
	HP	1	2.6	1.6	1.7	3.3	1.2	235	0.14	3.1
		2	2.3	1.3	1.2	2.5	0.9	216	0.16	3.9
Mean Control	LP	1	2.6	1.2	2.0	3.2	1.7	526	0.07	1.4
		2	2.5	1.1	1.3	2.4	1.1	402	0.06	1.7
	HP	1	3.0	1.4	2.3	3.7	1.6	486	0.08	1.5
		2	2.9	1.7	2.0	3.7	1.2	361	0.07	1.9

LP = Low protein

HP = High protein

1 = Primary infection (weeks 1-6)

2 = Reinfection (weeks 7-10)

Fig. 10: ^{125}I-labelled albumin disappearance in sheep following primary infection with H. contortus (•) and normal animals (o) maintained on low protein (LP ----) and high protein (HP ——) diets.

Fig.10

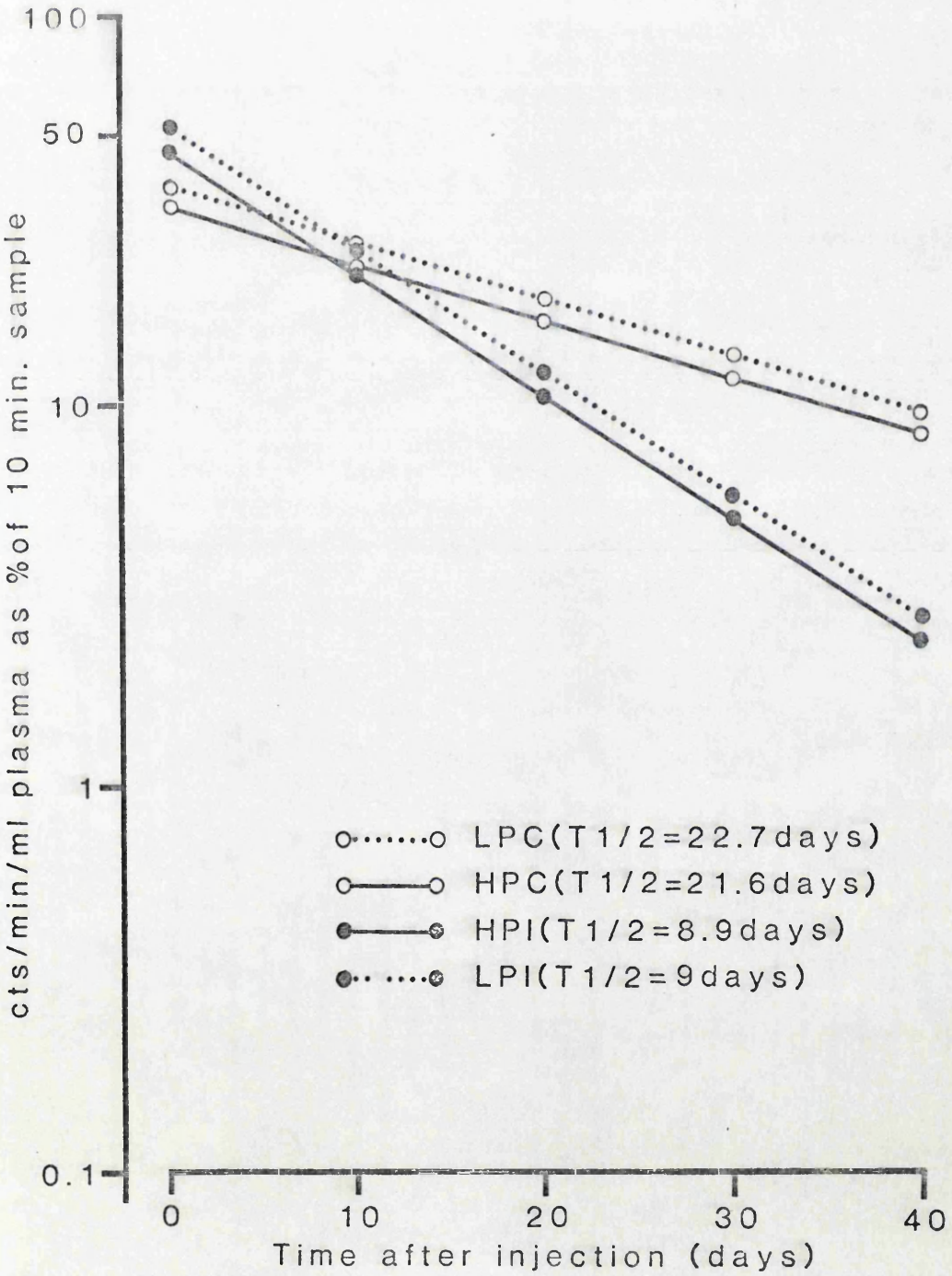
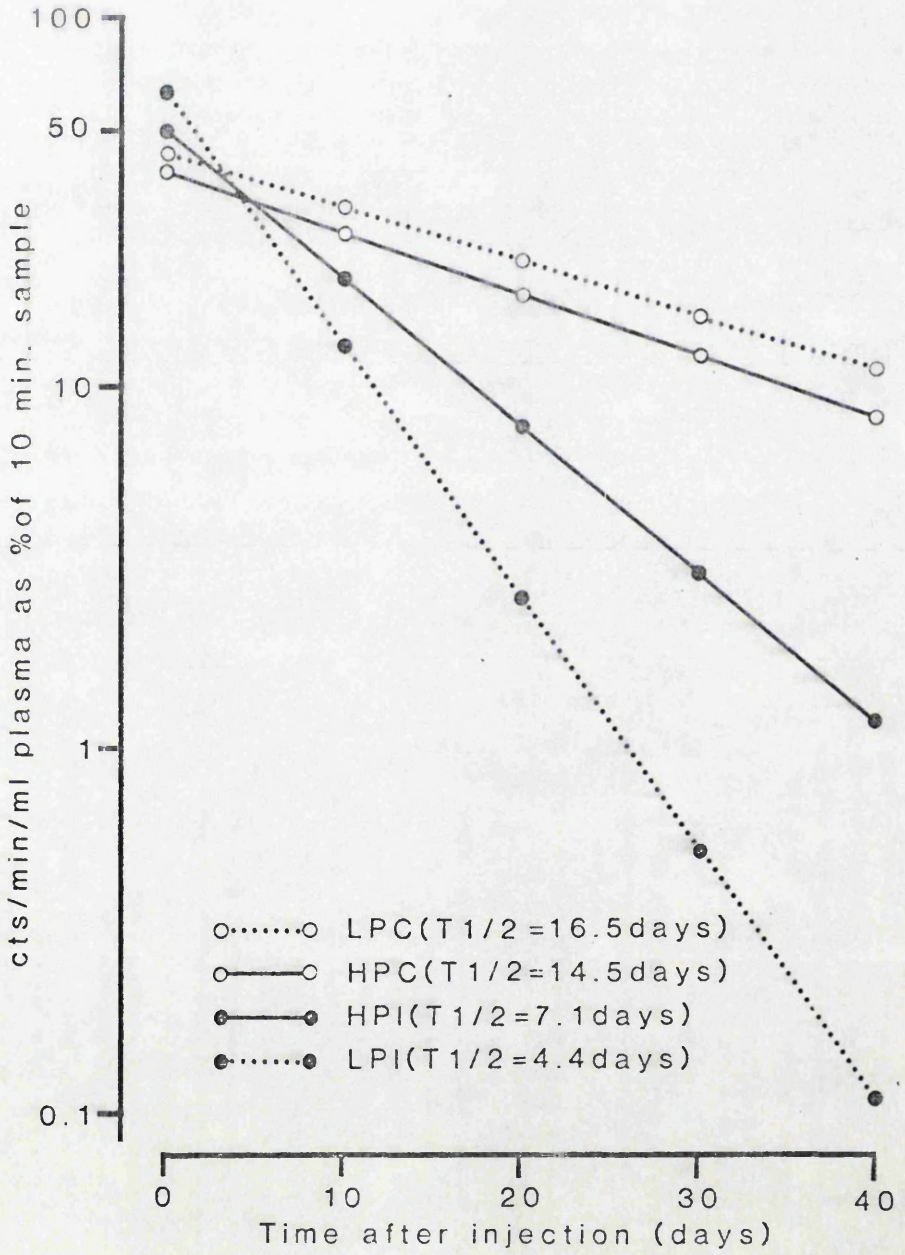

100
50
10
1
0.1
cts/min/ml plasma as %of 10 min. sample
LPC(T1/2=22.7days)
HPC(T1/2=21.6days)
HPI(T1/2=8.9days)
LPI(T1/2=9days)
0
10
20
30
40
Time after injection (days)

Fig. 11: ^{125}I-labelled albumin disappearance in sheep following a challenge infection with H. contortus (●) and normal animals (o) maintained on low protein (LP ----) and high protein (HP ——) diets.

Fig.11


100
50
10
1
0.1
cts/min/ml plasma as %of 10 min. sample
LPC(T1/2=16.5days)
HPC(T1/2=14.5days)
HPI(T1/2=7.1days)
LPI(T1/2=4.4days)
0
10
20
30
40
Time after injection (days)

of all body pools but these were more pronounced in the low protein group where the total body pool fell by 45% compared to 24% in the high protein group. Although both pools contributed to the depletion, the major loss occurred from the extravascular sites. In the low protein group, extravascular losses amounted to 50% and intravascular losses 40%. Corresponding losses in the high protein group were 19% and 29% respectively. EA/CA ratios were reduced due to the altered distribution of albumin. The only change in the control sheep was a drop in total body pool of the low protein group. The high protein group showed no increase in total body pool but intravascular pools rose while the extravascular pool decreased slightly. These differences were not significant.

(b) Albumin degradation:

From the measurements of albumin degradation, it is apparent that all infected animals experienced progressive hypercatabolism from about the 2nd week of infection. Initially the albumin half-lives were noticeably shorter in the infected animals relative to their controls (283 hours in the infected low protein group and 526 hours in the controls and 235 and 486 hours respectively in the high protein group). Fractional albumin catabolic rates showed similar changes at this stage.

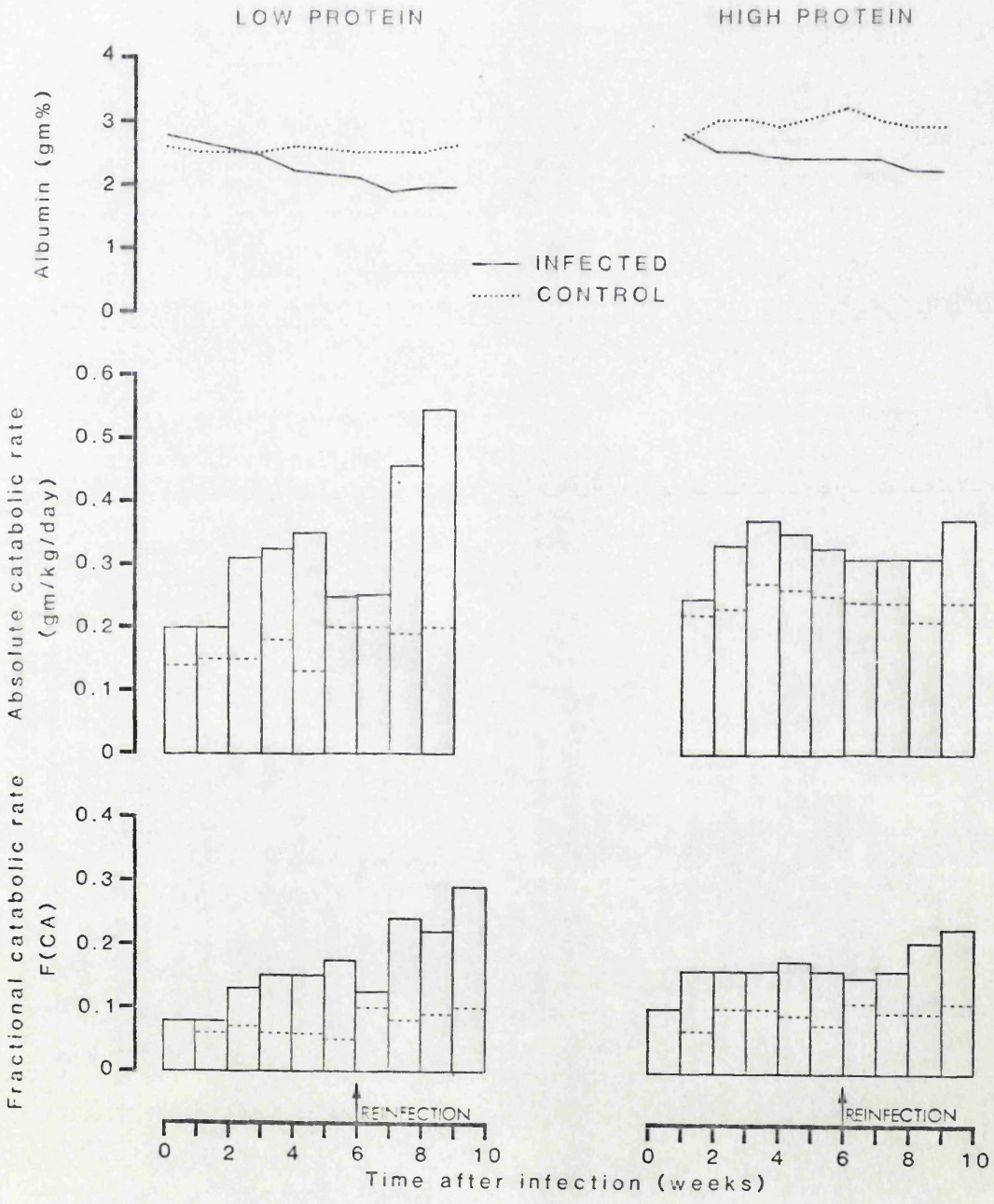
Following reinfection, the low protein group had a higher catabolic rate than the infected sheep on the high protein diet. Their mean albumin half-life was 160 hours compared to 216 hours in the high protein group and fractional catabolic rates were 22% and 16% respectively. These differences were also observed in the absolute amounts of albumin degraded (Fig. 12).

(c) Faecal plasma clearance:

The faecal activity/24 hours expressed as a plasma clearance (ml) was significantly higher in the infected sheep (4.7 ml and 3.1 ml/day in the/

Fig. 12: Serum albumin concentration and catabolic rate in sheep infected with H. contortus and normal animals maintained on low and high protein diets.

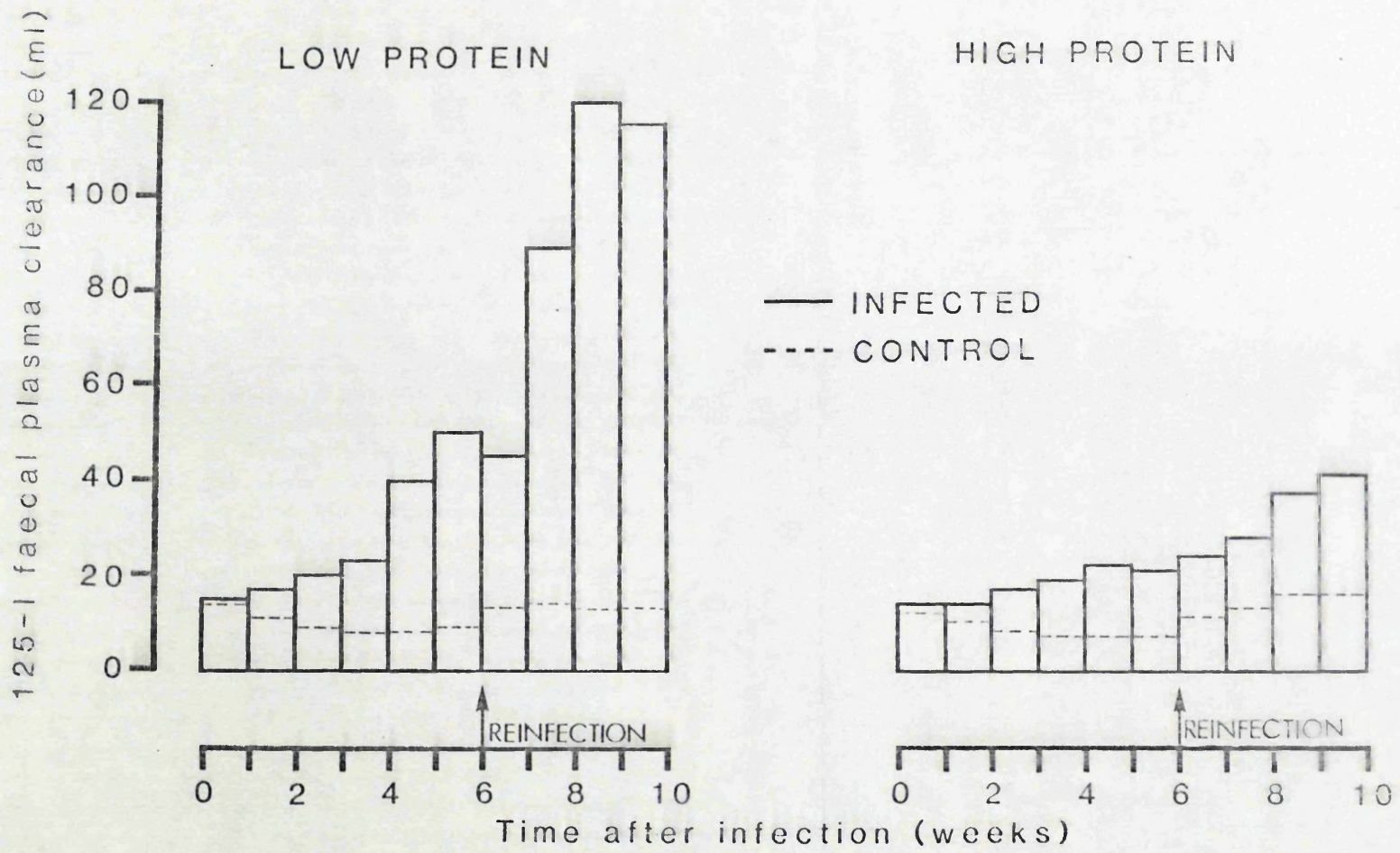
Fig. 12


LOW PROTEIN
HIGH PROTEIN
Albumin (gm%)
4
3
2
1
0
INFECTED
CONTROL
Absolute catabolic rate (gm/kg/day)
0.6
0.5
0.4
0.3
0.2
0.1
0
Fractional catabolic rate F(CA)
0.4
0.3
0.2
0.1
0
REINFECTION
REINFECTION
0 2 4 6 8 10
0 2 4 6 8 10
Time after infection (weeks)

the low and high protein groups respectively) compared to 1.4 ml and 1.5 ml in the respective control groups. Clearance figures were higher in the low protein group especially following reinfection (Figure 13). This suggests that the increased catabolic rate observed in the parasitised animals was due to loss of albumin into the gut. These figures are, of course, gross underestimates of plasma actually appearing in the gut since substantial breakdown and reabsorption of label is known to occur with ^{125}I-labelled plasma proteins.

Fig. 13: Gastrointestinal losses of ^{125}I-labelled albumin in sheep infected with H. contortus and normal animals maintained on low and high protein diets.

Fig 13


LOW PROTEIN
HIGH PROTEIN
125-I faecal plasma clearance(ml)
120
100
80
60
40
20
0
INFECTED
CONTROL
REINFECTION
REINFECTION
0
2
4
6
8
10
0
2
4
6
8
10
Time after infection (weeks)

SECTION F

NITROGEN BALANCE AND DIGESTIBILITY STUDIES

SECTION F - NITROGEN BALANCE AND DIGESTIBILITY STUDIES.

F. MATERIALS AND METHODS.

I - Collection and storage of samples:

Individual faecal and urinary outputs and feed and water intakes were recorded daily throughout the duration of the experiment. During the seven day nutritional balance study (Days 31-36) two ten per cent samples of the daily faecal outputs were retained and combined and at the end of the study suitable aliquots were analysed for moisture content, nitrogen, organic matter and ash content.

Urine was collected during this period in plastic buckets containing 100 ml of 5N-HCl and 10% of each daily output was retained and combined over the balance period for subsequent nitrogen determination. In some cases a 5% subsample was taken due to excessive urine output.

Random samples of each component of the compound feedstuff (sugar beet, pulp, siftings and soya bean meal) were collected for analysis.

II - Chemical Analyses:

(a) Dry matter: Samples of known weight of the feedstuffs and faeces were dried in a forced-draught oven at 100°C. to constant weight.

(b) Organic matter and ash: Samples of dried material of the faeces and feed were weighed into a crucible, charred using a bunsen flame and placed in a muffle furnace at 550°-600°C. for at least 5 hours. After cooling in an evacuated desiccator and re-weighing, organic matter and ash contents were calculated as the loss of weight and weight of residue respectively.

(c) Nitrogen (crude protein): Total nitrogen analyses were carried out by a Macro-Kjeldahl technique according to the recommendations of the Ministry of Agriculture, Fisheries and Food on the analyses of agricultural materials (1973) using an automatic Kjel Foss (Kjel Foss Automatic/

Automatic Model 16210).

Basically the procedure consisted of weighing approximately three grams of faeces accurately which was digested with sulphuric acid in a long-necked Kjeldahl flask with the addition of 2 copper Kjeldahl catalyst tablets (BDH Chemicals, Poole, England) and hydrogen peroxide. The mixture was then boiled automatically until the solution cleared. Sodium hydroxide was then added to the contents of the flask to make it alkaline and the ammonia distilled into another flask containing 2% boric acid solution and methyl red/methylene blue as indicator. This green solution was then titrated with hydrochloric acid of known molarity until the end point was reached. The nitrogen or crude proteins in the 3 gms. of faeces were read from the instrument and then converted to grams faecal dry matter.

Urine nitrogen was determined in a similar way to that described for faeces except that 10 ml samples were digested with the sulphuric acid.

(d) Result of analysis of diets: The high protein diet had a basic composition of 2 parts soya bean meal, 4 parts sugar beet pulp, 3 parts siftings and 0.18 part mineral and vitamin additives. On analysis this diet provided 84.7% dry matter, 14.9% crude protein and 6.4% ash on dry matter basis.

The low protein diet was made up of 4 parts sugar beet pulp, 2 parts siftings and 0.18 part mineral and vitamin mixture which, on analysis, yielded 86.1% dry matter, 8.6% crude protein and 6.5% ash.

III - Calculation and expression of results:

(a) Nitrogen balance: The protein status of each animal was calculated on the assumption that nitrogen enters the body exclusively via the diet and is lost primarily through the urine and faeces using the/

the formula:-

N balance = N intake - (faecal N + urine N).

Certain errors are inherent in this measurement (Wallace, 1959). Additional losses of N occur via the sweat, gases released from the alimentary tract, skin and hair which are difficult to quantify. Also overestimation of intake can occur through errors in the measurement of feed intake, faecal and urinary outputs and spillages. Moreover, the rate at which food passes down the alimentary tract is variable and the faecal material collected on any day is not necessarily derived from the food eaten the same day. This later error was minimised by ensuring that the animals were kept in cages on their respective diets for a long enough period before balance studies were started. Under careful experimental conditions these errors can be minimised and, since the values are needed for comparative purposes, useful deductions can still be made.

(b) Apparent digestibility coefficients (ADC): These were calculated for dry matter, organic matter, ash and crude protein by substituting the relevant data in the following equation:-

$$\text{ADC} = \frac{\text{Amount in feed - Amount in faeces}}{\text{Amount in feed}}$$

(c) Water balance: Water balance was estimated simply as:-

Water balance = (water drunk + food water) - (urine water + faecal water).

No corrections were made for metabolic water derived from the breakdown of nutrients within the body and for insensible water loss. These parameters are difficult to measure directly.

F. RESULTS/

F. RESULTS.

(a) Digestibility coefficients:

There were no significant differences seen in the digestibility of dry matter, organic matter and crude protein between infected animals and their pair-fed controls in each of the two protein treatment groups. However, in the low protein groups the control animals showed a crude protein digestibility of 0.54 compared with 0.48 in the infected animals suggesting that increased faecal nitrogen output may have resulted from plasma nitrogen loss in the infected animals. The higher values of about 0.75 digestibility for crude protein seen in the high protein groups is perfectly understandable and expected and is due to there being considerably less metabolic faecal nitrogen loss as a proportion of the food input (Table VIII).

(b) Nitrogen balance:

In the nitrogen balance study animals on the high protein diets showed no differences in any of faecal, urinary or retained nitrogen. However, there was an increased urinary N loss in infected animals (viz:- 5.6 compared with 4.2 g N/d) coupled with the increased faecal N loss (7.3 compared with 6.3 g N/d) in the low protein group. In this group the overall apparent retention of nitrogen was 0.78 in infected animals compared with 3.2 g. in the pair-fed control animals. Although not significant ($t = 2.2$) it is speculated that the small number of animals used in each treatment group (3 only) was the limiting factor and that repeated work with larger group sizes would show statistically significant differences.

(c) Water balance:

Water balance studies showed that infected animals on each protein treatment group retained slightly more water than their respective pair-fed controls. These differences were not significant. Between the two infected groups water retention was, however, similar.

TABLE VIII MEAN RESULTS OF DIGESTIBILITY, NITROGEN AND WATER BALANCE STUDIES IN EXPERIMENTAL H. CONTORTUS INFECTIONS IN SHEEP AND IN NORMAL SHEEP ON HIGH OR LOW PROTEIN DIETS.

Diet		Dry matter digestibility	Crude protein	Organic matter	Nitrogen balance	Water balance
Low Protein	Infected	0.6842 ± 0.002	0.4775 ± 0.0415	0.7199 ± 0.0033	+0.775 ± 0.9808	1331 ± 155
	Control	0.6720 ± 0.0089	0.5392 ± 0.0059	0.7047 ± 0.0119	+3.22 ± 0.534	1102 ± 70
	't' test	N.S.	N.S.	N.S.	N.S.	N.S.
High Protein	Infected	0.7055 ± 0.007	0.7593 ± 0.0130	0.7356 ± 0.0088	+5.283 ± 0.528	1341 ± 100
	Control	0.6975 ± 0.0097	0.7463 ± 0.0324	0.7259 ± 0.0087	+5.166 ± 0.377	1216 ± 257
	't' test	N.S.	N.S.	N.S.	N.S.	N.S.

DISCUSSION.

DISCUSSION.

Anaemia, hypoalbuminaemia and reduced weight gains are the major features of acute haemonchosis (Allonby, 1974). The results reported in this thesis clearly demonstrate that the severity of the disease, as judged by most of these indices, is influenced by the quality of the diet offered to the host. Well fed animals are less affected clinically than those on poor feed. This is illustrated in Section B by the more severe anaemia and hypoproteinaemia in sheep on the low protein diet which also had the higher faecal egg counts.

The reason(s) for the more severe manifestation of the disease in the low protein group remain largely undefined but two possibilities have been advanced. The first is that because poor nutrition adversely affects a number of host defence mechanisms (Vitale, 1974; Dobson and Bawden, 1974), such animals were immunologically less competent than their well fed counterparts and hence suffered the more severe abomasitis and haemorrhage associated with the establishment of a larger worm burden. Secondly, by virtue of their limited metabolic reserves, the poorer fed sheep were physiologically less able to counteract the pathogenic effects of their parasite burdens.

The second alternative seems less attractive in the light of the haematological observations made during the course of the study and because the well fed group held no obvious weight advantages over their poorer fed counterparts either at the start of the experiment or at any time in between. Moreover, as pointed out by Altaif and Dargie (1978a), weight differences as a measure of physiological advantages is only of limited value when resistance to parasite establishment is not constant since such advantages can be eroded by relatively small differences in worm burdens. Since animals on the poorer ration had consistently higher/

higher faecal egg output (total egg production) than the high protein group, this must have arisen from differences in worm egg production in the abomasum. Although the number of worms which established from the first infection were not measured, the differences in severity of the disease which developed following this initial infection in the two groups can only be explained by either differences in worm burdens or increased fecundity of the female worms which developed in the low protein group. Either of these possibilities might be associated with the immune response against the parasite. Similar observations were earlier made by Preston and Allonby (1978) and would seem to confirm the generally stated view that poor nutrition enhances susceptibility to helminthic infections (Scrimshaw et al, 1968; Reveron and Topps, 1970) and also exerts a major influence on the immune response (Good et al, 1976). This association between poor nutritional status and the immunological competence of hosts apparently does not operate in all helminthic infections, e.g. fascioliasis (Berry and Dargie, 1976).

From the haematological picture observed following primary infection, it is apparent that diet had a major influence, albeit an indirect one, both on the severity of the anaemic process and on its morphological characteristics. Whereas the low protein group developed a profound macrocytosis when PCVs fell below 20% with no impending signs of haemoglobin deficiency (MCHCs relatively normal), red cells of the high protein group remained virtually normal in size. Since macrocytosis is indicative of the presence of reticulocytes which enter the circulation only when erythrocyte production is greatly accelerated (Woodliff and Herrmann, 1973), these findings indicate an earlier response to haemorrhagic stress by sheep on the low protein group. Since the PCV of the high protein group did not fall below 20% at this stage, such

a/

a response was not observed. This difference is best explained by the 'minimum haematocrit deficit' theory first proposed by Ratcliffe et al (1969) and confirmed by Dargie and Allonby (1975) which states that the erythropoietic system of sheep is stimulated only when PCVs fall below a certain minimum level (20%-25%) and even at lower values there is a latency of 1-2 weeks depending on the severity of the anaemia before the host can reach the full potential for compensatory red cell synthesis. Further support for this theory can be found in the observations of Blunt et al (1969) that blood erythropoietin levels of sheep remain depressed until such a time as the animal is severely stressed and also by the work of Charleston (1964) and Allonby (1974) demonstrating significant anaemia prior to the appearance of changes in the distribution and extent of erythroid tissue in the bone marrow. The fact that PCVs were subsequently maintained at this low level for prolonged periods in both groups indicate that moderate worm burdens can be tolerated for a reaonable length of time.

Serum protein changes are essentially the same as those described by others (Dargie, 1973, 1975, 1980; Altaif and Dargie, 1978). However each of the major changes observed, i.e. hypoalbuminaemia, hypoproteinaemia with little or no change in serum globulins, was more pronounced in those suffering the greater haemorrhagic stress, i.e. the low protein group.

Most helminthic infections are associated with inappetence. According to Dargie (1973), haemonchosis, especially the acute form, appeared peculiar in that appetite was not depressed but rather increased. Similar observations were made in the work reported in this thesis. Since the infected sheep were eating the same amount as their pair-fed controls, the disease therefore must have developed from factors other than decreased/

decreased or increased feed intake. In this respect these findings are in agreement with previous observations that sheep infected with a variety of gastrointestinal helminth parasites develop more severe symptoms of disease than worm-free animals restricted to the same level of feed intake (Gibson, 1955; Roseby, 1973; Bawden, 1969; Owen, 1973

In Sections A and B it was shown that sheep infected with H. contortus experienced profound haematological and biochemical changes, the degree depending on the nutritional status of the host. To clarify the importance of this observation requires more detailed comparative information on the development of the parasite and the resultant disease. under the nutritional conditions adopted. This information cannot be obtained solely on the basis of clinical values and conventional techniques which by their very nature are merely indirect indices of worm activity and disease severity, i.e. the information obtained is of a static kind. For a proper appreciation of how the different diets affect the parasite and resultant disease, it is necessary to describe the development of each in terms of the functional disturbance taking place within the host's tissues. This was attempted in the subsequent sections by radioisotopic methods in an attempt to elucidate the pathophysiological factors that produce the anaemia and hypoalbuminaemia observed in acute haemonchosis.

The pathogenesis of haemonchosis is well documented. The present findings showing that the most rapid deterioration in the haematological picture of infected sheep coincided closely with the blood loss into the abomasum merely reinforce the view that H. contortus exerts its main pathogenic effect by blood sucking. The magnitude of the anaemia in each group correlated closely with the severity of the haemorrhage. Sheep on the low protein diet lost more blood and consequently became more anaemic. This initial rapid phase was superseded by a stage whereby the PCVs remained remained relatively constant. The isotopic measurements however/

however revealed that blood loss was continuous, thus the only way in which these sheep could maintain constant PCV in the face of continuous haemorrhage is by a greatly increased rate of red cell production. An index of erythropoiesis was obtained from the plasma iron turnover studies. Measurements made at six weeks post-infection testify to the marked increase in the rate at which iron was turned over through the plasma of the infected animals. This rate was again directly related to the magnitude of blood loss being almost twice as fast in the low protein group compared to those on high protein.

Another point of interest revealed by the isotopic measurements was that infected animals were suffering great losses of iron from the body without any significant reabsorption or signs of impending deficiency. This failure to reabsorb iron lost into the gut confirms earlier reports (Georgi, 1964; Dargie, 1973, 1975; Dargie and Allonby, 1975) and implies that a major consequence of this disease is progressive depletion of iron stores leading to frank iron deficiency and impairment of erythropoiesis. Such a situation characterised by low serum iron levels and reduced PITR was not recorded in the present studies. This, in part, suggests that erythropoiesis was not limited as serum iron levels were reasonably maintained. The only way the sheep, especially those on low protein diet, can cope with such iron losses from the body is by absorption from dietary sources. Unfortunately there is no information in the literature concerning the iron requirements of sheep or its availability from different feedstuffs and no attempt was made in these studies to measure dietary iron uptake or body iron stores. The obvious conclusion however is that dietary iron utilisation was not jeopardised and therefore erythropoiesis proceeded in accordance with the magnitude of blood/

blood loss. As pointed out by Dargie (1975), the second stage of the anaemic process in acute haemonchosis is characterised by, amongst other things, normal or slightly reduced serum iron concentrations. This was the stage reached during the course of the primary infection.

Previous work on the hypoalbuminaemia associated with haemonchosis have stressed the importance of hypercatabolism and excessive protein loss into the gut (Dargie, 1973, 1975, 1980). The work reported in this thesis confirms this general picture but also illustrates the more complex nature of the different processes and inter-relationships involved. Irrespective of dietary type, the essential features distinguishing infected sheep from their pair-fed controls were:- the high fractional and absolute rates of albumin catabolism, the loss of albumin into the gut of parasitised animals causing hypoalbuminaemia and the fact that a fall in the extravascular pool size was associated with hypoproteinaemia. Measurements made during the course of the primary infection failed to reveal any statistical difference in catabolic rate between sheep on high and low protein diets despite the fact that the latter were losing more albumin into the gut. If anything, catabolic rates were slightly higher in the high protein group. Since serum albumin loss were lower in the low protein group, enteric losses were therefore relatively larger. There was also a close correlation between the decline in serum albumin levels experienced by each group and the increased catabolic rates in terms of apparent half-lives and fractional albumin catabolism. It would therefore appear as if albumin catabolism is reduced with decreasing dietary protein as pointed out by James and Hay (1968) but the relationship is at best tenuous. This is because the differences in total albumin catabolism between infected sheep and their pair-fed controls were not significant. But since the high protein group suffered the less severe reductions in total body albumin pools, it must be concluded that the ability/

ability of infected sheep to replace lost albumin by synthesis was increased by higher protein intakes. The pathophysiological measurements indicate a more severe disease in the low protein group and since protein catabolism was not so severely affected, synthesis must have been proceeding at an accelerated rate in this group as well. No direct measurements of synthesis were carried out but, from the changes in pool sizes and catabolic rates, the indication is that synthesis was greatly accelerated in both groups.

From the nitrogen balance studies (Section F) increased urinary excretion of nitrogen occurred in the parasitised animals and was more noticeable on the poor plane of nutrition. This has previously been reported in H. contortus infections in sheep (Dargie, 1973) and in T. colubriformis infections (Roseby, 1973). This is not unexpected in sheep on two dietary protein planes and therefore different rates of protein turnover. The loss of similar quantities of blood proteins into the gut will result in relatively larger increases in protein turnover in the animals on the lower protein diet. However, the appearance of this protein in the urine and not in the faeces, as would have been expected, adds a further dimension to the loss. Firstly, it indicates that the lost protein is eventually processed and reabsorbed. Its ultimate excretion following degradation would lead to increased urinary losses. An alternative hypothesis in that the increased loss of nitrogen in the urine is a result of excessive catabolism of tissue and in particular muscle protein. This hypothesis postulates that the excessive passage of blood constituents and plasma into the gut will increase the amino acid requirements of organs such as the liver and bone marrow that manufacture blood proteins. Thus the requirement of these organs for amino acids for synthesis of essential proteins (albumin and haemoglobin) may result in the diversion of amino acids from less essential/

essential tissues such as skin and muscle. The increased catabolism at these non-essential sites could lead to increased urinary nitrogen losses. The finding of reduced synthesis and increased catabolism of skeletal muscle proteins in laboratory animals infected with gastro-intestinal nematodes would seem to support such a hypothesis (Symons and Jones, 1971, 1972).

The only interesting feature to emerge from the water balance study was the increased water intake of the majority of infected animals on either high or low protein relative to dry matter intake. Despite the increased water intake, the parasitised sheep exhibited only marginally larger water balances than their controls due to increased urinary output. Similar observations were made by Berry (1975) in sheep infected with Fasciola hepatica. The physiological significance of this observation is not clear.

The apparent digestibility coefficients determined in the study revealed that H. contortus had little or no ill effect on the overall digestive functions of sheep. The similarities of the various coefficients in the parasitised sheep and their respective controls indicate that the latter were able to digest and reabsorb the blood constituents and plasma lost through haemorrhage. As pointed out by Roseby (1973), one cannot ignore the possibility that the sites of digestion may be altered leading to a change in the relative proportion of the products of digestion which are eventually absorbed into the body.

A further aim of this study was to observe the influence of nutrition on the pathophysiological mechanisms following the self-cure phenomenon. This part of the experiment provided some intriguing features. Firstly, if self-cure is defined as an 80% reduction in egg counts over a three week period (Preston and Allonby, 1979a) then one can safely conclude that the animals failed to self-cure. Although some reduction in faecal egg counts was noticed in the week following reinfection, the/

the reduction fell short of the level expected if self-cure had occurred (40%).

Judging from the faecal egg count pattern, there appeared to have been a transient reduction in total egg output. This period also coincided with the change of diet to ad libitum feeding which led to increased faecal outputs. Perhaps the full expression of the self-cure was masked by this ad libitum feeding. However, the general picture observed following reinfection still falls within the last category of responses to be expected following experimental reinfection of animals, i.e. temporary suppression of egg laying followed by establishment of the new infection (Gordon, 1948). Secondly, no significant change in haematological indices and serum iron levels occurred following reinfection except that macrocytosis became established in the high protein group and more severe in the low protein group. Pathophysiological measurements presented a similar picture with the low protein group showing the greater blood loss and albumin catabolism. Thirdly, worm recoveries were essentially similar in both protein groups.

Various possibilities can be advanced for these conflicting observations. Firstly, it is obvious that the biotic potential and haematophagic activities of the worms in the reinfected sheep appear similar since the blood loss was not attended by more severe alterations in bodily condition. Secondly, although both groups harboured similar worm burdens, the high protein group experienced less functional disturbance. This is hardly surprising since they suffered less haemorrhage than the low protein group. Therefore it would be unwise to assume that the immunological status of the two groups are the same because of similar worm burdens. Losses of blood and plasma of the magnitude suffered by the low protein group will obviously affect the immune response since such losses represent depletion of circulating antibodies which could be important in the immune response. Whatever the nature of this immune response, be it directed against worm establishment/

establishment or suppression of egg laying ability, it was obviously more effective in the high protein group since they had fewer egg counts and also suffered the less severe pathophysiological changes following reinfection. If this response is classified as self-cure, it then confirms the original view of Stewart (1953), i.e. that self-cure can be produced by experimental reinfection of sheep. Sheep on the high protein diet can therefore be said to elicit a better immune response to the parasite.

REFERENCES.

Allonby, E.W. (1974)
In: 'Helminth Diseases of Cattle, Sheep and Horses in Europe', (Ed. G.M. Urquhart and J. Armour), pp. 59-63, Glasgow University Press, Scotland.

Allonby, E.W. and Urquhart, G.M. (1973)
Parasitology, 66, 45-53.

Altaif, K.I. and Dargie, J.D. (1978a)
Parasitology, 77, 161-175.

Altaif, K.I. and Dargie, J.D. (1978b)
Parasitology, 77, 177-187.

Bawden, R.J. (1969)
Australian Journal of Agricultural Research, 20, 589-599.

Berry, C.I. (1975)
Ph.D. Thesis, University of Glasgow.

Berry, C.I. and Dargie, J.D. (1976)
Veterinary Parasitology, 2, 317-332.

Berry, C.I. and Dargie, J.D.(1978)
Veterinary Parasitology, 4, 327-339.

Bishop, O.N. (1966)
Statistics for Biology, Longmans, Green & Co. Ltd., London.

Bothwell, T.H., Hurtado, A.V., Donohue, D.M. and Finch, C.A. (1957)
Blood, Journal of Haematology, 12, 409-427.

Blunt, M.H., Huisman, T.H.J. and Lewis, J.P. (1969)
Australian Journal of Experimental Biology and Medical Science, 47, 601-611.

Charleston, W.A.G. (1964)
Journal of Comparative Pathology, 74, 223-240.

Dargie, J.D. (1973)
In: 'Helminth Diseases of Cattle, Sheep and Horses in Europe', (eds. G.M. Urquhart and J. Armour), pp. 63-71, Maclehose & Co., Glasgow.

Dargie, J.D. (1974)
In: 'Helminth Diseases of Cattle, Sheep and Horses in Europe', (eds. G.M. Urquhart and J. Armour), pp. 63-71, Glasgow University Press, Scotland.

Dargie, J.D. (1975)
In: 'Pathogenic Processes in Parasitic Infections', Symposium of the British Society for Parasitology, 13, 1-26.

Dargie, J.D. (1980)
In: 'Digestive Physiology and Metabolism in Ruminants', Proceedings of the 5th International Symposium on Ruminant Physiology, (eds. Ruckebusch, Y. and Thivend, P.), M.T.P. Press Ltd., England, pp. 349-371.

Dargie, J.D. and Allonby, E.W. (1975)
International Journal for Parasitology, 5, 147-157.

Dobson, C. and Bawden, R.J. (1974)
Parasitology, 69, 239-255.

Ebaugh, F.G., Clemens, T., Rodnan, G. and Paterson, R.E. (1958)
American Journal of Medicine, 25, 169-178.

Edwards, K., Jepson, R.P. and Wood, K.F. (1960)
British Medical Journal, 1, 30-32.

Fourie, P.J. (1931)
17th Report of the Division of Veterinary Science and Animal Industry of the Union of South Africa, pp. 472-495.

Georgi, J.R. (1964)
American Journal of Veterinary Research, 25, 246-250.

Gibson, T.E. (1955)
Journal of Comparative Pathology and Therapeutics, 65, 317-324.

Good, R.A., Fernandes, G., Yunis, E.J., Cooper, W.C., Jose, D.C., Kramer, T.R. and Hansen, M.A. (1976)
American Journal of Pathology, 84, 599-606.

Gordon, H. McL. (1948)
Australian Veterinary Journal, 24, 17-45.

Gordon, H. McL. (1958)
Proceedings of the Australian Society for Animal Production, 2, 59-61.

Gray, S.J. and Sterling, K. (1950)
Journal of Clinical Investigation, 29, 1604-1606.

James, W.P.T. and Hay, A.M. (1968)
Journal of Clinical Investigation, 47, 1958-1972.

McFarlane, A.S. (1958)
Nature (London), 182, 53.

Ministry of Agriculture, Fisheries and Food (1973)
"The analysis of Agricultural Materials",
Technical Bulletin 27, H.M.S.O., London.

Owen, N.C. (1973)
South African Journal of Animal Science, 3, 1-4.

Owen, C.A., Bollman, J.L. and Grindlay, J.H. (1954)
Journal of Laboratory and Clinical Medicine, 44, 238-246.

Preston, J.M. and Allonby, E.W. (1978)
Veterinary Record, 103, 509-512.

Preston, J.M. and Allonby, E.W. (1979a)
Research in Veterinary Science, 26, 134-139.

Preston, J.M. and Allonby, E.W. (1979b)
Research in Veterinary Science, 26, 140-144.

Ratcliffe, L.H., Taylor, H.M., Whitlock, J.H. and Lynn, W.R. (1969)
Parasitology, 59, 649-661.

Reveron, A.E. and Topps. J.H. (1970)
Outlook in Agriculture, 6, 131-136.

Ritchie, J.D.S., Anderson, N., Armour, J., Jarrett, W.F.H., Jennings, F.W. and Urquhart, G.M. (1966)
American Journal of Veterinary Research, 27, 659-667.

Roche, M., Perez-Giminez, M.E. and Levy, A. (1957)
Nature (London), 162, 1278-1279.

Rodkey, F.K. (1965)
Clinical Chemistry, 11, 478-487.

Roseby, F.B. (1973)
Australian Journal of Agricultural Research, 24, 947-953.

Scrimshaw, N.S., Taylor, C.E. and Gordon, J.E. (1968)
Monograph No. 57, World Health Organisation, Geneva, "Interactions of Nutrition and Infection".

Smithies, O. (1955)
Biochemical Journal, 61, 629-641.

Snedecor, G.W. (1956)
'Statistical Methods', 5th Edition, Iowa State University Press.

Stewart, D.F. (1953)
Australian Journal of Agricultural Research, 4, 100-117.

Symons, L.E.A. and Jones, W.O. (1971)
Experimental Parasitology, 29, 230-241.

Symons, L.E.A. and Jones, W.O. (1972)
Experimental Parasitology, 44, 7-13.

Urquhart, G.M., Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., Mulligan, W. and Sharp, N.C. (1966a)
American Journal of Veterinary Research, 27, 1641-1643.

Urquhart, G.M., Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M. and Mulligan, W. (1966b)
American Journal of Veterinary Research, 27, 1645-1648.

Vitale, J.J. (1974)
American Journal of Clinical Nutrition, 27, 623-624.

Weichselbaum, T.E. (1946)
American Journal of Clinical Pathology, Technical Supplement 10, 40-49.

Whitlock, J.H. (1948)
Journal of the Council for Scientific and Industrial Research, 21, 177-181.

Woodliffe, H.J. and Herrmann, R.P. (1973)
Concise Haematology, Edward Arnold, London, pp. 216.