PATHOPHYSIOLOGY OF PARASITIC INFECTION

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PREFACE

Advanced changes in cells, tissues, and organs are the characteristic responses we expect following parasitic infections, and observations of such have contributed greatly to our knowledge of the parasitic process. These end results are the summation of a multitude of processes, often quantitative in nature, which lead to qualitative changes. It is only in the last decade or so that parasitic disease has been related to abnormal or altered biological processes which are finally expressed as the qualitative manifestations that represent the classical pathology of parasitic infections.

This symposium identifies some of the areas that have received intensive study. In almost all cases, it is obvious that the full implication of the disease process may be missed without the application of modern, sophisticated techniques of measurement. When these are applied, a dynamic process is revealed, the outcome of which may or may not be expressed as overt disease. Furthermore, the overt expression may be contrary to expectations, as for example in the case of gastrointestinal parasitism of ruminants. Here, despite morphological and physiological changes in the parasitized intestine, compensatory mechanisms operate to an extent such that malabsorption is not a major factor even in the most heavily parasitized animal. In sheep, for example, the effects of parasitism are expressed by the reduced incorporation of protein precursors into muscle and the like, rather than by malabsorption.

Studies of plasma protein metabolism have shown a dynamic state of synthesis and catabolism, resulting in an apparent normal balance, but which on investigation proves to be of great liability to a parasitized host should it have greater than usual demands placed on it. A similar situation exists with the anemias of parasitism. The etiology of parasite induced anemias is by no means simplistic, as we shall see in these proceedings, and undoubtedly we need much more study of the mechanisms that lead to and control this manifestation of parasitism, which is possibly the oldest and most widely recognized clinical sign of parasitic infection.

Immunological responses play a dual role in defense and injury mechanisms. The responses that we have so long regarded as beneficial to a host—immediate and

delayed hypersensitivity, antigen-antibody reactions, and activation of complement—now take on new meaning in terms of immunopathology. The delayed response in granuloma formation, antigen-antibody interactions with activation of complement and the role of this in immune glomerular and other vascular membrane associated disorders, and the contribution of immune events to the activation of pharmacologically active amines now constitute an important area of research and provide an impressive emphasis of the need to adopt a multidisciplinary approach to the study of parasitic infection.

The culmination of these various processes can be expressed as the "pathophysiology of parasitic infections." However, the parasites we study choose to inhabit the most unlikely locales of the vertebrate body. Those which parasitize the lymphatic and vascular systems are also endowed with a remarkable longevity and thereby are highly specialized, inducing specialized and often dramatic manifestations of their presence. It is possible that the epitome of parasitism is manifested by the filarial worms: our conference should provide a better understanding of these forms as well as others, which are the major pathogens of the world today.

As with other International Conferences of the World Association for the Advancement of Veterinary Parasitology, a theme, this time "Pathophysiology of Parasitic Infections," was the focus for invited papers by distinguished experts who have reviewed the present knowledge in their respective fields of interest. Supporting these are contributions selected from numerous short communications that are representative of the work in progress in the area of the pathophysiology of parasitic infections.

The art and science of medicine owe much to Greece, so that Thessaloniki provided the historical background, the scholarly perspective, and the organizational success that with the Macedonian hospitality made this, the Seventh International Conference of the WAAVP, a memorable occasion. To Professor Himonas and his colleagues we owe our grateful thanks.

As editor of the volume, I wish to thank the contributors for their cooperation in providing manuscripts—some required a little extra prodding—and to the staff of Academic Press, I extend my special thanks for their patience during the preparation of the manuscripts. The production of such a volume requires understanding, diligence, and energy on the part of the office staff, and I thank Ms. Margo Bradford and Ms. Cleola Taylor for their assistance.

As is usual, my wife, Annette, has provided the moral support and incentive to undertake this task: I thank her.

E.J.L. Soulsby,
Philadelphia, 1976

Introduction to the Seventh International Conference of the World Association for the Advancement of Veterinary Parasitology on Pathophysiology of Parasitic Infections

Dr. Georg Lämmler, President
Institute for Parasitology and Parasitic Diseases of Animals
Justus Liebig University, Giessen
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Minister, Mister Dean, distinguished guests, ladies and gentlemen:

It is my special duty and high honor to open the Seventh International Conference of the World Association for the Advancement of Veterinary Parasitology. The scientific program of the conference again follows the endeavor and intent of the Association to focus on topics of importance and contemporary interest in the field of parasitology.

Since the first conference twelve years ago in Hanover (1963) on the "Evaluation of Anthelmintics," which was the first formal meeting of the Association, various subjects have been selected and discussed: "Biology of Parasites" (Philadelphia, 1965), "Reaction of the Host to Parasitism" (Lyon, 1967), "Pathology of Parasitic Infections" (Glasgow, 1969), "Immunology of Parasitic Diseases" (Mexico City, 1971), and "Parasitic Zoonoses" (Vienna, 1973). These have been topics of major contemporary interest and with a major impact on modern parasitology.

The previous conferences have demonstrated in a very positive manner the broad spectrum of interest and the scientific plurality in parasitology, and the highly beneficial influence of ideas and techniques from other scientific areas. Thus, veterinary parasitology, which is the basic interest of our association, has received immeasurable benefit from the fruitful interaction with the various disciplines in the natural sciences.

The topic of this Seventh International Conference, "Pathophysiology of Parasitic Infections," has been designed to serve the same objective. The invited papers and short communications will give authoritative accounts on our present knowledge and experience in the field of pathophysiology of parasitism. We hope they will stimulate fruitful discussions and lead to new ideas for research.

Pathophysiology, or pathologic physiology, as the term implies, is concerned with disturbances in normal physiology, the mechanisms producing these functional abnormalities, and the way in which they are expressed as symptoms and clinical signs. The basis of the discipline is the knowledge and understanding of the normal physiological, biochemical, physical, and morphological processes, and therefore the topic represents the scientific basis of preventive and curative medicine.

Pathophysiological abnormalities may accompany or result from anatomical defects, but more frequently they occur in the absence of any pathological anatomical change. Often only "biochemical lesions" are present, which express cellular disfunction before the process is severe enough, or has been present long enough, to cause detectable damage at the macroscopic or microscopic level. Pathophysiologic disturbances represent the mechanisms whereby etiologic agents effect their damage, and include the many phases of pathogenesis of diseases.

Pathophysiology elucidates the reaction pattern between the causative agent and the host and is, therefore, a connecting link permitting a synthesis between theoretical and practical medicine. In the field of parasitology, pathophysiology represents a relatively new discipline, although a variety of parameters of different body systems and organs have already been investigated in various parasitic diseases.

Some of the problems of the evaluation of the pathophysiologic responses of the host to parasitic agents result from the multiplicity of parasitic species and of their infective stages that enter the body in very different ways. The differences in the subsequent development, migration, or multiplication in a variety of cells, tissues, and organs are also important considerations in the pathogenic mechanisms produced by the various causative agents.

Because of the major importance of parasitic infections in most parts of the world, and the widespread occurrence of diseases in domestic animals caused by helminths, protozoa, and arthropods, it is probable that further important advances will be made in the field of pathophysiology in the future. These should provide a better understanding of the impact of parasitic diseases on the host, both at the cellular level and at the whole body level. In addition, they should give more information on the differential mechanisms that disturb normal physiology.

It is important also to know more about the functional interaction between host and parasite and to understand better and more precisely how the host is able to eliminate the parasite or to limit its establishment and development in the host. The inclusion of the topic "pathophysiology" in the program of this conference will, we hope, stimulate further developments in this special field of parasitology and give rise to further close cooperation between parasitologists and scientists of other natural and medical science disciplines.

I would like to thank especially the President of the Local Organizing Committee and Director of the Institute of Applied Helminthology and Entomology, Professor Himonas, the other members of the Organizing Committee from Athens and Thessaloniki and all their helpful co-workers for the hard work they have undertaken to make this conference a success. In addition, we thank the Hellenic Ministry of Culture and Science for the sponsorship of the conference and the industrial

companies for their very helpful financial assistance.

There is much to discuss in the conference and I wish you a successful conference, scientifically and socially, and I hope you have a memorable stay in beautiful Thessaloniki.

DETERMINANTS OF PARASITISM: FACTORS IN PATHOGENESIS

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INTRODUCTION

The factors that determine the pathogenesis of an infectious disease are often unclear: this is particularly so with parasitic infections. The present topic is an attempt to dissect the mechanism(s) of pathogenicity from the structural and physiological processes which may be expressed as the pathophysiology of parasitic infections. We know a great deal more about the mechanisms of the pathophysiological changes than the mechanisms of pathogenesis and it is probable that a fuller comprehension of the latter will come only when we understand more of the factors which permit a parasite to maintain itself in the host niche to which it has become adapted.

The functional and morphological changes which follow parasitic infections are frequently non specific, the parasitized host responding in a limited number of ways to a variety of aetiologic agents. Examples of this are the response of the bowel mucosa to a variety of noxious stimuli, the catabolism of protein in various parasitisms, the various anaemias in several parasitic infections and the immunopathologic events to a similar array of infections.

Nevertheless, it is obvious also that a thorough knowledge of what makes an individual parasite pathogenic for its host is lacking and until we understand the determinants of this we will continue to lack the knowledge essential for the control of these infections.

FACTORS DETERMINING HOST-PARASITE COMPATABILITY

One of the unanswered questions of modern biology is that of the molecular basis of host-parasite specificity. Under natural conditions each parasitic species has a relatively well defined host range. It is certain that there is a molecular basis for this and it is probable that the evolution of this basis occurred as host and parasite co-evolved. The degree of integration between host and parasite possibly

explains the breadth or the narrowness of the host range. It is generally considered that the evolution of host parasite associations occurred over an enormous span of time and number of generations of hosts and parasites (Cameron, 1964). However, the role of short term influence has received little study and it is possible these are also determinants of host parasite compatibility. An example of this might be the recent marked increase in equine hydatid infections in Great Britain (Thompson and Smyth, 1974). Epidemiological and experimental studies indicate that the major definitive hosts for this strain of *Echinococcus granulosus* are dogs belonging to hunting packs (Thompson and Smyth, 1975). Protoscolices of this strain differ in their *in vitro* requirements from the sheep strain (Smyth and Davies, 1974) and human infection with the horse strain appears rare. It is interesting to speculate whether the determinants of host specificity co-evolved with the association of horse and dogs of hunting packs (hounds), which, on an evolutionary scale, must be a negligible period of time or whether changes in the determinants of host specificity are related to dietary practices of hunt kennels, which, because of very recent economic pressures, lead to an increase in the feeding of raw horse flesh and offal to hounds (Thompson and Smyth, 1975).

Studies of the molecular basis for host-parasite compatibility have progressed further with the protozoa than with the helminths. For example, the basis of red cell susceptibility to *vivax* malaria (*Plasmodium vivax*) may be associated with Duffy blood group determinants (Fy^a or Fy^b) which may function as erythrocyte receptors for the parasite (Miller, Mason, Dvorak, McGinniss and Rothman, 1975). Evidence that Duffy blood group negative human erythrocytes are resistant to infection with *Plasmodium knowlesi* has been correlated with the resistance of West Africans and American blacks to *Plasmodium vivax* which corresponds to the distribution of Duffy negative erythrocytes in the world. If this factor, alone or in association with another determinant, serves as a receptor for this merozoite of *P. vivax*, then the uniqueness of host-parasite compatibility becomes evident since other human malarias are not dependent on this factor, in that persons resistant to *P. vivax* are susceptible to other species of the genus.

A further example of the molecular basis for host-parasite compatibility is the induction of enzyme activity in host cells for the biochemical needs of the parasite. Thus Oelshlagel, Sander and Brewer (1975) have produced evidence for the introduction of a pyruvate kinase isozyme into host red cells, by malaria parasites, in amounts sufficient to alter

red cell glycolysis. They consider this direction favourable to the parasite because it will increase red cell adenosine triphosphate (ATP) which is needed by the parasite. These authors also speculate that "pyruvate kinase perturbations" may be a general phenomenon in host-parasite relationships and if so, then the role of the parasite in the determination of its destiny becomes more obvious than hitherto.

Alterations of function of host cells which determine the outcome of the infection are seen in the interactions between *Toxoplasma gondii* and cells (Hirsch, Jones and Len, 1974). These organisms have the ability to induce phagocytosis in cells which are not ordinarily phagocytic and subsequently they are able to block the delivery of lysosomal constituents into the phagocytic vacuole in which they live. Consequently they create for themselves a microenvironment which permits their replication and in which they are sheltered from the effects of antibody. The situation with *T. gondii*, where the parasite inhibits fusion of lysosome with the phagosome is evident also with *Mycobacterium tuberculosis* and other organisms (Hirsch *et al.*, 1974). However, with other protozoa different mechanisms are employed to deal with the digestive enzymes produced by the lysosome. Thus with *Leishmania* species fusion of lysosomes with the phagocytic vacuole occurs, but the organism resists the enzymes and the low pH which results. On the other hand the membrane of the vacuole in which the invasive stage of *T. cruzi* is found after invasion of a cell, is quickly lost and the parasite then lives in the cytoplasm of the cell where it is free from the effect of lysosomal enzymes (Trager, 1974).

The need for a recognition factor, or receptor, has been referred to above, with respect to malaria. With the developmental stages of *Leishmania donovani* Dwyer, Langreth and Dwyer (1974) have demonstrated polysaccharides in the surface membranes. Various carbohydrates (e.g. α -1,4 and α -1,6 glycan bonded polysaccharides) are distributed randomly on the pellicular and flagellar membranes and it has been suggested that the negative charge of the promastigote membrane may induce phagocytosis and internalization of the organism and, in addition, the surface saccharides may impact a resistance in lysosomal enzymes. The loss of certain saccharides during *in vitro* culture is associated with a loss of infectivity, in the case of *Leishmania braziliensis* (Davidowicz, Hermandez, Infante and Convit, 1975).

The relationship of these surface saccharides to the antigens which are modulated on the surface of promastigotes and amastigotes by immune serum, resulting in a "capping" phenomenon, (Doyle, Behin, Mauel and Rowe, 1974) is not known.

However, in part they might serve as a mechanism to evade the host response by antigenic modulation or they may assist in recognition and invation of the host cell, possibly aided by the various receptors for subunits of immunoglobulin or complement present on macrophages.

The factors which determine host-parasite compatability for helminths are largely unknown. The majority of the infective stages of helminths have a low resting metabolism and are in a state of semi-dormancy (Lackie, 1975) requiring some specific stimulus for activation. The majority are ingested and hatching, excystment or ecdysis are now generally considered to be active processes on the part of the parasite induced by physical and chemical factors present in the portion of the bowel where these forms commence their parasitic existence. The various mechanisms of this have been reviewed by Lackie (1975), and it is clear that the receptors for these environmental factors which initiate the parasitic process are just as finely tuned as those for some of the protozoa. The factors which initiate exsheathment of infective larvae of *Haemonchus contortus* and *Trichostrongylus axei* are examples of such fine tuning and furthermore the result of these exsheathment stimuli is the production of leucine amino peptidases which are highly specific and which will attack the substrates in the sheaths of their own infective larvae only (Rogers, 1966).

Conceptionally, the major determinant(s) of host parasite compatibility must be expressed at the interface of the initial contact between host and parasite. The evidence to date indicates that they are.

FACTORS DETERMINING PATHOGENICITY

Again we need to turn to the protozoa for molecular explanations of pathogenicity. Extensive studies have been undertaken by Honigberg and his colleagues of the pathogenesis of *Trichomonas* species (Honigberg, 1973). Thus avirulent strains of trichomonads are richer in antigens and are capable of stimulating a stronger immune response than the virulent strains, such as the Jones' Barn strain. This property of avirulence, with its increased number of antigens is maintained in culture.

Comparison of several strains indicates that the virulent Jones' Barn (JB) strain had a more limited capacity to stimulate antibody production than others of lesser virulence. The increase in the number of antigens occurs in non-virulent organisms irrespective of whether they are fresh isolates or whether they have been rendered avirulent by *in vitro* cultivation (Stephvanski and Honigberg, 1972).

In some protozoans, the need for concomittant bacterial infection to induce virulence is well known (e.g. *Entamoeba histolytica, Histomonas meleagridis*). They are, however, anomalies in this situation since whereas the presence of bacteria is necessary for the pathogenic effects of these organisms, the composition of the bacterial flora is not critical and pathogenic as well as non pathogenic bacteria are associated with this effect. It has been thought that bacteria produce a "suitable environment" (Neal, 1971) to support amoeba. However, the findings of Wittner *et al.* (1970) have suggested that direct contact between amoeba and bacteria is necessary and Honigberg (1973) has suggested that an "episomal factor" capable of phagocytosis to produce a protozoan-bacterial relationship is essential for successful parasitism. Such factors may also pertain to *Histomonas meleagridis* of turkeys where enterohepatitis is not produced in germ free chickens or turkeys, but is regularly produced in "conventional" hosts.

In the majority of cases, *in vitro* cultivation of organisms results in loss of virulence, but in many cases pathogenicity can be restored by serial passage through susceptible hosts. The virulence factors which are rescued by this passage procedure are unknown, as are the mechanisms of their rescue, nevertheless this is evidence for a degree of instability of the factors responsible for pathogenicity (Neal, 1971). Perhaps, however, this is interpretable on the basis that environmental influences play a special part in determining pathogenicity and this is expressed as a spectrum of responses when the host range of the parasite is increased. With the protozoa, variations of pathogenicity are well known and according to the host, members of the genus *Trypanosoma*, express very different capabilities of pathogenesis (Losos and Ikede, 1972).

Parasite factors which determine pathogenicity of helminths, as those which determine host-parasite compatibility, are poorly understood. With the forms which remove blood or essential body nutrients the obvious mechanism is a well adapted oral structure and a powerful oesophagus. Apart from such general information acquired decades ago, we are no nearer to a rational explanation of a mechanism of pathogenesis on the part of the majority of helminth parasites.

SURVIVAL OF PARASITES IN HOSTS

The survival of parasites in their hosts is a topic of increasing interest. The majority of parasites can survive for weeks or months and some may survive for years in hosts which have become sensitized to them. Various mechanisms

are used to accomplish this, some being based on the inability or an altered ability of the host to respond effectively to reject the parasite and some being concerned with the adaptation of the host environment. This general topic is reviewed by Ogilvie and Wilson (1976) under the concept of evasion of the immune response by parasites and a further review of the subject at this time is unnecessary. Notwithstanding, mechanisms such as unresponsiveness in the young, unresponsiveness induced by lactation, immuno-depression induced by antibody or by suppressor cells on the part of the host and antigenic variation (as seen in trypanosomes) and the occurrence of host antigens on, for example, the tegument of schistosomes are entities which variously determine the success or otherwise of an infection.

The "host like antigens" on the tegument of schistosomes are associated with the protection of schistosomula and adult schistosomes against damage by antibodies. These have been shown to be red cell antigens determinants, but it is yet unclear whether such antigens are acquired directly from the host or are synthesized by the parasite. The role of host derived, or host like, antigens in the survival of other parasitic helminths and parasitic protozoa is still unclear. Vickerman (1974) has reported that *Trypanosoma vivax* may bind host serum protein to its surface and this may then disguise the organism from the host response, though in the trypanosomes the ability of the trypanosome population to change its antigenic surface appears to be this principal mechanism whereby the parasite evades the host response.

With the nematodes a hypothesis for prolonged survival, for example, of *Ascaris* has been suggested based on the occurrence of blood group and heterophile antigens on the surface of the larvae (Soulsby, 1971). It has been suggested that the union of specific immunoglobulin with such receptors provides the host protein coat by which these organisms effect their disguise.

A further feature of the survival of parasites in their hosts is the reduced reactivity of parasites upon their entry into a host. Leventhal and Soulsby, (1976) have shown that the early larval stages of *A. suum* have reduced reactivity in terms of binding of immunoglobulins, activation of complement and the attachment of phagocytic cells such as polymorphonuclear leukocytes. This could be interpreted as a mechanism whereby a parasite achieves an initial advantage on invasion of the host, temporary though this may be.

Another aspect of modulation of recognition of a parasite by a host is that reported by Ogilvie (1974) where adaptation of *Nippostrongylus brasiliensis* worms in immune rats is

associated with a changed acetylcholinesterase isoenzyme pattern and which is apparently induced by antibodies to this enzyme, present in the host.

However, as with the factors which regulate host-parasite compatibility, more long range influences may be concerned in the mechanisms of survival of parasites in their hosts and such survival is not simply an interaction between parasite and host. It is increasingly clear that external environmental factors may play an important role in the determination of the duration of the prepatent period of many nematodes.

There is a growing body of evidence that parasitic nematodes (and possibly other forms) at the boundaries of their geographical distribution, or under local conditions where environmental influences serve as limited factors in transmission, acquire survival characteristics which are synchronized with other biological events of the ecosystem which together permit progression of the species. This is manifest usually as an arrest in development in the host and release from arrest when ecosystem factors are satisfactory for further development. The most extensively studied is the seasonal arrest or "hypobiosis" of gastro-intestinal helminths of ruminants (Armour, Jennings and Urquhart, 1969, Blitz and Gibbs, 1972a) associated with lowered environmental temperatures in the fall, but with release of these larvae from arrest in the host during the early part of the following year (Blitz and Gibbs, 1972b, Michel, 1974). The release from arrest of ruminant nematodes is associated, in general, with parturition and lactation, the latter being the relevant physiological event (Connan, 1968). The immunological implications of lactation have been studied in depth by Dineen and Kelly (1972), Ogilvie and Love (1974) and recently by Ngwenya (1976) using rodents with various nematodes. Nevertheless pregnancy, parturition and lactation of ruminants are not the sole determinants for such re-activation (which is manifest as the "spring rise" "post-parturient rise" or "lactational rise" in faecal worm egg counts of sheep) since a similar rise in faecal egg output, albeit of a lower order, occurs in non pregnant females, males and castrated males. Michel (1974) explains this on the basis that resumption of development of nematodes occurs at a specific time of year in all animals, but the subsequent fate of worms is determined by parturition or lactation, possibly by a suspension of the rejection mechanism. This explanation would fit the facts in ruminant nematode infections and experimental infections with nematodes in rodents. It is not so readily applicable to the situation in man where similar inhibition of development is evident with hookworms,

where, for example, in India, arrested development is regarded as an adaptation to a seasonally unfavourable external environment (Schad *et al.*, 1973).

SUMMARY

Though the mechanisms of pathogenicity in parasitic infections are poorly defined at present there is an increasing preoccupation with such phenomena since parasitic infections provide opportunities to study the interrelationship between the determinants of pathogenicity and pathophysiological mechanisms which are somewhat unique.

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MALABSORPTION

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In many infections of the gastrointestinal tract the lesions, particularly of the mucosa, are striking. If they occur in the duodenum or jejunum, where a large part of digestion and absorption of important constituents of the diet take place, it is tempting to conclude that malabsorption must occur, and is the probable explanation of the major effects of the disease. Although it is not uncommon to find such statements about malabsorption in sections on pathogenesis in textbooks of parasitology, it is surprising how few critical measurements of digestion and absorption are quoted, or have actually been made. Many of the measurements that have been made and the conclusions derived from them may be criticized because the methods were inadequate, or they examined one region of the intestine rather than the tract as a whole, or over too short a time. It must not be overlooked that the functional reserve of the intestine is large, so that disturbance in one section may be compensated for in the organ as a whole.

Because of their limitations, it is relevant to this discussion to examine some of the methods used to measure digestion and absorption.

Digestibility of the constituents of the diet, particularly of protein, has often been measured by the classical balance study method. This technique may provide a useful indicator of the nutritional economy of the host, but the assumption made by some that a negative nitrogen balance, for instance, indicates malabsorption of protein is not justified. It does not distinguish between faecal excretion of exogenous or endogenous nitrogen. Endogenous nitrogen may include serum proteins lost into the intestine and unabsorbed, or constituents of epithelial cells that are shed at a faster rate in the infected animal. In a ruminant, the bacterial breakdown of nitrogenous compounds in the caecum or large bowel may be affected by any change in the physical nature of their contents.

Other methods that give clinical or diagnostic rather then critical assessments of absorption have been used for man and

domestic animals. These include measurements of faecal nitrogen or fat, or concentrations of test substances such as D-xylose in blood or urine. These are clearly indirect methods of measuring absorption. Others have used more direct methods such as the passing of tubes with two or more lumena, through which substances may be placed into or withdrawn from specific regions of the intestine. Most have important limitations, for they only assess absorption over a limited period of time and/or in a limited region of the intestine.

These tests have been used to determine whether malabsorption occurs in common parasitic infections of man, e.g. hookworm disease, strongyloidiasis and ascariasis. Brandborg (1971) concluded that, despite the difficulties of interpretation between lesions and absorption, the consensus of opinion was that malabsorption does not occur in hookworm infection, whereas it can occur in strongyloidiasis and certain protozoal infections. Perhaps, however, the details of these investigations are not strictly relevant to a symposium on veterinary parasitology.

One might expect that the effects of helminth parasites upon digestion and absorption would have been studied to greater depth and with more sophistication in domestic animals than is possible in man. Nevertheless, more critical experiments need to be done, despite the fact that these infections may seriously affect the production of commercially important meat and wool, etc. Earlier work with domestic animals has already been reviewed (Symons, 1969). This review includes reports, some of them conflicting, of reduced digestibility in intestinal nematode infections of sheep.

A number of experiments that illustrate the care that must be taken with the interpretation of many measurements of absorption, and which, it is believed, enabled an assessment to be made of the importance or otherwise of malabsorption to the pathophysiology of parasitic disease, were carried out at the McMaster Laboratory of the Commonwealth Scientific and Industrial Research Organization. Most of the work was done with the jejunal infection of the rat with *Nippostrongylus brasiliensis*.

When segments of the jejunum of the infected rat were perfused *in vivo* with solutions of NaCl it was found that there was a net movement of water and sodium and chloride ions into the intestine. The use of ^{24}Na showed that this was due to inhibition of absorption from the intestine and not to hypersecretion (Symons, 1960a).

Similarly, there was shown to be jejunal malabsorption of D-glucose, the amino acids methionine and histidine, palmitic

acid and bromosulphalein. These include substances that are actively absorbed as well as those absorbed by diffusion. The degree of malabsorption was related to the severity of the infection (Symons, Gibbins and Jones, 1971). There was also jejunal malabsorption of the long chain fatty acids stearic and oleic in the same infection (Gallagher, Playoust and Symons, 1971).

A warning of the care that is necessary when measuring absorption in even one region of the intestine was the finding that the difference between the rates of absorption depended upon the parameters used. Absorption expressed in terms of mucosal dry weight (the tissue involved with absorption) indicated greater malabsorption than found if absorption was expressed in terms of unit length of intestine. This was due to hypertrophy of the mucosa, so that there was actually more mucosal tissue per unit length of intestine in the rat with nippostrongylosis, despite villous atrophy.

Although there was convincing evidence of malabsorption in the jejunum, the site of the infection in the rat, there was no evidence of derangement if absorption was measured over the entire length of the small intestine. In these experiments the substrates were glucose, histidine, maltose and protein (Symons, 1960b, 1966). The digestion and absorption of protein was measured in mice infected by *Nematospiroides dubius* and sheep by *Trichostrongylus colubriformis* as well as rats with nippostrongylosis. Radioisotopically labelled proteins were used to avoid the possibility of confusion from endogenous excretion of protein (Symons and Jones, 1970).

The experiments on digestion illustrated how important it is to measure these gastrointestinal functions over sufficient time. The rates of digestion and absorption of maltose and protein in the infected rat appeared to be depressed in the early stages in the infected animal when maximum amounts of the test meals were passing from the stomach to the intestine, but the difference between the infected and uninfected animals disappeared on time went on (Table I). It was only in animals so severely affected that they would have subsequently died that detectable amounts of maltose or glucose passed into the large bowel, and this was a small fraction of the original meal.

When absorption from the unparasitized distal ileum of the infected rat was measured it was found that the rates of absorption of glucose and sodium were increased (Symons, 1961). This apparent compensatory rate of absorption could explain, at least in part, the report that there was little or no malabsorption at the site of the infection (Table II).

TABLE I

Combined digestion and absorption of protein by rats infected by Nippostrongylus brasiliensis *and killed one hour after feeding in one experiment and 17 hours in another*

	Infected		Uninfected
	Percentage Absorbed		
After 1 hour	50.0	p = 0.005	31.1
After 17 hours	77.5	ns	78.5

TABLE II

Absorption of glucose by jejunum, distal ileum and entire small intestine of the rat infected by Nippostrongylus brasiliensis

	Infected		Uninfected
Jejunum m.moles/hr/g dry mucosa	0.88 ± 1.02 (S.D.)	p < 0.01	3.92 ± 2.12
*Distal ileum m.moles/hr/g dry tissue	2.18	p < 0.001	1.38
Entire Small Intestine mg/40 min	200 ± 20	ns	181 ± 42

*Means of four separate experiments pooled for statistical purposes: See Symons (1961).

An important study of infection of sheep by *Ostertagia circumcincta* that supports some of the work with nippostrongylosis of the rat was described by Parkins, Holmes and Bremner (1973). They found that the negative nitrogen balance in this infection was due to lower food consumption and a significantly higher urinary nitrogen excretion. They also reported that greater negative nitrogen balances occurred in animals fed low levels of crude protein.

A further interesting observation of an apparent depression of digestibility was reported by Steel (1974). It is a common observation that the degree of inappetence of sheep with gastrointestinal nematode infections is directly related to the severity of the disease. Steel reported that the amount of nitrogen leaving the small intestine was linearly related to the amount entering from the abomasum in both worm-free and sheep infected with *T. colubriformis*. However, with increasing food intake the flow of nitrogen from the ileum increased at a more rapid rate in the infected sheep. Because it is the more lightly affected sheep that have the greater food intake, it follows from this observation that it was these animals that had a significantly lower apparent digestibility. Other experimental evidence suggested that this apparent paradox of lower digestibility by the less affected animals was due to the loss of serum proteins and other endogenous protein such as desquamated epithelial cells. This endogenous nitrogen was readily reabsorbed by the relatively inappetent more severely affected sheep, whereas in the marginally affected the capacity for complete reabsorption was exceeded.

The work of Castro and his associates, who worked with *Trichinella spiralis* infections of the guinea pig must be mentioned, because not only is this an example of experiments that examined aspects of the problem with care, but also because their results do not entirely support those with nippostrongylosis of the rat. They measured glucose absorption by the guinea pig both *in vivo* by infusion and *in vitro* with the everted sac technique (Castro, Olson and Baker, 1967). With 10,000 larvae, but not with 5,000,absorption was depressed, firstly in the upper 3/4 of the intestine and then in the fourth quarter at a later stage of the infection. Malabsorption returned to normal in the same order as the infection had developed.

Before finally assessing the importance of malabsorption to the pathophysiology of parasitic disease, it is pertinent to look for the cause of malabsorption of the wide range of substances that has been described.

An obvious approach is to correlate malabsorption with the

severity of the lesions as has been done, for example, with hookworm disease of man. The opinions as to whether this is important are conflicting (Symons, 1969).

Castro *et al* (1967) pointed out that from their observations with trichinosis, it could be assumed that malabsorption in early infection was associated with a loss of absorptive area due to flattening of the mucosa and fusion of villi. In a later phase of the disease it was observed that the only form of abnormality accompanying malabsorption was the replacement of the normal columnar cells with abnormal flattened or cuboidal epithelial forms. From time to time it has been suggested that derangement of enzyme activity might account for malabsorption. It was reported that although the total activity of trypsin and amylase in the jejunal contents was not reduced by the infection of the rat by *N. brasiliensis*, the activities of the jejunal mucosal enzymes maltase, leucine aminopeptidase and akaline phosphatase were about a quarter of their normal values (Symons and Fairbairn, 1963). Despite this deficiency of maltase, there was no significant failure of maltose digestion or absorption (Symons, 1966).

The activities of a number of disaccharidases in the intestinal phase of trichinosis of the guinea pig have also been examined by Castro and Gentner (1972). The activities of maltase, sucrase, trehalase and palatinase were severely reduced by 10 to 35-fold in the upper small intestine. They concluded that weight loss and diarrhoea in this infection might be due to impaired digestion and absorption. Their reports of glucose malabsorption referred to above appeared to support this. However, as the *in vivo* measurements were made over only 30 minutes, it would be interesting to continue this study over a longer period because, as has already been stated, measurements of absorption over a short term may be misleading.

The activities of chymotrypsin and trypsin were found to be depressed in chickens infected by *Ascaridia galli*. Yet despite a reduction of 30-40 *per cent* in proteolytic activity, this had no effect upon protein absorption (Hurwitz, Shamir and Bar, 1972; Hurwitz, Shamir and Nitsan, 1972).

In conclusion, it can be said that although enzyme deficiencies may affect digestion and absorption at the site of infection, there is no unequivocal evidence that they are a major cause of malabsorption.

The observation of Castro *et al.* (1967) quoted above, that abnormalities of villi and their epithelial cells may explain malabsorption, is relevant to some work on jejunal malabsorption in nippostrongylosis of the rat (Symons *et al.*, 1971).

Firstly, it was shown that the respiratory activities of

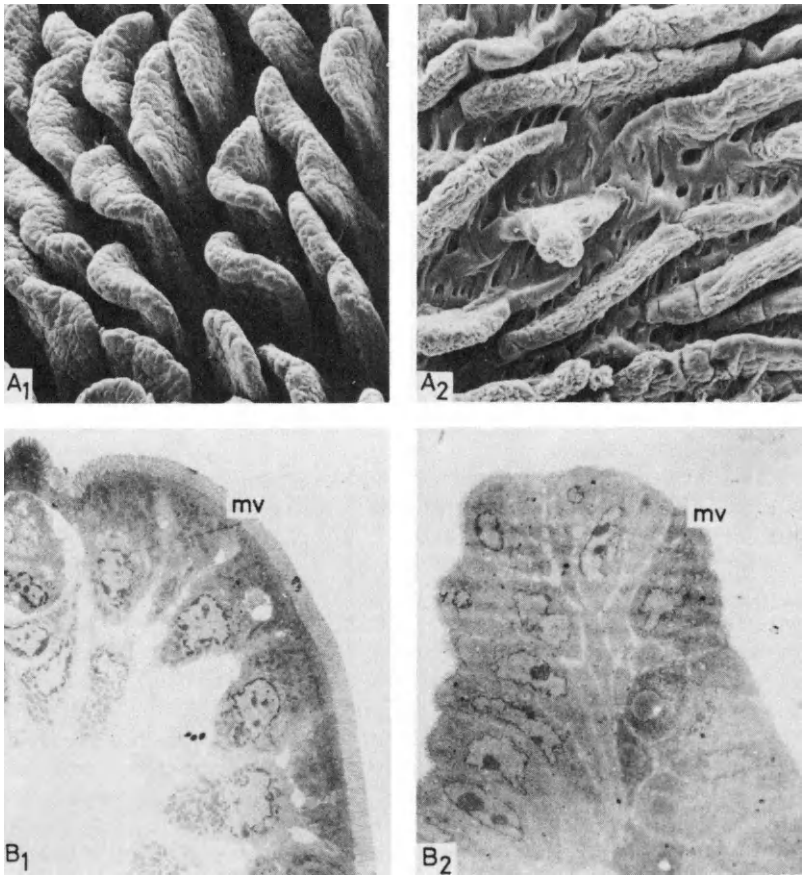

Fig. 1. Electorn microscopy of the rat intestine illustrating the loss of surface area due to villous atrophy and abnormal brush borders when infected by Nippostrongylus brasiliensis.

A_1 Scanning electron microscopy (SEM) of normal villi. (X125)

A_2 SEM of mucosa from infected rat. (X125)

B_1 Transmission electron microscopy (TEM) of a normal villus. MV = microvilli of brush border. (X5,200)

B_2 TEM of a villus with abnormal microvilli on crypt-like epithelial cells from infected rat. (X2,350)

isolated epithelial cells or mucosal mitochondria were not affected by the infection. But by transmission electron microscopy it was shown that a variable proportion of jejunal villous epithelial cells were immature and cryptlike, with poorly developed brush-borders. Crypt cells are known to be deficient in enzymes associated with digestion and absorption. These cells migrated from crypts to the villi without differentiation when the rate of turnover and proliferation of epithelial cells is increased in this infection (Symons, 1965).

Later it was clearly shown by scanning electorn microscopy that the villous surface area of this region of the intestine is decreased by villous atrophy (Symons, 1975). This finding supports that of Barker (1973) who also showed by scanning electron microscopy a more severe degree of villous atrophy and reduction of surface area in sheep infected with *T. colubriformis*.

It was concluded, therefore, that jejunal malabsorption in nippostrongylosis is due to a combination of the appearance of undifferentiated non-absorbing epithelial cells on the villi and a reduction of surface area due to villous atrophy (Fig. 1).

One is now left with two questions, firstly, whether parasitic malabsorption exists at all and, secondly, if it does, is it an important explanation of poor growth and production? In answer to the first question one can say that apart from malabsorption at the site of infection the evidence so far presented that it is, is not convincing. From the work at the McMaster Laboratory it is unlikely that malabsorption is a major symptom of these diseases and that what evidence there is for malabsorption must be interpreted with care. The explanation by Parkins *et al.* (1973) for low digestibility in sheep infected with *O. circumcincta* has already been referred to. Al-Rabii and Platt (1964) concluded that the severely reduced nitrogen balances by dogs infected by *Toxocara canis* when fed low protein diets were due to faster catabolic rates and not to poor digestibility.

Does malabsorption explain poor growth and production? Consider the fact that sheep infected in the abomasum by *Trichostrongylus axei* and calves by *Ostertagia ostertagi* in the same organ, sheep with *T. colubriformis* in the small intestine and calves with *Oesophagostomum* spp. in the distal ileum and large bowel, may all lose weight or grow poorly. These are infections of three different regions of the gastrointestinal tract, each with a different function. It is most unlikely that malabsorption would be a symptom common to each of these infections.

If malabsorption cannot account for the important clinical manifestations of intestinal parasitic infection, is there an alternative explanation?

Recent experiments have shown that the rate of skeletal muscle protein synthesis is depressed in mice infected by *Nematospiroides dubius* and in guinea pigs and sheep by *T. colubriformis*. The rate of whole-body protein catabolism was increased in infected mice. Concurrently with these changes the rate of liver protein synthesis is evalated (Table III).

TABLE III

Skeletal muscle protein synthesis and whole-body protein catabolism in mice infected by Nematospiroides dubius

	Uninfected (Ad-lib)	Infected (Anorexic)	Uninfected (Ad-lib)	Uninfected (Reduced intake)
Incorporation of ^{14}C-L-leucine (dpm/mg N)	581±176	370±77	632±171	351±143
		$p = 0.03$		$p < 0.001$
Protein turnover (^{75}Se-seleno-methionine) $t_{\frac{1}{2}}$ (days)	26.2±4.2	17.4+5.5	-	13.5±1.8
		$p < 0.001$		ns

Anorexia is the major cause of the changes of muscle protein metabolism, whereas the faster rate of liver portein synthesis is confined to the production of serum proteins, which is stimulated by their loss into the intestine. The rate of synthesis by wool follicles is also depressed in sheep infected with *T. colubriformis*. This is consistent with poor

wool growth in infected sheep (Symons & Jones, 1971, 1972, 1975; Symons, Jones & Steel, 1974).

Following this work, it is suggested that anorexia exacerbated by the loss of serum proteins that has been shown to be a feature of several gastrointestinal infections, and both of which affect protein metabolism, is the most important pathophysiological response of the host to gastrointestinal infection. These and the unpublished results of recently completed experiments indicate, however, that another undefined factor besides anorexia is also responsible for poor growth. If malabsorption were present it would aggravate the demand by these changes upon the economy of the host.

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PATHOPHYSIOLOGY OF PARASITIC INFECTION
PLASMA PROTEIN METABOLISM

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Introduction

The ways in which parasites exert their effects upon the host animal are manyfold, e.g. blood sucking, tissue destruction during larval migration or feeding, mechanical or chemical irritation of contact surfaces, liberation of toxic metabolites, obstruction of excretory ducts, air passages, blood vessels, etc. Whether the structural alterations caused by the parasites will result in functional disturbances severe enough to be observed as parasitic disease will depend, not only upon the mass of infection but also upon environmental and management factors such as, for example, the level of nutrition, climate, grazing procedures, etc. Further, organs vary considerably in their ability to compensate for loss of functional tissue cells. Thus, the functional reserve of the liver is so large that as much as 60-70 per cent of the liver tissue may be lost before dysfunction becomes evident (Jubb and Kennedy, 1970). The compensatory ability of the gastrointestinal tract is also very considerable. Thus, Symons (1971) found that rats infected with *Nippostrongylus brasiliensis* compensated for malabsorption in the parasitized location of the small intestine (jejunum) by an increased rate of absorption in the distal ileum so that the absorption from the small intestine as a whole remained unchanged. On the other hand, organ systems with a more limited functional reserve - such as the lungs or the large intestinal arteries of the horse - have a lower tolerance level and dysfunction may be evident at an early stage of the parasitic infection.

When discussing the pathophysiology of parasitic infections this question of the relationship between structure and function is a very important one. To what extent is the tissue damage mirrored by the functional tests by means of which we try to assess it? A partial answer to this question may be derived from the fact that new methods are constantly being developed with the purpose of permitting a still deeper insight into the host-parasite interactions. Improved technology is a *conditio sine qua non* if we are to obtain infor-

mation of, for example, the early effects of a parasitic infection upon the host - and *vice versa*. If we were to study plasma proteins only by means of Kjeldahl analysis and electrophoresis we would soon reach a limiting point; for example, we would not be able to state whether a low plasma level of a protein was due to a decreased synthesis or an increased catabolism. Even a normal level might, in fact, mask a situation of abnormality, e.g. an accelerated catabolism by an equally accelerated synthesis. Only when synthesis can no longer keep pace with catabolism would our static parameters reflect the abnormality and this event would probably occur late in the course of infection. The rapid accumulation of knowledge that has characterized the last few decades has only been possible because of the continuous advent of refined technical tools, such as radioactive tracers, electron microscopy, immunochemical protein analyses and improved methods for isolation and purification of individual proteins.

GENERAL SURVEY OF PLASMA PROTEIN METABOLISM

A healthy 500 kg cow will have a total plasma protein concentration of approximately 7 grams/100 ml, of which approximately 3 grams are albumin and 2 are immunoglobulins, the remaining 2 grams being represented by some thirty different proteins. With a plasma volume of 20 litres (4 per cent of the bodyweight) the vascular pools of albumin and immunoglobulins are approximately 600 and 400 grams, resp. The total pools (vascular + extravascular) are approximately 1,500 grams (albumin) and 800 grams (immunoglobulins). Thus, approximately 30 per cent of the total albumin pool is circulating in the plasma. For the immunoglobulins, the extravascular: intravascular ratio varies with the molecular size: Fifty per cent of the total IgG pool is vascular, the corresponding figure for IgM being only 25 per cent (Jensen 1969, Nansen 1970).

The example above is an indication of the large number of plasma proteins that are involved in the metabolism of an average non-pregnant and non-lactating cow. For each protein there is a characteristic turnover rate, with a continuous breakdown counterbalanced by synthesis so that vascular and extravascular pool sizes are kept constant. By means of proteins labelled with radioisotopes and injected into the blood stream it is possible to follow, from the decline in plasma radioactivity and the radioactivity appearing in the excreta, the fate of the injected molecules and from this all relevant kinetic parameters may be calculated. Table I Lists some metabolic data for albumin and immunoglobulin-G in

Species	Calculation method[+])	Immunoglobulin-G					Reference
		Half life, days	Fractional catabolic rate, %/day	Preparation	Half life, days	Fractional catabolic rate, %/day	
Cow	S	20.7		Mixed	21.2		Dixon *et al.* (1953)
	M	20.6 (16.5-28)	8.2 (6.4-10)				Nielsen (1966a)
	N			IgG-1	7.9	14.1	Nansen (1970)
	N			IgG-2	17.6	6.6 (5.4-9.3)	Nansen (1970)
	M	24.0 (19.26.8)	6.7 (5.9-8.3)				Halliday *et al.* (1968)
Sheep	Excr.	20 (14-29)	7.5 (4.4-10.0)				Campbell *et al.* (1961)
	M	19.4 (14-23.9)	7.7 (7.1-8.7)				Nansen *et al.* (1968)
	N			IgG-1	9.5 (7.5-12.2)	12.7 (10.2-15.5)	Nansen & Aalund (1972)
	N			IgG-2	10.6 (7.8-14.8)	12.0 (8.5-16)	Nansen & Aalund (1972)
	Excr.	23.4 (21-29)	6.2 (4.7-7.8)				Holmes & MacLean (1971)
Goat				IgG-1	12.6 (9.5-16.5)	-	Aalund (1968)
				IgG-2	23.2 (15.5-30.5)	-	Aalund (1968)
Horse	S	19.4 (18.2-20.5)	3.6 (3.4-3.8)	Mixed	11.0 (9.0-13.5)	6.5 (5.1-7.7)	Mattheeuws *et al.* (1966)
	N			IgG-2	14.3 (12.4-16.4)	7.9 (7.5-8.1)	Nansen & Riising (1971)

[+]) S: Sterling (1951) M: Matthews (1957) N: Nosslin (1966) Excr.: Based upon excretion of radioactivity (see text)

Table I. Normal albumin and immunoglobulin-G metabolic parameters in domestic animals Mean values and ranges (parentheses).

various domestic animals. A detailed account of the methodology involved is outside the scope of the present survey. Methods and calculations in general use have been reviewed and discussed by Jarnum (1963), Andersen (1964) and Nansen (1970). Most authors have preferred the two iodine isotopes, ^{125}I and ^{131}I in tracer studies with plasma proteins. Both are relatively safe to handle and suitable methods for trace-labelling have been worked out so that denaturation of the protein is avoided.

It should be borne in mind that the various methods for calculating plasma protein turnover data do not always yielding comparable results. Calculation is based upon measurement of the catabolic rate of the protein, either from a mathematical treatment of the plasma disappearance curve over the weeks following injection (calculation methods of Matthews, 1957 and Nosslin, 1966) or from measurement of radioactivity excreted in faeces and urine over the experimental period (method of Pearson *et al.*, 1958). The latter method is seldom employed in studies with large animals where quantitative collection of urine and faeces is extremely difficult; the method is more applicable in studies with laboratory animals. Another problem with this method is associated with the existence of a free iodide pool into which iodine liberated at the catabolic site is distributed before ultimate excretion via the kidneys or intestine. In cattle and sheep excretion of iodide from this pool is very slow, appr. 50 per cent per day (Pedersen, 1973) compared with approximately 160 per cent per day in man (Rossing and Andersen 1965). Accordingly, there is a rather considerable delay between liberation of iodine and its ultimate excretion and since the catabolic rate is calculated from the equation:-

$$\frac{\text{excreted radioactivity (faeces and urine)}}{\text{plasma radioactivity on the day of collection}}$$

it follows that catabolic rates calculated by this method tend to be overestimated, and this more accentuated when the rate of catabolism is faster. Thus, Nansen (1970 found a fairly good agreement between catabolic rates calculated by the method of Pearson *et al.* (1958) and by Nosslin's method (1966) when studying IgG2 metabolism in normal cattle whereas with IgG1, which is metabolized at a much faster rate,

turnover data are greatly over estimated with the former method. This should be considered in metabolic studies with parasitized animals because parasitic infections are usually associated with an accelerated catabolism.

Metabolism of a plasma protein occurs as a first-order reaction, i.e. a constant fraction is catabolized and synthesized per time unit. Functionally, synthesis and breakdown occur in, or in close proximity to, the plasma compartment, i.e. newly synthesized protein and breakdown products are released into the blood stream before further distribution and excretion. Andersen (1964) has shown that metabolic data are only valid under this assumption. However, this does not imply that the plasma is the *anatomical* site of synthesis and catabolic breakdown. These processes may well occur elsewhere - the gastrointestinal tract, kidney or other sites - provided that these locations are in a state of rapid equilibration with the plasma compartment.

Status (number of animals)	Vascular Pool (mg/kg)	Fractional catabolic rate (%/day)	Half life (days)
IgG-2 deficiency (n = 3)	0	4.6	27.1
Normal (n = 14)	238	6.6	17.6
Chronic pyogenic infection (n = 9)	1239	8.7	13.2

Table II. The relationship between vascular pool size and metabolic rate of immunoglobulin-G2 (after Nansen, 1970).

For albumin and immunoglobulin-G there is a direct correlation between the plasma concentration, or size of the vascular pool, and catabolic rate. Subjects with a high plasma level have a high catabolic rate and a correspondingly short plasma half life. This was first known in mice made hyperimmunoglobulinaemic by large infusions of the various immunoglobulin classes (Fahey and Sell 1965). In cattle with increased IgG2 levels due to pyogenic infections, Nansen and Nielsen (1966) observed the same phenomenon: the higher the serum-IgG level the higher the catabolic rate. That low IgG2 levels are associated with a low turnover rate was shown by Nansen (1970) in cattle with a heriditary deficit of this

immunoglobulin (Table II). Similar findings have been reported for albumin metabolism (Gordon *et al.*, 1959, Picou and Waterlow 1962, Montgomery *et al.*, 1962). It is not yet known whether IgG1 metabolism in ruminants is regulated in the same way. In the bovine, IgG1 is a secretory immunoglobulin appearing in colostrum and other secretions (Aalund, 1968). The fact that (at least in cattle and goats) IgG1 is metabolized at a much faster rate than IgG2 might be related to this particular function.

PLASMA PROTEIN METABOLISM IN HELMINTHIC INFECTIONS

Abomasal trichostrongyles

When, 2-3 decades ago, suitable methods for protein analysis became available, the new techniques were soon applied in parasitological work. One of the first detailed reports on chronic infection with the medium stomach worm *(Ostertagia ostertagi)* in cattle was that of Martin *et al.* (1957). Clinical and pathological features were described and it was shown that affected animals were severely hypoproteinaemic. Leland and co-workers (1959, 1960a,b) infected calves, sheep and lambs with *Trichostrongylus axei* larvae and observed reduced plasma volumes and hypoproteinaemia, due mainly to a decline in albumin. These authors tentatively suggested that these alterations might be ascribed to a decreased protein synthesis and, possibly, a loss of plasma protein through the abomasal lesions. A study of chronic abomaso-enteritis in cattle (Nielsen and Aalund, 1961) confirmed that animals with clinical parasitic abomasitis (ostertagiasis) had severe hypoalbuminaemia. The authors discussed their findings in light of the then newly discovered syndrome of "protein-losing gastroenteropathy" in man, where a gastric leakage of plasma proteins had been demonstrated in a variety of disorders, e.g. in hypertrophic gastritis, a condition that is patho-antomically very similar to chronic ostertagiasis (type II or winter ostertagiasis) in cattle. A gastric leak of macromolecules was demonstrated by Citron *et al.* (1957) and Schwartz and Jarnum (1959) who showed that patients with hypertrophic gastritis had a marked albumin hypercatabolism because albumin passed from the circulation to the gastric lumen in considerable quantities. Over the next few years a large number of papers were published on protein-losing gastroenteropathies and today the list of diseases with a proven gastrointestinal loss of plasma proteins comprises more than 40 entities. Several of these entities show pathological lesions rather similar to various diseases of domestic animals, e.g. hypertrophic abomasitis/gastritis in ruminants and swine (ostertagiasis and hyostrongylosis), hypertrophic enteritis (Johne's disease), regional enteritis of pigs, and others, It would

seem logical, therefore, to examine whether a similar pathophysiological abnormality could be demonstrated in animals with gastrointestinal diseases, and ostertagiasis was the first entity to be studied. Nielsen (1962) was able to demonstrate plasma albumin in abomasal digesta taken from animals with chronic ostertagiasis. These animals had very low levels of plasma protein (3.8 - 5.5 gr./100 ml) and albumin (0.5 - 1.6 gr/100 ml). This, although suggesting abomasal leakage of plasma protein, was not definite proof and, in fact, Cornelius *et al.* in a report from the same year (1962) failed to find evidence of hypercatabolism of ^{131}I-labelled albumin in cattle with parasitic gastroenteritis. However, the albumin preparation used by these authors was obviously denatured which made it difficult to draw valid conclusions. Mulligan *et al.* (1963) were the first to publish results from studies with ^{131}I-albumin where it was proven beyond doubt that heifers with ostertagiasis had a marked albumin hypercatabolism, thus indicating that bovine ostertagiasis is a true protein-losing gastropathy. This was confirmed in subsequent studies with cattle (Nielsen 1966a, Halliday *et al.*, 1968) and sheep (Holmes and MacLean, 1971). Nielsen's study comprised various other disease entities such as Johne's disease and simple catarrhal abomaso-enteritis and he presented evidence that the functional state of the gastrointestinal tract might be an important factor in the pathogenesis of the protein leakage. Thus, when patients were studied during a period with marked clinical symptoms, such as diarrhoea, the albumin and immunoglobulin turnover rates were greatly accelerated whereas patients studied during a quiescent period or during convalescense had normal or even subnormal turnover rates (Table III and IV; Figure 1). This finding, although not unanimously accepted by other authors, has received support from studies with ^{131}I-IgG carried out by Nansen (1970). Further, Nielsen and Dich (1965) studied the transfer of ^{131}I-albumin and ^{125}I-immunoglobulin G into the gut of normal calves fitted with re-entrant loops at various sites of the small intestine. This study confirmed that part of *normal* albumin and immunoglobulin breakdown occurs in the gut and when large volumes of fluid - magnesium sulphate or intestinal digesta - were instilled through the loops the transfer of both plasma proteins into the loop lumen increased greatly. From these results it seems justified to conclude that the plasma protein leakage is influenced by intestinal transit time and perhaps other physical or chemical factors in the intestine or intestinal digesta. Nor would it be surprising that an aggravation of clinical symptom should add quantitatively to the protein loss.

TABLE III

Animal	Clinical state	Total plasma protein gr/100 ml	Plasma albumin gr/100 ml	Fractional catabolic rate, per cent/day	Plasma half life, days
1	Diarrhoeic	5.7	1.29	15.5	11.0
2	"	5.3	2.04	13.8	10.9
3	"	5.1	1.94	17.6	8.0
4	"	4.0	0.81	9.9	11.5
5	"	5.2	1.48	11.0	10.0
6	Quiescent	5.0	1.48	6.5	20.1
7	"	4.4	1.39	5.6	22.0
Eight normal controls (mean)		7.4	2.84	8.2	20.6

^{131}*I-albumin metabolism in 7 bovine patients with chronic ostertagiasis (Type-II ostertagiasis). Note difference in fractional catabolic rates and plasma half lives between diarrhoeic and non-diarrhoeic animals. (after Nielson, 1966a).*

TABLE IV

Diagnosis	Status	Fractional catabolic rate (%/day)		Plasma half life (days)	
		^{131}I-alb.	^{125}I-IgG	^{131}I-alb.	^{125}I-IgG
Ostertagiasis	Diarrhoeic	13.8	12.4	10.9	8
Ostertagiasis	Quiescent	5.6	5.8	22.0	19.0
Control	Normal	8.5	8.3	20.4	14.7

Effect of clinical status upon metabolism of ^{131}I-albumin and ^{125}I-immunoglobulin G in chronic ostertagiasis. The immunoglobulin preparation contained both IgG1 and IgG2 (after Nielsen, 1966a).

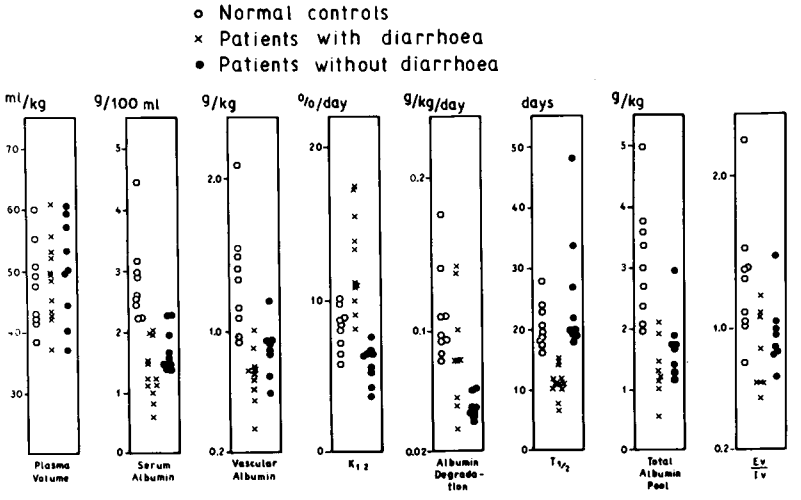

Fig. 1. Diagramatic survey of ^{131}I-albumin turnover in cattle with chronic gastrointestinal disorders, including Type II ostertagiasis, Johne's disease and chronic catarrhal abomaso enteritis. Explanation of symbols: $K_{1\,2}$ = fractional catabolic rate. $T_{\frac{1}{2}}$ = plasma half life. $\frac{Ev}{Iv}$ = extravascular intravascular ratio. Calculation method: Matthews (1957). (after Nielsen 1966a).

Hyostrongylus rubidus *infection in pigs*

This trichostrongylid causes a gastric lesion that is rather similar to the abomasitis caused by *O. ostertagi* in ruminants. Nielsen (1966b) studied two cases with ^{131}I-albumin and failed to find evidence of hypercatabolism. One of the animals was studied during clinical remission. On the other hand, Dey-Hazra *et al.* (1972) in studies with pigs inoculated with 500 and 1,000 larvae/kg bodyweight showed that 25 days post infection the faecal radioactivity following intravenous injection of ^{51}Cr-albumin was three to five times higher in infected pigs than in the controls. Titchener *et al.* (1974) confirmed this finding. After inoculation of 60,000-350,000 infective larvae there was a decline in total plasma protein and albumin levels on day 16, the lowest values being reached at day 20. With the larger doses of larvae, clinical diseases (anorexia, vomiting, diarrhoea) was noticed in half of the pigs. Studies with ^{131}I-albumin revealed marked hypercatabolism 15-25 days post infection. There was a distinct difference between clinically affected pigs and pigs without symptoms, the degree of hypercatabolism being most pronounced in the former group. Both groups had an increased faecal excretion of radioactivity after intravenous injection of ^{131}I-PVP (polyvinylpyrrolidone) and again the excretion was higher in the diarrhoeic pigs. It seems that the leakage of proteins is associated with the period when larvae are leaving the gastric pits and migrating onto the surface of the stomach, an event that is accompanied by profound cellular damage.

Other gastrointestinal parasites

Evidence has been presented that *Strongyloides ransomi* infection in pigs causes an enteric loss of plasma proteins (Dey-Hazra *et al.*, 1972). In *Nippostrongylus brasiliensis* infection in the rat the protein leak appears to be linked with the immunological events taking place at the time of expulsion of the worm burden (Murray *et al.*, 1971, Murray, 1972).

It is highly probable that increased plasma protein leakage will occur in parasitic infections other than those mentioned above. Infections with *Haemonchus* spp. in ruminants and *Strongylus* spp. in the horse would appear to be logical possibilities. Infections accompanied by a frank blood loss e.g. hookworms and coccidiosis - have not been considered in the present survey.

Morphology of the gastrointestinal mucosa during protein loss

Waldmann and co-workers described severe abnormalities of

the intestinal lymph vessels in patients with protein-losing gastroenteropathies (Waldmann *et al.* 1961, 1962, Waldmann 1966). The lesion is characterized by a dilatation of the chylus vessels, so-called *intestinal lymphangiectasia.* An identical lesion was described in cattle by Nielsen and Andersen (1967) (Fig. 2.).

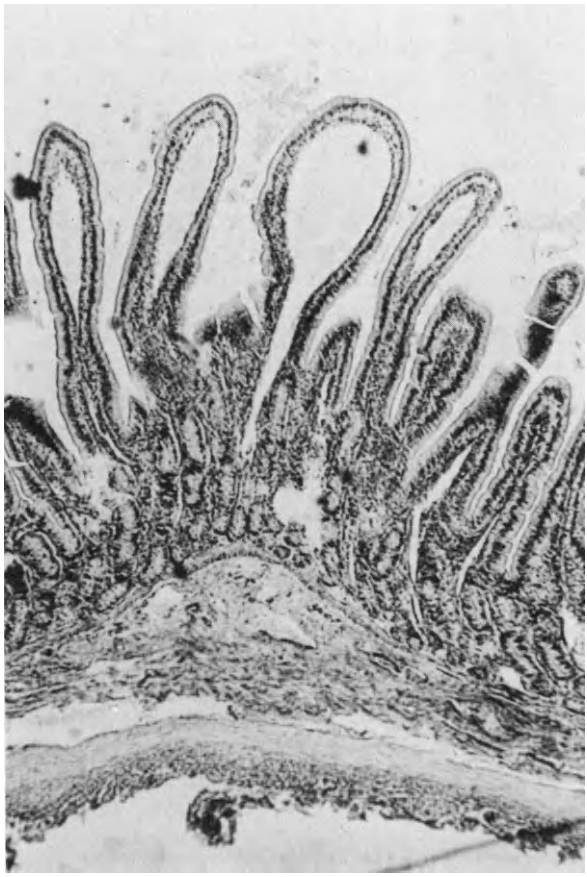

Fig. 2. Intestinal lymphangiectasia in a heifer with Type II ostertagiasis. Jejunal villi are deformed due to distension of chylus ampoules (x 80). (After Nielsen and Andersen 1967)

Oozing of lymph through rupture of such dilated lymph vessels would seem to be a logical explanation of the macromolecular leakage. In this context it should be mentioned that several studies have shown that the macromolecular leakage involves

not only albumin and IgG but other plasma proteins, for example, IgM, IgA, coeruloplasmin and transferrin (Strober *et al.* 1967, Jarnum and Jensen 1972, Jensen 1973). The study of Strober *et al.* (1967) in 18 patients with intestinal lymphangiectasia is particularly intersting. Most patients and decreased plasma levels of albumin, IgG, IgM and IgA due to accelerated catabolic rates. A loss of lymph through ruptures of the intestinal chylus vessels was considered likely and further sustained by the fact that the patients had very low blood lymphocyte counts and showed various signs of a reduced immune response such as hyporeactivity to tuberculin, low serum antibody titers in response to injection of various bacterial antigens, a decreased ability to reject skin grafts and a high incidence of infections. It seems that this lesion may cause a profound general depletion of the immune system, through a loss of antibodies (immunoglobulins) and immunocompetent cells (lymphocytes).

Murray (1969) and Murray *et al.* (1970, 1971), in electron microscopical studies, demonstrated lesions of the intercellular junctions between epithelial cells in abomasa from cattle with ostergiasis and in the intestine of rats infected with *Nippostrongylus brasiliensis*. In ostertagiasis, an electron-dense material, probably plasma proteins, was seen in the *zona occludens* between the epithelial cells. In *N. brasiliensis* infection, macromolecules (horseradish peroxydase) injected intravenously could be seen flooding through the damaged junctional complexes into the lumen of the gut.

Fascioliasis

Chronic fascioliasis is associated with profound alterations in the plasma protein profile. Total protein levels usually remain within the normal range but electrophoresis reveals hypoalbuminaemia and hyperimmunoglobulinaemia (Simesen *et al.* 1968). Immunoquantitation has revealed that the raised immunoglobulin level is entirely due to an increase of IgG1 whereas IgG2 remains in the normal range (Nansen 1970). Recent studies by Movsesijan *et al.* (1974, pers. comm.) have shown that the increase in IgG1 is paralleled by a rise in circulating antibodies directed against *F. hepatica* antigens. A significant proportion of this antibody activity resides in the IgG1 molecule.

Studies of experimental fascioliasis have revealed several interesting features of plasma protein and erythrocyte kinetics. It was shown in rabbits that turnover rates of albumin and erythrocytes are not altered significantly during the prepatent period when the fluke larvae migrate through the liver tissue. However, as the infection becomes patent in the bile

ducts, turnover rates and faecal excretion of radioactivity increased markedly, showing that there was a loss of protein through the inflamed bile ducts (MacLean *et al.* 1968, Dargie et al. 1970, Dargie and Mulligan 1971). ^{131}I-albumin and IgG2 studies in cattle and sheep confirmed that a patent *F. hepatica* infection causes a plasma protein loss (Nansen *et al.* 1968, Holmes *et al.* 1968, Nansen 1970, 1971), (See Table V).

Table V

Animal	Plasma IgG-1 (gm/100 ml)	Plasma IgG-2 (gm/100 ml)	IgG-2 fractional catabolic rate (%/day)	IgG-2 Half life time (days)
1	1.47	0.82	11.8	8.7
2	1.73	0.66	10.8	11.2
3	1.61	0.74	10.4	10.6
4	1.83	0.99	13.0	8.3
5	1.11	0.82	8.1	12.9
6	1.90	0.00+)	7.4	16.2
7	1.73	0.71	12.5	7.1
8	1.18	0.71	8.4	11.2
9	0.89	0.11	10.4	9.4
Mean	1.49	0.70	10.3	10.6
14 normal controls	0.73	0.66	6.6	17.6

+) Not included in the mean

Metabolic data of IgG1 and IgG2 in 9 animals with chronic fascioliasis (after Nansen, 1970).

Obviously, a substantial part of this loss is associated with blood sucking but Dargie *et al.* (1970) presented evidence that the macromolecule leakage is greater than can be accounted for entirely by the blood loss. Apparently, there

is a continued passage of plasma after the fluke has finished its blood meal. Histology of the smaller bile ducts reveals enormous enlargement of the surface area due to epithelial hyperplasia, i.e. a lesion that would be liable to contribute to this additional plasma leakage (Nansen 1970).

As mentioned above, plasma levels of albumin decrease during fascioliasis whereas IgG levels increase. Since albumin and IgG catabolism are increased at comparable rates the explanation could be different abilities of the synthesizing tissues to compensate for the protein loss. In fact, Nansen (1970, 1971) has shown that synthesis rate for albumin was normal or slightly decreased whereas IgG2 synthesis was greatly accelerated and thus able to maintain normal or increased plasma concentrations. The insufficient albumin synthesis may be explained by fibrotic lesions and atrophy of hepatic tissue, which would reduce the number of cells synthesizing albumin, the only synthesis site for this protein being the liver. IgG synthesizing cells (lymphocytes and plasma cell), on the other hand, are more numerous and proliferative and, furthermore, Nansen (1970) found evidence of a local immunoglobulin synthesis in the fluke-infected liver and histological studies revealed an abundance of lymphocytes and plasma cells in the smaller bile ducts. It would seem worth while to conduct studies on IgG1 synthesis in *F. hepatica* infections. As mentioned earlier, there is a distinct rise of this immunoglobulin and evidence has been presented that circulating IgG1 carries antibody against fluke antigens (Movsesijan *et al.* Pers. Comm.). No answer has yet been given to the question, whether these antibodies are functional i.e. "protective". However, attempts to protect animals against *F. hepatica* by means of immune sera have mostly been negative and, furthermore, a number of immunoglobulins, including IgG1, have been demonstrated in bile from infected animals. Thus, the flukes are actually surrounded by a fluid containing antibody which does not appear to affect them in any way (Nansen (1970).

Other trematode infections.

Dargie *et al.* (1973) studied albumin turnover in sheep with an experimental *Schistosoma mattheei* infection. From the 6th week post infection albumin levels decreased and immunoglobulins increased, i.e. similar findings as reported for fascioliasis. Albumin catabolic rate was increased and isotope (^{131}I) was excreted in increased quantities in the stools. Thus, it was demonstrated that the infection caused an intestinal albumin loss.

CONCLUDING REMARKS

Studies of plasma protein kinetics have contributed to our knowledge of the pathogenesis and pathogenic effects of a number of parasitic infections. The use of isotopically labelled proteins and other metabolites (amino acids, carbohydrates, etc.) may be expanded in an attempt to gain more insight in the metabolic alterations caused by parasites in their host or, for that matter, alterations caused by hosts in their parasites. It seems relevant to examine, for example, the extent to which the metabolic parameters of the various immunoglobulin classes are associated with the immune response of the host animal. Examples have been quoted from *Nippostrongylus* studies in the rat, where the intestinal protein leakage is associated with expulsion, an immunological event triggered by release of vasoactive substances and resulting in increased mucosal permeability which allows antibodies to reach the target site, the gut lumen. Sudden, or gradual, elimination of worm burdens is a very common phenomenon and it might be worth while to do similar studies in domestic animals. Consideration might also be given to studies with IgG1 in fascioliasis with the view of elucidating its possible role in the immunology of this very important parasitic infection. Another obvious field of study would be *Strongylus vulgaris* infection in the horse. The metabolic strain upon the host animal during the migration of larvae in the mesenteric arteries is incompletely understood and studies with labelled proteins, lipids or carbohydrates would be indicated.

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THE ANAEMIAS OF PARASITIC INFECTIONS

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INTRODUCTION

An animal is regarded as being anaemic when the packed cell volume or the haemoglobin concentration per unit volume falls below the accepted normal range for that particular species. This is a static measurement as it only measures these indices at a particular time and makes no allowance for haemoconcentration or haemodilution due to changes in the plasma volume. Changes in the plasma volume are now becoming recognised as contributing to the anaemia in certain parasitic infections such as trypanosomiasis (Clarkson, 1968) and schistosomiasis (Dargie & Preston, 1974).

The conventional haematological indices may also give little indication of the reactivity of the erythropoietic system, as these may be normal and yet the animal may be under considerable haemorrhagic stress as in chronic haemonchosis (Allonby & Dargie, 1975).

It is obviously beyond the scope of this review to deal exhaustively with all the anaemias associated with parasitic infections; therefore it is intended to deal in greater detail with those that the author has personal experience of, while the others will be discussed much more superficially and the reader should refer to more recent reviews, e.g. Holmes & Dargie, (1975).

ANAEMIA OF ECTOPARASITE INFESTATIONS

In the context of animal health the ectoparasites, such as fleas, lice and ticks, are usually considered mainly as disease vectors and not as being directly responsible for the anaemias. However, there is no doubt that they can be responsible for the development of anaemia *per se* if the infections are severe enough. Schalm (1965) described the case of a dog in the veterinary clinic at the University of California suffering from a severe infestation of fleas. This dog had a very low PCV which recovered to normal after removal of the flea population.

Lice infestations are also common throughout the world, and usually go unnoticed unless the parasites are present in

large numbers. Anaemia, unthriftiness and lack of vigour were noted in bovines, heavily infested with the short-nosed sucking louse *Haematopinus eurysternus*, on ranches in Alberta and Sasketchewan (Shemanchuk *et al.*, 1960). They reported that the infested animals had erythrocyte counts ranging from 2.1 to 5 millions/mm^3 compared to the normal parasite-free animals of 6.5 to 8.5 millions/mm^3. They also concluded that the anaemia could be attributed directly to lice, since when these were eradicated the anaemic condition of the animals began to improve immediately, and they returned to normal health in 36-50 days.

The association of ticks with anaemia has been better documented and it has usually been attributed to the rapid engorgement of the female ticks with blood. There is however the possibility that other factors may be involved, such as substances secreted by the salivary glands causing either a dyshaemopoietic effect or a stimulation of red cell destruction but this has still to be fully investigated.

Critical and even fatal anaemias associated with heavy tick infestations have been reported by various writers. Nuttall & Warburton (1908) in their monograph on ticks quote Lounsbury in reference to *Argus persicus* infestations in chickens as follows: "The victims die entirely from loss of blood and inflammation produced by the excessive parasitism". A rapidly fatal anaemia in the horse experimentally infested with "blue ticks", *Boophilus decoloratus* has been reported by Theiler (1921) and Fenstermacher and Jellison (1933) found severe anaemias in the moose, *Alces americana*, infested with winter ticks, *Dermacentor albipictus*.

Jellison and Kohls (1938) have shown that experimental infestations of *Dermacentor andersoni* may cause anaemia in rabbits due primarily to exsanguination by the rapidly engorging female ticks. These authors attempted to measure the amount of blood withdrawn from the host animal during the period of attachment. They calculated that each tick withdrew approximately 1.7 to 2 grams of blood in engorging, or each gram of engorged tick represented a blood loss by the host of between 3.6 to 4 grams of blood.

Riek (1957) in his studies on the cattle tick *Boophilus microplus* estimated that the female tick contained approximately 0.5 ml of blood after engorgement, no allowance being made for any blood excreted by the tick prior to this.

Little (1963) in his investigations on the effect of cattle tick infestations on the growth rate of cattle found that animals with an average daily tick count of 60 gained on average, 22.8 lbs. less than their corresponding tick-free controls. He regarded this level of infection as being relatively light,

and infestations of 4,000 to 5,000 engorged females for a short period were not uncommon, and at times infestations have been estimated up to 20,000 ticks. If Riek's figure of 0.5 ml per tick is used for the calculation this would mean a blood loss equivalent to 10 litres or more of blood. Indeed, Kitaoka (1961) has estimated that *B. microplus* may remove 0.7 to 0.8 ml of blood, and this implies that an animal with 20,000 ticks would lose nearly 20 litres of blood. It seems remarkable that heavily infested animals in the field, dropping about 1,000 engorged adult female ticks per day are able to withstand the effects of this parasite for long periods without succumbing (Riek, 1957).

The sheep tick *Ixodes ricinus* in Great Britain is confined mainly to hill pastures and has been implicated in the anaemia of sheep by McLeod (1933) and Philips *et al.*, (1935). Apart from the specific diseases carried by ticks it has been stated (McLeod, 1938, 1939) that the ticks cause damage to the sheep by irritation and primary anaemia due to removal of blood. The actual estimation of the amount of blood consumed by a tick is difficult to assess, as according to Lees (1943, 1946) the tick passes blood through the alimentary canal almost unchanged prior to the actual process of engorgement. Heath (1951) by estimating the total iron content of both the faeces and the engorged tick calculated that the blood loss of a sheep due to a tick infection of approximately 265 ticks would amount to something in the region of 1,250 ml, i.e. 4.7 ml/tick. He concluded however that this loss of blood spread over the tick period of several months would be unlikely to have any significant influence on the condition of the sheep.

There are many other insects which require blood meals from the host, e.g. mosquitoes, but it is unlikely that these ever consume sufficient blood to cause anaemia (Tatchell, 1969). Tatchell reports observations by Standfast who collected nearly 1,500 mosquitoes in 1½ hours from an area of 2 square feet from a cow in Central Queensland. From this figure it can be assumed that for an average sized animal at least 72,000 mosquitoes would bite in this period. He also reports from a personal communication from Dyce collecting in Southern Queensland, who caught 3,272 *Culicoides* feeding on a cow in one hour and estimated that at least 10,000 fed each night.

In general, therefore, it is considered that the arthropod parasites are more important as disease-carrying vectors than the primary cause of anaemias. However, the diseases which are carried by these arthropods are in many instances responsible for severe anaemias in the host.

THE ANAEMIAS OF HELMINTH INFECTIONS

Trematodes

Fascioliasis

Fascioliasis has a world wide distribution and affects all species of domestic animals. It is generally recognised that the disease occurs in two forms, acute and chronic. The acute disease, usually fatal, is caused by the ingestion of a large number of *F. hepatica* or *F. gigantica* and death occurs while the young, immature, flukes are migrating through the liver parenchyma. Anaemia may occasionally occur prior to death, invariably due to haemorrhage into the peritoneal cavity caused by rupture of the liver; this is probably more common in the older animal.

The chronic form is caused by the adult flukes after they have become established in the bile ducts and is due mainly to anaemia caused by their haematophagic activities.

It would appear that in the sheep, acute infections of *F. gigantica* are more important than chronic infections. Kendall (1954) reported that it was impossible to keep sheep and goats in the worst affected areas of Pakistan. Also Condy (1962) reported that outbreaks of *F. gigantica* in Rodesia were nearly always of the acute type. Infections of approximately 5 metacercariae/kg live weight have been reported to produce acute infections (Grigoryan, 1953; Davtyan, 1953).

In *F. hepatica* infection, the chronic disease is the more common, and provided the burden of adult flukes is sufficiently high, a characteristic anaemia results. This can be excerbated by a poor level of nutrition (Berry & Dargie, 1975).

According to most workers, the anaemia is haemorrhagic. Railliet (1890) injected plaster of paris containing a blue dye intravenously into an infected sheep and recovered both plaster and dye from the caecum of the flukes. Hsu (1939) and Stephenson (1947) observed intact red blood cells in the caecal contents and Todd & Ross (1966) concluded by spectroscopic examination that the caecal contents of *F. hepatica* were due to haemoglobin breakdown products.

The first attempts to measure quantitatively the amount of blood loss caused by *F. hepatica* was by Jennings, Mulligan and Urquhart (1956) who, using ^{32}P-labelled red cells, calculated a figure of 0.2 ml. of blood loss per fluke/day. This figure was an underestimate and when other red cell labels were used (^{51}Cr and ^{59}Fe) the red cell loss per fluke was found to be nearer 0.5 ml/day (Jennings, 1962; Holmes *et al.*, 1968; Holmes, 1969; Holmes and Maclean (1971). This represents a considerable loss of red cells and proteins each day, and although much of this protein is broken down and reabsorbed as amino

acids, it does mean that the animal has to resynthesis large amounts of protein each day.

Iron is also lost into the alimentary tract, but only in the most severe infections is any significant amount of this reabsorbed (Holmes and MacLean, 1969). It is, therefore, not surprising that this continual loss of iron from the body Dargie, 1975a) may lead to a relative deficiency and eventually to a reduction in the amount of haemonglobin produced by the animal. This may be the reason for the many reports that dyshaemopoiesis is important in the anaemia of fascioliasis (Sinclair, 1962, 1964, 1965, 1967).

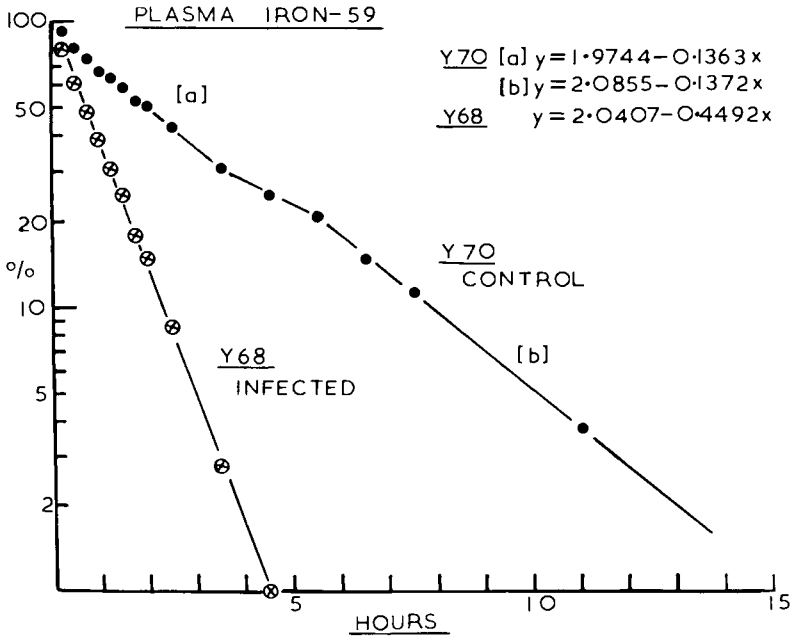

Fig. 1. Disappearance of ^{59}Fe citrate from the plasma of a sheep infected with F. hepatica *(Y68) and an uninfected control (Y70).*

As an illustration of the blood loss a sheep with a burden of 160 flukes, when injected with ^{59}Fe ferric citrate removed this very rapidly from the plasma (half-life of 42 mins. compared to 119 mins. for the normal sheep) and incorporated it quickly and efficiently into the red cells Fig. 1. However, instead of maintaining a constant level of red cell radioactivity, which the normal animal maintains due to re-incorporation of the ^{59}Fe from red cell breakdown, the activity of the red cells in the infected animal fell rapidly (half-life 9.6 days). By estimating the amount of ^{59}Fe in the faeces

and relating this to the blood activity, it was calculated that this sheep was losing at least 134 ml of blood per day (Fig. 2). In other words, this sheep maintaining a PCV of 25 was turning over its entire red cell volume each month. It had been infected for a period of 11 months when the investigation was carried out.

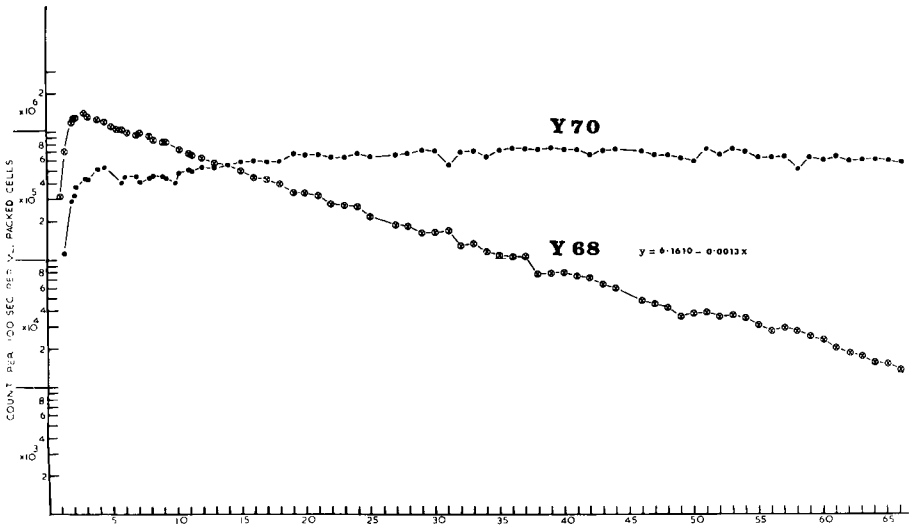

Fig. 2. The levels of ^{59}*Fe maintained in the red cells over a period of 65 days after the injection of* ^{59}*Fe-citrate into an* F. hepatica *infected sheep (Y68) and an uninfected control (Y70).*

Anaemia associated with chronic infections of *F. gigantica* is similar to that of *F. hepatica* (Bitakaramire, 1969; Hammond, 1973) and although detailed kinetic studies have not been done to determine red cell production and destruction, it is likely that the underlying pathophysiological mechanisms of the anaemia are similar.

An anaemia is also associated with infections of *Dicrocaelium dendriticum* (Soulsby, 1965), although no information is available on its aetiology.

In heavy infections of paramphistomes the first clinical signs appear within one or two weeks of ingestion of metacercariae, and it is at this stage that anaemia may develop; however, if the animal survives this acute phase of the disease then the anaemia regresses (Soulsby, 1965).

Schistosomiasis

Although schistosomiasis is widespread in cattle and sheep in many areas of the world, it is generally not regarded as a common cause of overt disease. However, reports of acute field outbreaks in both species, with accompanying anaemia have been reported (Le Roux, 1929; Strydon, 1963; Hurter and Potgieter, 1967).

Recent studies on *S. mattheei* infections of sheep, involving ferrokinetic and other studies on the anaemia, (Preston, Dargie and MacLean, 1973a,b; Preston and Dargie, 1974; Dargie, 1975b, have shown that the normocytic normochromic anaemia is due mainly to blood loss into the alimentary tract caused by the schistosome eggs passing out through the wall of the intestine. It is however complicated by haemodilution and reduced red cell production; the former has also been noted in humans with schistosomiasis (Fiorillo *et al.*, 1954; Jamra, Maspes and Meira, 1964).

Mahmoud and Woodruff (1972) working with mice infected with *S. mansoni* concluded that the anaemia was in part autoimmune in origin. Certainly it has been postulated (Foy and Nelson, 1963) that the anaemia in the early stages of the disease is caused primarily by blood loss while in the more chronic stages hepatosplenomegaly probably constitutes the more important aetiological factor. Splenomegaly has been cited as the cause of anaemia *per se* (Zuckerman, Abzug and Burg, 1969) and this coupled with an increased mononuclear phagocytic system and increased erythrophagocytosis might lead to increased red cell breakdown. Although Preston *et al.*, (1973a) did not find any evidence of haemolytic anaemia in sheep infected with *S. mattheei*, they were dealing with acute infections, where presumably a haemorrhagic blood loss was dominant; in addition it has been reported that sheep show little evidence of splenomegaly even in long-standing infections (McCully and Kruger, 1969; Preston *et al.*, 1973a).

Nematodes

Abomasal parasites

The abomasal parasites of ruminants which have been associated with anaemia are, *Haemonchus placei* in cattle (Delaune and Mayhew, 1953) and *H. contortus* in sheep. Infections of *Trichostrongylus axei*, *Ostertagia ostertagi* and *O. circumcincta* have also been reported to be associated with anaemia but in this, it is much more likely to be a marginal nutritional anaemia associated with inappetance and excessive loss of plasma proteins into the alimentary tract (Mulligan, Dalton and Anderson, 1963; Holmes and MacLean, 1971; Parkins *et al.*, 1973).

One of the earliest reports on *H. contortus* is by Railliet (1895) who noted the blood-sucking activities of the parasites, but the first detailed work was by Fourie (1931) who described the anaemia and attributed its aetiology to abomasal haemorrhage.

It has only been relatively recently that the sequential development of this anaemia has been studied using quantitative techniques to study red cell removal and production (Whitlock, 1950; Baker and Douglas, 1957a,b; Georgi, 1964). Clark, Kiesel and Goby (1962) using red cells labelled with ^{51}Cr and ^{59}Fe estimated that the average blood loss per parasite was 0.05 ml and showed that the labelled blood appeared in the faeces 6-12 days (average 8.6 days) after the sheep were infected with *H. contortus*, i.e. prior to the appearance of eggs in the faeces.

Dargie and Allonby (1975) working with Merino sheep and with fairly large experimental infections of 10,000 *H. contortus* larvae considered the anaemia to follow three stages. The first, which occurred during the pre-patent period during the first 3 weeks was characterized by a fairly dramatic fall in PCV; serum iron at this stage was normal. This was considered to be the result of blood loss due to the developing but immature *H. contortus*. Although at this stage the blood loss in absolute volumes was not as large as when the parasites were mature, the haemopoietic system of the host was not fully mobilised to produce red cells in quantities sufficient to meet the needs of the animal. This is analogous to the findings of Scott *et al.*, (1971) who reported that sheep from which small quantities of blood had been repeatedly removed prior to infection were more 'resistant' to the pathogenic effects of *H. contortus* infection. Presumably the haemopoietic system of these animals had been fully mobilised and could produce red cells immediately there was a demand for them.

The second stage of the anaemia occurred at the early post-patent phase of the disease. During this, quantitative measurement showed a maximum blood loss, not necessarily associated with any further drop in PCV due to the mobilisation of the haemopoietic system and the high serum iron levels. Since, however, the capacity of the sheep infected with *H. contortus* to reabsorb haemoglobin iron is limited (Dargie and Allonby, 1975) the iron reserves of the animal became seriously depleted, and led progressively to the third stage of the anaemia, i.e. a low serum iron was accompanied by a marked drop in PCV indicating a dyshaemopoiesis due to iron deficiency. This is possibly complicated by a relative failure of globin synthesis due to the persisting protein-losing gastroentero-

pathy.

A number of factors have been shown to influence the course of *H. contortus* infections and the severity of the associated anaemias. In addition to the nutritional status and the ability of the animal to respond quickly to haematological stress, the immunological status of the animal and the innate resistance determines the proportion, if any, of a given dose of infective larvae which will become established. These, may be genetically linked and resistance to *Haemonchus* infections have been shown by Whitlock (1958); Whitlock and Madsen (1958); Madsen and Whitlock (1958); Ross, Lee and Armour (1959). The original suggestions of Evans, Blunt and Southcott (1963) that sheep with haemoglobin A were more resistant to *H. contortus* infections could not be substantiated by Bradley and his colleagues (Jilek and Bradley, 1969; Radhakrishnan, Bradley and Loggins, 1972) working with Florida sheep. More recently, however, Allonby and Urquhart (personal communication) obtained field data from Merino sheep in East Africa which supported the original suggestion of Evans *et al.*, (1963). Since then observations on two breeds of sheep have confirmed the superior haematological performance of HbA type sheep when subjected to experimental infections of *H. contortus*, Altaif, Dargie and MacLean (in preparation). The reasons for this are still under study, but perhaps one factor is that haemoglobin A type sheep under conditions of severe haemorrhagic stress have the ability to produce a new type of haemoglobin (HbC)(Blunt and Evans (1963); van Vliet and Huisman (1964); Braend, Efremov and Helle, 1964; Gabuzda *et al.*, (1968)), which has an improved oxygen-carrying capacity (Dawson and Evans, 1962).

The other abomasal parasites of importance such as *Ostertagia ostertagi* infections of bovines are generally not associated with any marked degree of anaemia (Threlkeld and Johnston, 1948; Ross and Todd, 1965; Ritchie *et al.*, 1966). If anaemia does occur it is usually associated with Type II ostertagiasis due to maturation of a population of inhibited larvae (Martin *et al.*, 1957; Anderson *et al.*, 1965, 1969) and is probably due to a low plane of nutrition during the winter superimposed on abomasal dysfunctions characteristic of the disease (Mulligan, Dalton and Anderson, 1963; Jennings *et al.*, 1966).

In the case of *O. circumcincta* infections of sheep there are conflicting reports on the incidence of anaemia associated with this parasite. Horak and Clark (1964) originally reported anaemia in Merino sheep with infections of the order of 300,000 larvae, but this was not confirmed in a later experiment (Horak *et al.*, 1965). Certainly, Armour *et al.*, (1966)

working with Scottish Blackface sheep infected with 100,000 larvae produced no evidence of anaemia, and Holmes and MacLean (1971) using 300,000 larvae in the same breed failed to find any evidence of anaemia. However, Bezubik *et al.*, (1975) working with Polish Long-Wool sheep obtained significant drops in PCV with infective doses of 50,000 or 400,000 larvae. This may be another illustration of the importance of breed in resistance to the pathogenic effects of parasitic infections.

Parasites of the Small and Large Intestines

A number of parasites whose predilection sites are in the small intestine are implicated directly or indirectly with anaemias. The most important of these are the hookworm infections in man and animals, and it is with these infections that the greatest amount of quantitative work has been carried out. Roche and his co-workers (1957) were some of the first to use ^{51}Cr-labelled red cells for the measurement of blood loss in human hookworm infections, and they estimated that for *Necator americanus* the blood loss per day per hookworm was in the order of 0.03 ml. per day, while the blood loss due to *Ancylostoma duodenale* was much higher at approximately 0.2 ml. per hookworm per day. The latter figure is similar to that found by Miller (1966a) for *A. caninum* infections in dogs. Miller (1966b) also found that *A. brasiliensis* caused only insignificant losses of blood of the order of 0.001 ml in young pups, and 0.002 ml per worm per day in kittens.

The anaemias of hookworm infections are essentially similar to the anaemias of haemonchosis in that initially it is a macrocytic normochromic anaemia, which later develops, due to loss of iron from the body into a macrocytic hypochromic anaemia. Indeed, in human hookworm infections iron therapy (Layrisse *et al.*, 1959; Rhoads *et al.*, 1934) is apparently as important as anthelmintic treatment, although it has been shown that at least some part of the haemoglobin iron extracted by the hookworms may be reabsorbed (Roche, Perez-Gimenez and Levy, 1957).

As well as *Ancylostoma caninum*, *Uncinaria stenocephala* which cause a haemorrhagic anaemia in dogs, the other hookworms of importance in domestic animals are *Bunostomum trigonecephalum*, *B. phlebotumum* and *Gaigeria pachyscelis*. They also produce anaemias although the kinetics of the red cell production have not been studied.

The intestinal bleeding associated with *Oesophagostomum radiatum* in calves has been investigated by Bremner (1969a,b, c, 1970a,b). Bremner and Fridemanis and Fridemanis (1974) found that an infection of 2,500 *O. radiatum* caused a maximum

blood loss of 39 ml of packed red cells per day; intestinal leakage of plasma was much higher, amounting to 800 ml per day (Bremner, 1969b,c). *O. columbianum* infections of sheep also produce anaemia (Horak and Clark, 1966; Dobson, 1967) but studies of the erythrokinetics have still to be carried out. It has, however, been shown that a low plane of nutrition exacerbates the disease (Bawden, 1969a,b).

Other nematodes of the alimentary tract are also incriminated in the production of anaemias. Thus, a severe haemorrhagic anaemia occurs during the developmental stages of *Chabertia ovina* in lambs (Wetzel, 1931; Ross, Dow and Purcell, 1969; Herd, 1971) and in calves this parasite has also been reported (Ross and Todd, 1968) as producing severe microcytic hypochromic anaemia.

In the large intestine both natural and experimental infections of the *Trichuris* spp., are frequently accompanied by bloody diarrhoea. This has been reported in *Trichuris trichiura* of man (Fernan-Nunez, 1927); *T. vulpis* in dogs (Miller, 1939); *T. ovis* in sheep (Powers, 1961) and *T. suis* in pigs (Mozovoi, 1952; Batte, McLamb and Vestal, 1975). In the absence of this symptom blood loss appears to be insignificant, thus Beer, Sansom and Taylor (1974) using ^{59}Fe-labelled erythrocytes failed to show any appreciable loss of red cells in infected pigs. Similarly, Mahmood (1966); Lotero, Tripathy and Bolanos (1974) reported that there was only an insignificant blood loss in children and Otto (1935) also could find no evidence of anaemia attributable to *Trichuris* infections in a large group of children in Louisiana.

Although all these parasitic infections so far mentioned have been implicated as a cause of haemorrhage into the alimentary tract, or of direct consumption of blood, there are other intestinal parasites where the mechanism of the anaemia remains obscure. Although *T. colubriformis* infections cause a loss of protein into the intestine (Barker, 1973) there is no suggestion that red cells are lost. The anaemia is, therefore, not haemorrhagic in aetiology, and has been variably attributed to digestive disturbances (Gallacher, 1963), to inhibition of iron uptake, to its reduced transport and storage resulting from changes in the intestinal epithelial cells (Barker, 1973) and to a toxin produced by the parasites which depresses erythropoiesis (Horak, Clark and Gray, 1968). The latter has been given some credence by Baker and Douglas (1966) who found evidence of impaired erythropoiesis as measured by iron transfer rates and liver and spleen scanning techniques in cattle infected with trichostrongyles other than *Haemonchus*. On the other hand, they found (Baker and Douglas, 1957) that a six month old bovine suffering from

chronic trichostrongylosis, in this case mainly *Ostertagia ostertagi*, had a shortened life-span of the circulating erythrocytes coupled with an increased rate of haemoglobin production of approximately four times normal. In spite of this, they maintain that there was still evidence of impaired erythropoiesis as they contended that the bone marrow should be capable of responding even more to the anaemic stimulus than it did. They may be true, but consideration should, in these cases, be given to the duration of the "chronic" infection as the constant hypercatabolism of plasma proteins could conceivably lead to a relative failure of protein (i.e. haemoglobin) synthesis. Until sequential erythrokinetic studies are carried out on these types of anaemia, possibly coupled with studies on protein catabolism, the true aetiology of the anaemia will still be in dispute.

Cestode Infections

The classic example of *Diphyllobothrium latum* causing pernicious anaemia (Von Bonsdorff and Gordon, 1952-53) due to its utilising vitamin B_{12} in the diet has now been well documented, and has led many investigators to wonder if a similar phenomenon exists in other parasitic infections. Davtyan and Movsesyan (1958) found that liver flukes contained 3 to 5 times as much vitamin B_{12} as the liver which they parasitised, but Sinclair (1967) was unable to show that parenteral administration of the vitamin B_{12} (or iron) had any effect on the anaemia of sheep infected with *F. hepatica*. Downey (1965, 1966) found no relationship between cobalt status and infection of *H. contortus* and *T. axei* in lambs in Ireland, and Gardiner (1966) found no changes in the serum B_{12} levels of sheep infected with a combination of *H. contortus*, *T. colubriformis* and *Ostertagia* spp. irrespective of a high or low plane of cobalt nutrition.

It does seem possible that the severe abomasal lesions and loss of abomasal function associated with acute ostertagiasis (Jennings *et al.*, 1966) might lead to a defective secretion of intrinsic factor and a temporary loss of B_{12} absorption. However, due to the temporary nature of the lesion and the adequate reserves of B_{12}, a megaloblastic anaemia has not been reported.

ANAEMIA IN PROTOZOAL INFECTIONS

Erythrocytic Parasites

Anaemia in protozoal infections where the parasite is on the surface of the cell or intraerythrocytic is understandable. In the case of malaria, rupture of the red cells by

the escaping parasites may cause *ipso facto* an anaemia, and the same can be argued for babesiosis. However, many reports present evidence of an anaemia greater than that which might reasonably be due to parasitised erythrocytes alone (see review by Schroeder and Ristic, 1968) and therefore additional mechanisms for red cell removal from the circulation have been postulated. However, the anaemias associated with protozoal infections can all be classified as predominantly haemolytic in aetiology, although haemolysis need not necessarily occur intravascularly. The underlying mechanisms are probably similar and will be dealt with jointly at the end of the section.

Anaplasmosis

Anaplasmosis is an infectious disease of cattle and sheep manifested by a rapidly progressive anaemia, and transmitted predominantly by arthropod vectors (Anthony and Roby, 1966; Piercy, 1956). The disease is manifested by anaplasma bodies within a proportion of the circulating red blood cells, and these are destroyed rapidly by the mononuclear phagocytic system, especially the spleen (Baker, Osebold and Christensen, 1961; Kreier, Ristic and Schroeder, 1964). According to Kreier *et al.* (1964), there was no evidence of any failure of erythropoiesis and erythrophagocytosis, initiated by parasitic damage to the erythrocytes and an anti-erythrocyte autoantibody phenomenon was suggested as the probable cause of the anaemia in both *Anaplasma marginale* and *A. ovis* infections of calves and sheep. Certainly, Ristic, Lykins and Morris (1972) have demonstrated autohaemagglutinins and erythrocyte-bound autoantibodies, the autohaemagglutinins being presumably an IgM antibody as their activity was destroyed by mercaptoethanol.

A striking feature of the disease is the speed of the red cell destruction which may increase up to ten times the normal (Baker *et al.*, 1961) even in the splenectomised calf. Increasing anaemia persists for 5-10 days, and during this time the animals frequently lose as much as 60-70% of their circulating red blood cells (equivalent to 13.6-27.3 ℓ of whole blood) (Jones and Brock, 1966). Despite this, haemoglobinaemia and haemoglobinuria have not been noticed, and the red cell destruction must be presumably due to erythrophagocytosis.

Eperythrozoonosis

Although *Eperythrozoon ovis* infections have been observed concurrently with *Anaplasma* (Splitter, Twiehaus and Castro, 1955), *E. ovis* in its own right has been reported to be re-

sponsible for anaemia and general unthriftiness in lambs in South Africa (Neitz, 1937) and Australia (Littlejohns, 1960; Sheriff, Clapp and Reid, 1966; Harbutt, 1969a,b). In the United Kingdom, Foggie (1961), Foggie and Nisbet (1964) and Rouse and Johnson (1966) have all reported this parasite in conjunction with anaemia in sheep as have Delpy (1936) in Iran and Overas (1962) in Norway. The aetiology of the anaemia has not been established but is presumed to be similar to anaplasmosis.

Haemobartonellosis

Haemobartonella bovis, which has been recorded in Algeria, Palestine, U.S.A., Iran, Australia, Ruanda, Kenya and Spain (see review by Kreier and Ristic, 1968), has also been found associated with other protozoan diseases in the United Kingdom (Brocklesby, 1970; Barnett and Brocklesby, 1971) but has rarely been implicated directly in the incidence of anaemia.

Haemobartonellosis in laboratory rodents, although generally regarded as non-pathogenic, produces a severe anaemia if the animals are splenectomised and it is important to check that animals are free of this parasite if studies of anaemia are being conducted. Similarly, *H. canis* in the dog is generally non-pathogenic and usually only becomes apparent after splenectomy. On the other hand, *Haemobartonella felis* causes a severe clinical anaemia in domestic cats (feline infectious anaemia).

Babesiosis (Piroplasmosis)

Although infections of *Babesia* are largely confined to domestic animals, there have been at least six reported cases of babesiosis in man three of which died (Anderson, Cassaday and Healy, 1974); these were diagnosed as *B. bovis*, *B. divergens* (bovine), *B. microti* (rodent) and a query *B. equi* (equine). Normally, however, *Babesia* are regarded as species specific and thus unlikely to spread between unrelated animal species.

The most important species in the bovine is *B. bigemina* which occurs in tropical and sub-tropical areas throughout the world. In more temperate climates *B. divergens* is the causative agent of "Redwater" in cattle, and it is this haemoglobinuria that denotes the haemolytic aetiology of the anaemia in the acute disease. Haemoglobinuria also occurs in *B. canis* infections of dogs. As in other protozoan diseases, however, the degree of anaemia need not necessarily be correlated with the level of parasitism, and other factors such as splenomegaly, erythrophagocytosis and autoimmune haemolysis may also play a role especially in the more chronic stages of

the disease.

Malaria

Malaria due to *Plasmodium* infections are of prime importance in man and other primates, but are of minor importance in domestic animals. The occurrence in *P. falciparum* malaria of "Blackwater Fever" has been compared to canine babesiosis. Christophers and Bentley (1908) pointed out that in both diseases the anaemia occurred after the peak parasitaemia and that in Blackwater Fever the anaemia could occur suddenly in the absence of observable parasitaemia.

Plasma Parasites

Trypanosomiasis

Of the protozoal parasites which neither penetrate the red cells nor attach themselves to the erythrocyte surface, the trypanosomes are the greatest importance. The anaemia they produce is preceded by a haemodilution effect and plasma volumes may be increased by as much as 50% (Clarkson, 1968; Naylor, 1971; Mamo and Holmes, 1975; Holmes and Jennings, 1975). The characteristics of the anaemia, which has been studied in greatest detail in the laboratory rodent, are macrocytosis, reticulocytosis, normoblastic hyperplasia of the bone marrow and spleen and increased haemosiderin deposits and erythrophagocytosis in the spleen. These, together with the shortened circulating half-life of ^{51}Cr-labelled red cells the accelerated urinary excretion of ^{51}Cr from the labelled red cells and the retention of injected ^{59}Fe in the body are all indicative of an anaemia of haemolytic origin.

In addition, no evidence of intravascular haemolysis, such as jaundice, increased bilirubin and haemoglobin in the serum has been found as a consistent feature, and it would appear that extravascular destruction of the red cells is a more likely explanation for the anaemia. However, in a small proportion of rats, an acute haemolytic crisis occurred with gross haemoglobinuria and haemoglobinaemia which terminated fatally in a few hours; the reason for this in unknown (Jennings *et al.*, 1974).

The mechanism whereby red cells are "processed" for inceased erythrophagocytosis in all the protozoal diseases is still a matter for speculation and there are a number of hypotheses to account for this phenomena. Six of these are discussed below relating particularly to trypanosomiasis but they may be equally applicable to the other blood-borne protozoal infections.

1. Trypanosome antigen may become attached to the surface of the red cell and thus make it susceptible to erythrophagocytosis. The first part of the process, i.e. the attachment to the red cell has been shown to occur by two groups of workers (Woo and Kobayashi, 1975; Herbert and Inglis, 1973) (Fig. 3).

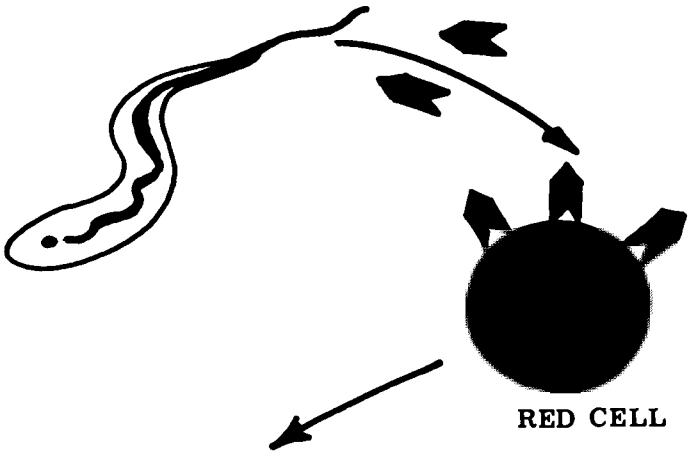

Fig. 3. Trypanosome antigen fixing to the surface of the red cell and promoting erythrophagocytosis.

2. Trypanosome antigen once attached to the surface of the red cell may combine with anti-trypanosome antibody from the circulation. This may make the red cells susceptible to phagocytosis or alternatively, to lysis if complement is fixed to the immune complex (Woo and Kobayashi, 1975). This would explain the positive Coombs' anti-globulin test detected in some sleepy sickness patients (Woodruff *et al.*, 1973) (Fig. 4).

3. Once complement has become activated and bound to erythrocyte surfaces, antibodies to complement (mainly the C_3 fragment), immunoconglutinins, may be produced and exacerbate the haemolytic process. High levels of immunoconglutinins have been found in trypanosomiasis of experimental animals (Ingram and Soltys, 1960; Woodruff *et al.*, 1973; Woodruff, 1973) (Fig. 5).

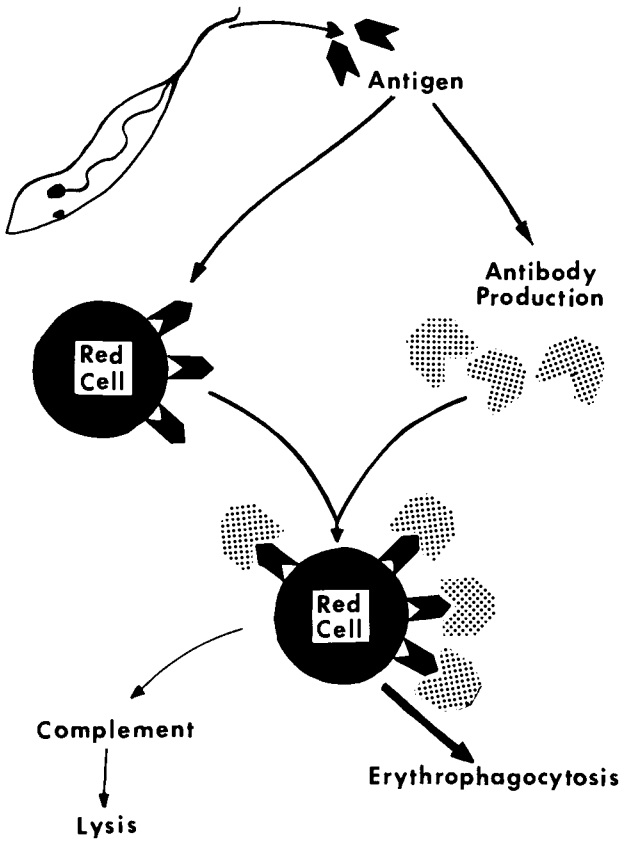

Fig. 4. Trypanosome antigen on the surface of the red cell combining with anti-trypanosome antibodies to promote erythrophagocytosis or lysis of the red cells.

4. Another possibility which is receiving attention at the present time is that trypanosomes produce a haemolytic factor which causes direct haemolysis of the circulating red cells. This was first noted by Fiennes (1954) who found that if cells from normal bovines were incubated with infected plasma then the cells were haemolysed. Recently, workers in Geneva, (Nguyen Huan *et al.* (1975), Murray *et al.*, (1975) have found that trypanosomes produce a small molecular weight protein which if incubated with normal red cells is capable of causing lysis. This is an attractive hypothesis because of its

simplicity and would explain the rapid onset of the anaemia, i.e. in 2 to 3 days in many experimental infections. One can visualise, in the case of very high parasitaemias, that sufficient of this haemolysin would be produced to cause anaemia; however, it is more difficult to envisage this happening where parasitaemias are very low (Fig. 6).

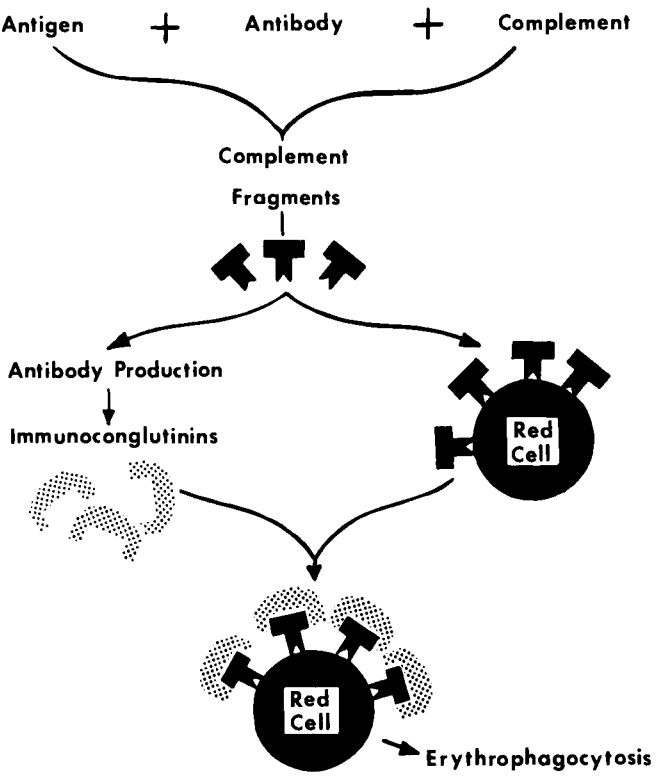

Fig. 5. Antibody-antigen reactions producing activated complement fragments which cause the production of immunoconglutinins and increased erythrophagocytosis of the red cells.

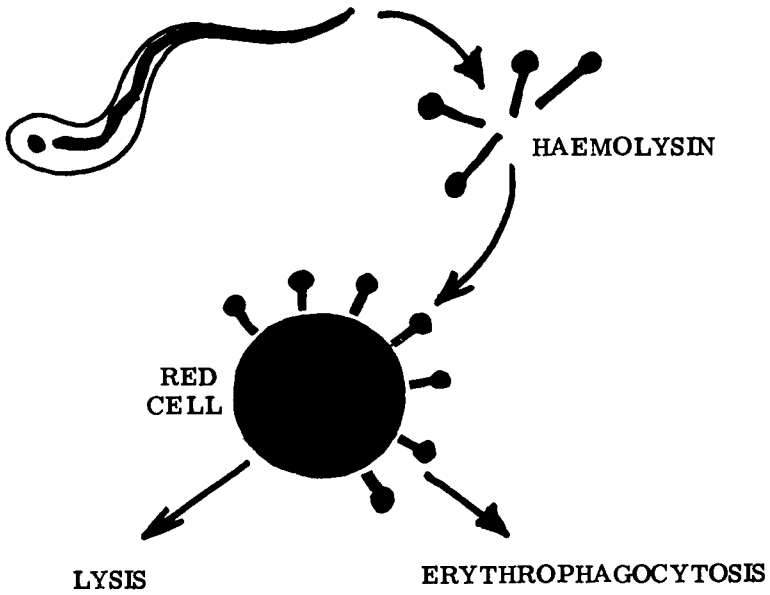

Fig. 6. Production by the trypanosome of a haemolysin which lysis the red cells directly or promotes erythrophagocytosis.

5. Trypanosomes are highly active and it is conceivable that they compete with the red blood cells for essential metabolites; certainly there have been suggestions of a hypoglycaemia especially in the terminal stages of the disease. If the red cells are unable to regenerate ATP their surfaces will be altered and they may become susceptible to phagocytosis (Maruta and Mizuno, 1971). This also is an attractive theory if the parasitaemias are high, but one frequently encounters anaemic animals with scanty parasitaemias.

However, whatever the alteration of the red cell surface, if cells from infected and anaemic donors are labelled with ^{51}Cr and transferred to normal recipients then the "half life" of the cells are normal, so "infected" cells are not permanently damaged and can recover in a normal environment (Jennings *et al.*, 1974; Anosa, 1975).

6. The aetiology of the anaemia in some cases of trypanosomiasis at least has been attributed to thrombocytopenia with

disseminated intravascular coagulation; this has been observed to occur in human patients (Barrett-Connor *et al.*, 1973) and in rats (Davis *et al.*, 1974) and it is also known that increased levels of fibrinogen and their degradation products are common in trypanosomiasis (Boreham, 1968; Boreham and Pacer (1974).

There are obviously some parasites which are implicated in anaemias which have not been mentioned in this review such as Coccidia which are neither erythrocytic nor plasma parasites yet are responsible for severe haemorrhage and death in birds. *Eimeria tenella* and *E. necatrix* are the two species in poultry which cause haemorrhage and death, the survivors are markedly anaemic, but this anaemia regresses rapidly. Other parasites of birds also implicated in anaemia are *Capillaria*, *Amidostomum*, *Ascaridia* and *Ornithostrongylus* infections (Soulsby, 1965).

SUMMARY

The anaemia of parasitic infections are either:

Haemorrhagic in nature due to direct blood loss, which may lead eventually to a failure of erythropoiesis due to an iron and/or protein deficiency.

The parasites which are not implicated in direct blood loss may also cause anaemia probably by causing some marginal nutritional deficiencies concurrent with increased catabolism of proteins.

The anaemias of protozoal infections are mainly haemolytic in nature, especially during the acute phases of the disease. The exact aetiology of these haemolytic anaemias has still to be completely understood and may be partially autoimmune in nature. Most are associated with increased erythrophagocytosis and enlarged mononuclear phagocytic systems.

In some cases there may be some complications due to haemodilution or haemoconcentration and it is therefore important that kinetic measurements of red cell production and destruction are carried out on these anaemias. In only a few instances, e.g. schistosomiasis has a genuine dyshaemopoietic effect being demonstrated, but as methods of determining erythrocyte production improve more marginal effects may become apparent in other parasitic diseases.

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LEVELS OF SERUM ENZYMES AND ELECTROLYTES ASSOCIATED WITH SWINE TRICHURIASIS

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INTRODUCTION

Infection of young pigs with the swine whipworm *(Trichuris suis)* resulted in severe clinical manifestations. These included anemia, anorexia, diarrhea with mucus and blood in the feces, weight loss and death (Beer and Lean, 1973). Batte (1972) reported that the diarrhea began 17 to 21 days post-infection and did not respond to antibiotic therapy. Histological examination of infected pigs showed moderately severe cecitis and colitis with marked catarrhal inflammation.

Kuchyukas and Medzyavichyus (1968) found that the enterokinase and alkaline phosphatase activities of the feces were increased in pigs given 2,000 or 6,000 ova of *T. suis*. Medzyavichyus, Titishkite and Kuchyukas (1968) followed the serum protein patterns in pigs given 2,000 or 6,000 *T. suis* ova. They observed a rise in the total protein level and the alpha, beta and gamma globulin fractions but a fall in the albumin fraction in comparison to non-infected animals.

In 1974, Beer, Sansom and Taylor infected pigs with 15,000 40,000 or 100,000 *T. suis* ova and used whole-body counting technique to measure the loss of ^{59}Fe labeled erythrocytes. The average of loss of erythrocytes was correlated to the rate of infection. A transitory macrocytosis occurred at the medium level and a decrease in serum concentration was found at the high level.

Sansom, Beer and Kitchenham (1974) did not detect clinical signs or significant biochemical changes in pigs infected with 15,000 *T. suis* ova. Pigs given 100,000 ova had a decrease in serum sodium concentration and an increase in serum globulin. Infection with 50,000 ova resulted in a decrease in serum sodium, phosphorus and albumin with an increase in urea and globulin.

Batte (1972) infected 12-week old pigs with 25,000, 50,000, 100,000, and 400,000 ova. There was a decreased alkaline phosphatase activity but increased SGOT activity and serum protein, cholesterol, globulin, uric acid and glucose. Total white blood cell counts were increased. Most of these changes occurred during the period of profuse diarrhea.

MATERIALS AND METHODS

Feces were collected from donor animals harboring a pure infection of *T. suis* . The ova were harvested by flotation in 40% sugar solution and then by centrifugation. The sediment was washed with distilled water until free of sugar and debris. The ova were incubated at 37°C with continuous aeration for a minimum of 30 days.

Parasite-free pigs confined in sanitized isolation units were given 50,000 or 100,000 infective *T. suis* ova when they were 9 weeks of age. Blood samples were collected twice weekly from infected and non-infected littermates. Three pigs were in each dose level or control group and there were 3 replicates.

Electrophoretic patterns were determined by the Gelman Sepratex® system and a Digiscreen recorder.

Serum enzymes were determined by use of prepackaged reagents* and a Beckman Model 25 spectrophotometer.

Serum electrolytes were quantitated by use of a Jarrell-Ash flame photometer.

RESULTS

Pigs given infective ova developed profuse diarrhea 17 to 21 days post-infection (P.I.). The fluid feces were blood-tinged with flecks of free blood. Infected pigs exhibited anorexia, retardation of growth and emaciation beginning 21 days P.I.

There was a sharp drop in albumin beginning 10 to 14 days P.I. as shown in Fig. 1. The values plotted are the average of all animals in that group. The albumin level in the pigs given 50,000 ova dropped from 54% on day 7 to 38% on day 28. Pigs given 100,000 ova had a decline from 55% on day 10 to 37% on day 28.

The average value of alpha globulin began increasing 14 days P.I. (Fig. 2). The average value at 14 days P.I. for the pigs givin 100,000 ova was 14%. This value was doubled or 28% by 28 days P.I. The average value of the pigs given 50,000 ova rose from 16% on day 14 to 23% on day 28.

Beta globulin levels varied, but at both levels of infection they exhibited an abrupt increase at 24 days P.I. (Fig. 3). Pigs given 50,000 ova had an increase beginning on day 14 with a maximum of 24% on day 17 which was the day diarrhea began. Another maximum of 24% occurred at day 28.

*Boehringer Mannheim Corporation, New York, N.Y.

Fig. 1. Average serum albumin levels in groups of pigs infected with 50,000 or 100,000 eggs of Trichuris suis, *or not infected.*

Fig. 2. Average serum alpha globulin levels in groups of pigs infected with 50,000 or 100,000 eggs of Trichuris suis, *or not infected.*

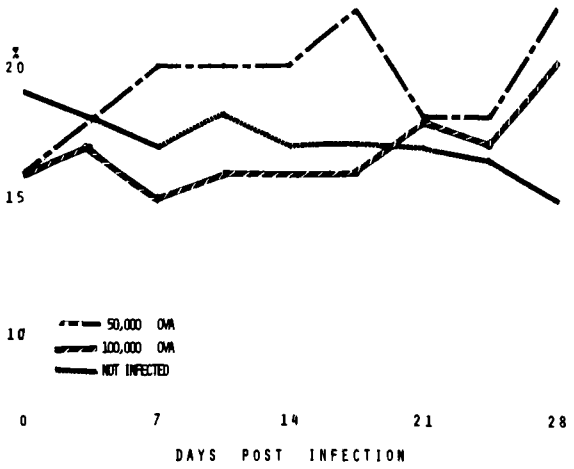

Fig. 3. Average serum beta glovulin levels in groups of pigs infected with 50,000 or 100,000 eggs of Trichuris suis, *or not infected.*

Pigs given the higher dose of ova exhibited an increase of beta globulin beginning at day 17. The increasing trend continued to day 28.

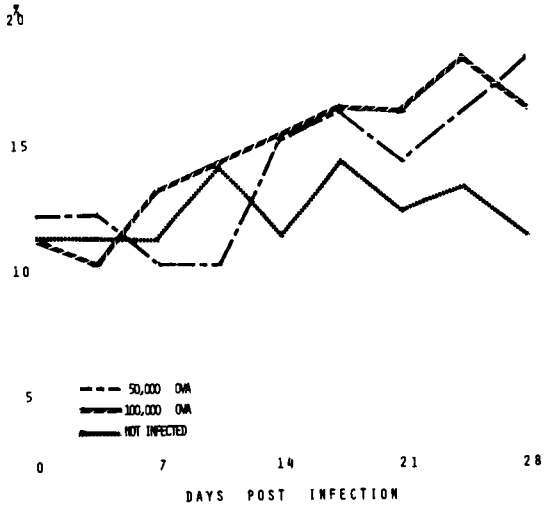

Fig. 4. Average serum gamma globulin levels in groups of pigs infected with 50,000 or 100,000 eggs of Trichuris suis, *or not infected.*

The gamma globulin began to increase 7 to 10 days P.I. (Fig. 4). The gamma globulin level of pigs given 100,000 ova began a steady increase on day 7 that continued until day 17. The pigs given the lower level of infection had an increase in gamma globulin level beginning at day 10. The trend was an increase in value until day 28.

Depressed serum calcium levels were closely correlated to the rate of infection. The decrease in calcium began 14 to 17 days P.I. There was no correlation of zinc levels to the rate of infection, and similarly, there were no correlated changes in serum creatinine, SGPT, blood sugars, hemoglobin, bilirubin or BUN of infected or non-infected pigs.

DISCUSSION

Changes in serum enzymes and electrolytes were closely related to clinical signs. Most of the changes were apparent 14 to 21 days after infection which coincided with the onset of the profuse diarrhea.

The hypoalbuminaemia could be caused by albumin loss into the lumen of the large intestines resulting from destruction of mucosa by the parasite. This mechanism has been suggested by Mulligan (1971). Increased synthesis to replace the lost albumin renders the pig vulnerable to secondary infections under conditions of nutritional and environmental stress. Invasion of opportunistic microorganisms due to weakened resistence may explain the high mortality rate in field outbreaks.

The increase in serum alpha, beta and gamma globulin fractions are probably a reflection of activation of the immune mechanism. The beta globulin are known to contain immunoglobulins (Putman, 1965).

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PATHOPHYSIOLOGICAL STUDIES OF EXPERIMENTAL *FASCIOLA HEPATICA* INFECTIONS IN SHEEP AND RABBITS

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INTRODUCTION

A number of workers have studied the pathophysiological effects of *Fasciola hepatica* infection in sheep and rabbits (Kearney, Connelly and Downey, 1967; Ross, 1967; Simesen, Nielsen and Nansen, 1973; Thorpe and Ford, 1969). In previous experiments (Banting *et al.*, unpublished data; Le Bars and Banting, 1975) the serum levels of OCT, alkaline phosphatase, GOT, total cholesterol and albumin were determined in aged ewes artificially infected and treated with different flukicides. Individual variations were very marked but despite this, OCT and alkaline phosphatase showed significant differences between the various groups. Data obtained from such studies have been useful in assessing the effects of the infection and also the consequences of therapy. Nevertheless, because of the marked variation which may occur, it was necessary to undertake studies in a more homogeneous group of sheep. In addition, because questions have been raised whether the rabbit can be used as an alternate experimental animal to the sheep the present studies compared the responses of the sheep and the rabbit to infection with *F. hepatica*.

MATERIALS AND METHODS

Studies with rabbits.

Two groups each of 8 female rabbits were used. One group served as controls, and animals of the other were each infected with 25 metacercariae of *Fasciola hepatica*. Blood was taken from the marginal vein of the ear and the determinations of the serum levels of asparate amino-transferase: glutamicoxalacetic transaminase (GOT), of alanine amino-transferase: glutamic pyruvic transaminase (GPT), of ornithine carbamyl transferase (OCT) and of albumin were made by the use of a Technicon Auto-Analyzer (Cerietti, 1971; Levine and Hill, 1966). The animals were killed at the end of the experiment (100 days after infection) and the number of flukes was counted.

Studies with Sheep

Twenty, fifteen months old, ewes of the same breed and weight, were divided into two groups. One group served as controls and the other group was infected with 200 metacercariae of *Fasciola hepatica* per animal. Serum OCT, albumin, urea, plasma triglycerides and SGOT were determined by the use of a Technicon Auto-Analyzer. Blood was taken from the jugular vein prior to and after infection at the times indicated in the graphs, except that such measurements for the control animals were made only until the 66th day of the infection.

RESULTS

Studies with Rabbits

In the rabbits an average of 60% of the infection was recovered, and this varied from 45% to 100%. The mean levels of the various serum components for infected and control rabbits are presented in Figs 1, 2, 3 and 4.

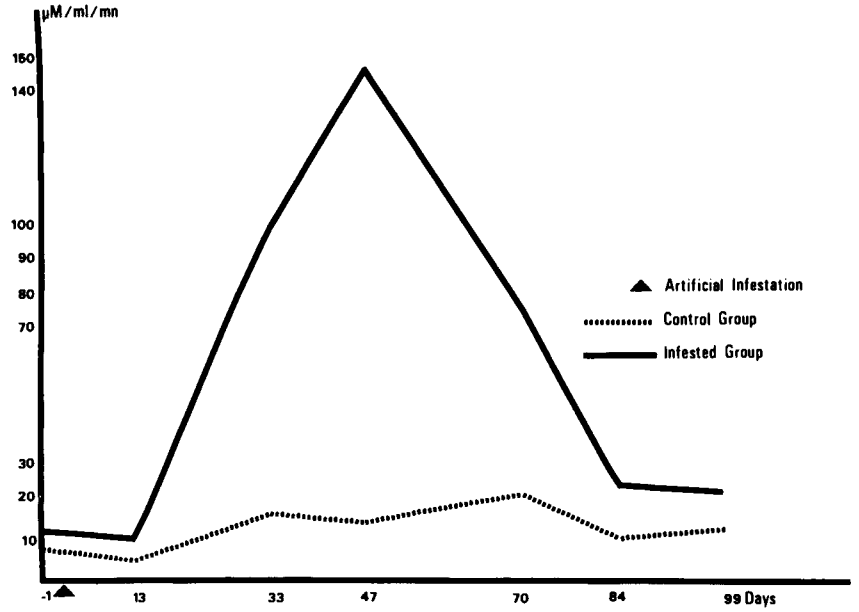

Fig. 1. Mean orithine carbamyl transferase (OCT) levels in rabbits infected with F. hepatica *at intervals after infection. Solid line, infected animals; broken line control animals.*

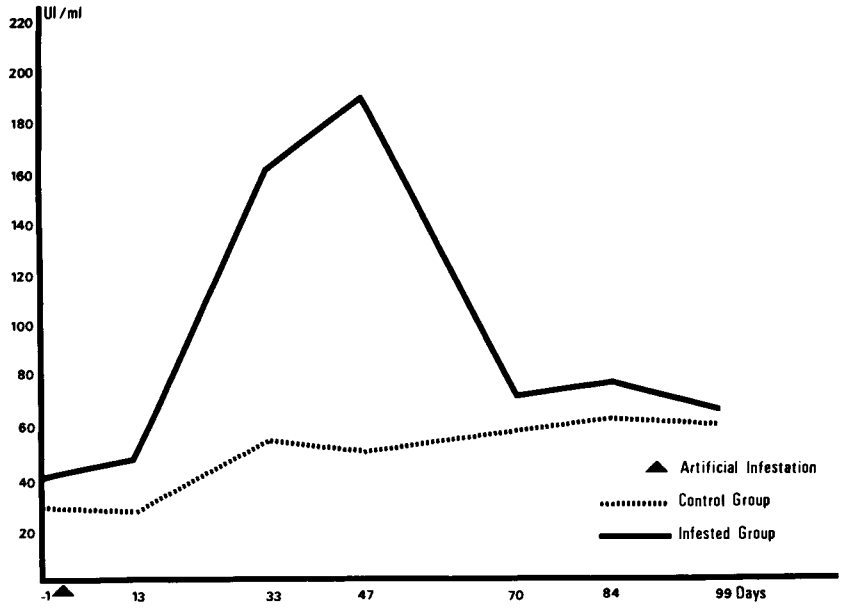

Fig. 2. Mean serum glutamic pyruvic transaminase (SGPT) levels in rabbits infected with F. hepatica *at intervals after infection.*

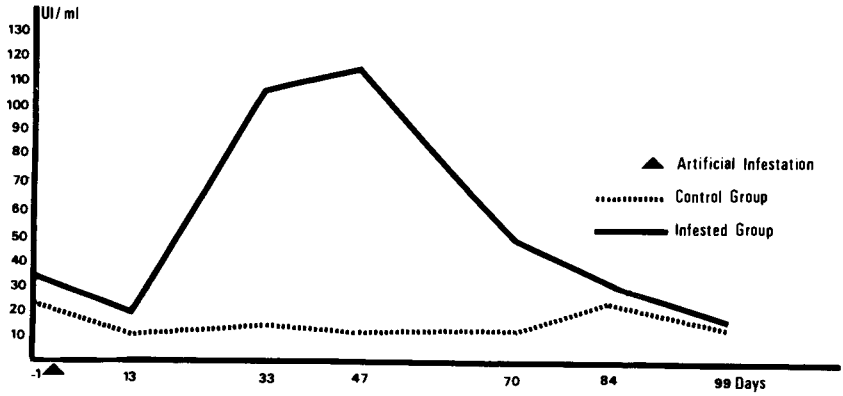

Fig. 3. Mean serum glutamic oxalacetic transaminase (SGOT) levels in rabbits infected with F. hepatica *at intervals after infection.*

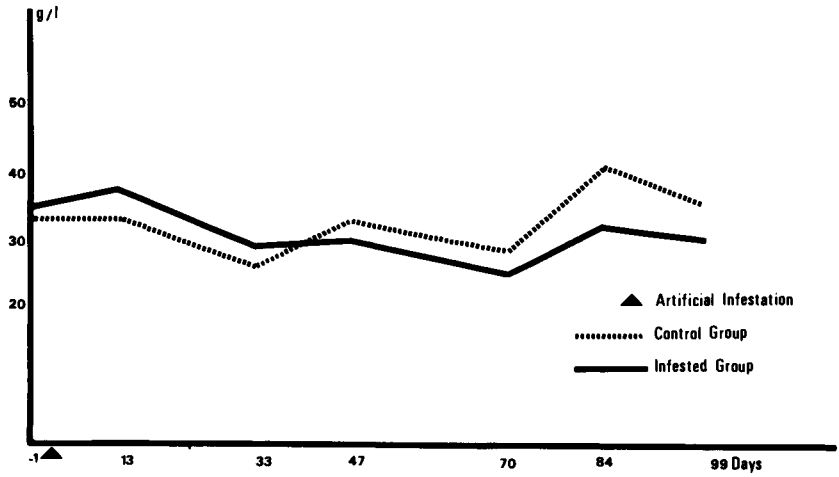

Fig. 4. Mean serum albumin levels in rabbits infected with F. hepatica *at intervals after infection.*

The maximum levels of the OCT and the transaminases (SGPT) and SGOT) were reached in the serum of infected animals at 47 days after infection. The individual variations of control animals were low, though a greater variation was evident with the SGOT in control rabbits.

Studies with Sheep

The mean number of parasites recovered per animal was 55.

The mean levels of the various serum components for infected and control sheep are presented in Figs. 5, 6, 7 and 8.

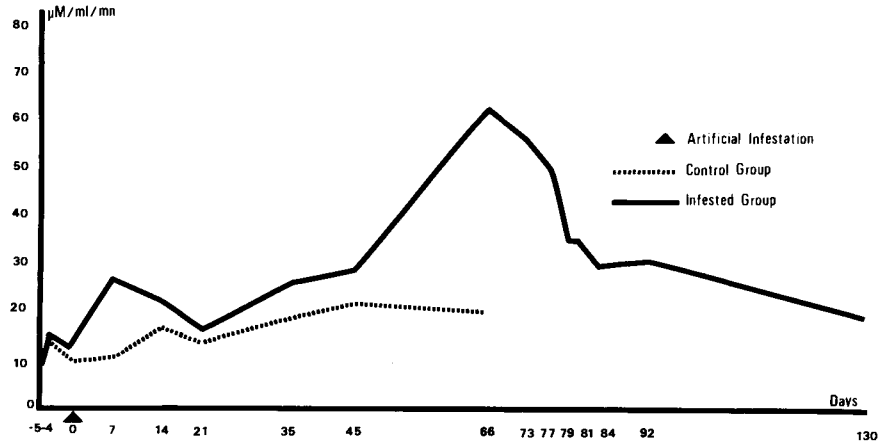

Fig. 5. Mean serum ornithine carbamyl transferase (OCT) levels in sheep infected with Fasciola hepatica *at intervals after infection.*

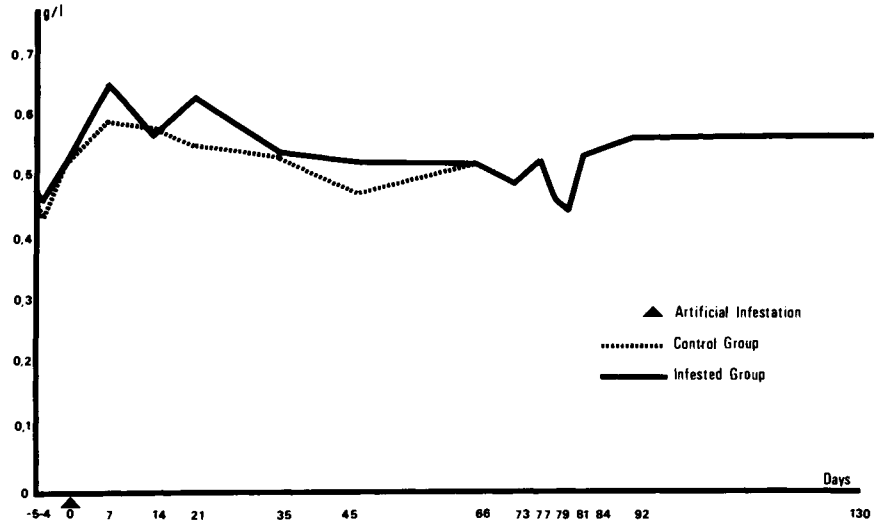

Fig. 6. Mean serum urea levels in sheep infected with Fasciola hepatica *at intervals after infection.*

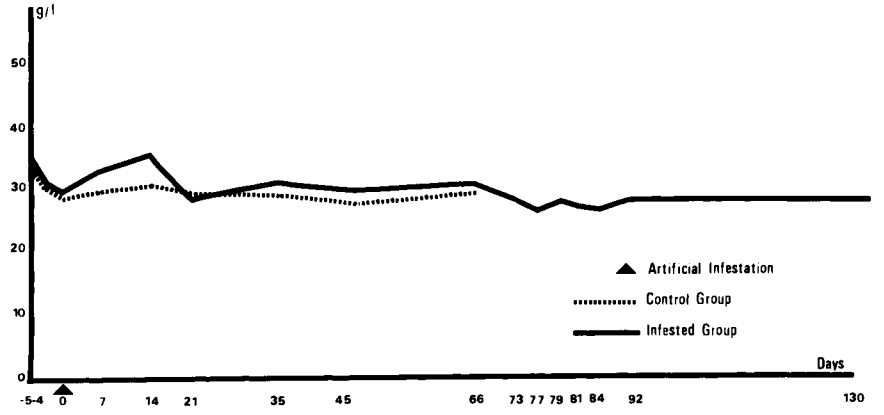

Fig. 7. Mean serum albumin levels in sheep infected with Fasciola hepatica *at intervals after infection.*

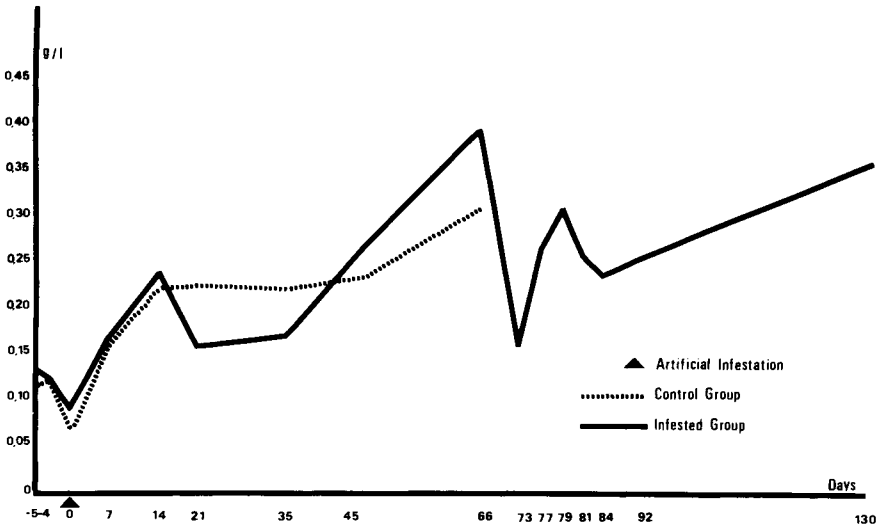

Fig. 8. Mean plasma triglyceride levels in sheep infected with Fasciola hepatica *at intervals after infection.*

The plasma triglyceride level was modified compared to control animals on days 21 and 66 after infection. Consequently, *F. hepatica* seems to modify lipid transport in differing ways according to the developmental stage of the parasite. It should be noted, however, that in these studies, for unexplained reasons, the plasma triglyceride levels of the control animals also increased, but this occurred in a regular manner.

For the levels of OCT, albumin and urea, an increase was evident on the 7th day after infection. Following the initial reflection of liver damage, the OCT levels generally increased until the 66th day of infection, thereby reflecting the hepatic changes during the infection. The albumin and urea levels were not significantly changed subsequent to the response on the 7th day after infection.

No significant differences were observed for SGOT values of control and infected sheep.

DISCUSSION

Studies with Rabbits

Although an increase in serum GOT is attributed to infection by *F. hepatica*, other factors can bring about variations

in the level of this transaminase in the rabbit. Individual variations in the controls are important and it seems that, in certain cases, blood sampling and the associated manipulations can account for part of the increase in observed serum GOT in both control and infested animals. (An example of this is seen in Fig. 3, day 84 for control animals). Consequently this parameter cannot be considered as satisfactory in the rabbit experimental model. This "non liver" specifity of SGOT is in agreement with the studies of Ross, Dow and Todd (1967), Sewell (1967) and Thorpe and Ford (1969). It is considered that measurement of OCT is a more useful parameter for infection than others. For example, OCT is more specific than GPT as has also been noted by Truhaut *et al.* (1967). Nevertheless, it seems that GPT and OCT levels are the best parameters to use to evaluate the effects of a flukicide on a parasitized liver providing the product is given during the first six weeks of infection. In the rabbit, serum levels of OCT and GPT become normal six weeks after infection. On the other hand, any hepatotoxic effect of a flukicide would be evident using these estimations.

Studies with Sheep

In the rabbit an increase in hepatic protein synthesis was evident at the beginning of the infection. This was more evident in sheep: a similar stimulatory effect was seen with serum urea levels. In sheep the increased serum OCT levels are interpreted as due to an increased leakage of cellular OCT to serum as a result of higher intracellular levels of this enzyme. In all the results suggest that*F. hepatica* infection of the sheep results in stimulation of hepatic function rather than a suppression of it.

It is concluded that the rabbit and sheep do not react in the same way physiopathologically to infection with *F. hepatica*. Two distinct phases are distinguished in sheep at the beginning and at the end of the parasitic cycle, whereas, in rabbit, the main pathophysiological reaction appears during the second third of the infection. Consequently, the rabbit cannot be used as a model for the study of the pathophysiological effects of *F. hepatica* infection.

Our previous studies (Banting *et al.*, unpublished data; Le Bars and Banting, 1973) have shown that even in unfavorable experimental conditions measurement of OCT is a very satisfactory measurement of hepatic function. Similarly measurement of alkaline phosphatase is a satisfactory parameter of pathophysiological effect in fascioliasis, but previous studies as well as the present work indicates that measurement of SGOT levels does not provide a satisfactory evaluation of hepatic function in fascioliasis. SGPT has

already been abandoned by many authors because of inconstant quantities found in the liver and also because the kinetics of synthesis and catabolism are difficult to study in the ruminant.

The parameters chosen to assess hepatotoxicity are particularly important. The four parameters used in this study (plasma triglycerides, OCT, albumin and urea) appear to be satisfactory to measure fluke hepatotoxicity, but perhaps inclusion of sorbitol dehydrogenase and glutamate dehydrogenase (Hughes, Trecher and Harness, 1973, 1974) measurements would assist in understanding the pathophysiological mechanisms in infected ruminants.

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BLOOD PEPSINOGEN ESTIMATIONS AND PRODUCTION RESPONSES IN TRICHOSTRONGYLID PARASITISM OF RUMINANTS

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INTRODUCTION OF PAPSINOGEN ESTIMATION TO VETERINARY PARASITTOLOGY

Although plasma pepsinogen concentrations had been used as a tool in human medicine (Mirsky *et al.*, 1952), the technique did not attain any significance in veterinary medicine until clinical investigations began at the Glasgow Veterinary School on the problem of seasonal gastritis in calves. This problem had been recognised as a parasitic disease due to the trichostrongylid *Ostertagia* Sp. (Hirschowitz, 1957; Martin, *et al.*, 1957; Mirsky *et al.*, 1952).

Anderson *et al.* (1965) published the first definitive clinical study of *Ostertagia* infection in the field, characterising the disease by differing epidemiological origins into two types (I and II). The first was the classic infection on which veterinary medicine had attempted to base its solutions. This originates with direct development of newly ingested larvae to egg laying adults. The second origin follows the erratic resumption of development by large numbers of previously ingested larvae whose development had been inhibited and which had been dormant in the gastric mucosa. The known occurrence of such stages had previously been disregarded by pathologists as a zoological curiosity. In the cool temperate climate of the Scotland observations this latent infection persisted in animals which were housed over winter.

Plasma pepsinogen estimations were shown to be reliable indicators of clinical disease, with mean levels in affected groups of calves elevated to around 3000 to 4000 mU (milli Units, equivalent to 1000x micromoles of tyrosine liberated per minute per litre of test plasma). Nematode free calves with inhibited larvae only had around 1000 mU. Because some laboratories perform the estimations on serum, the term blood pepsinogen is used to mean either in the following discussion.

Work published in the succeeding 10 years has emphasised the importance of *Ostertagia* infections in calves. Further,

gastric infection by trichostrongylid nematodes is a source of economic loss in cattle and sheep production throughout the world where ever it has been investigated.

SEASONAL VARIATION OF PEPSINOGEN ESTIMATIONS IN RELATION TO CLIMATE

Most observations of the pathology in natural cases of parasitic gastritis (review, Michel, 1969) have been reported from animals grazing in the warm to cool temperate rain regions of the world, where the period of predominant pasture growth is in summer. Attempts to transpose these results to situations with animals in more arid or more seasonally contrasting climatic zones have not lead an adequate explanation of the factor leading to disease. Observations in different major zones are likely to show varying clinical pathophysiology in terms of epidemiological interaction. Analyses of the features in common will contribute to understanding the mechanisms. These climatic zones are shown in Table I, based on the British glossary (McIntosh, 1972) and with reference to Lansberg *et al.* (1965). Readers requiring further details of a particular locality should consult Lansberg (1969 *et seq.*).

TABLE I

MAJOR CLIMATIC ZONES
Modified by whether under Oceanic (Maritime) or Continental Influence

	rainfall not seasonal	alternation of wet and dry seasons
HOTTER AREAS*	EQUATORIAL (RAIN FOREST) CLIMATE	TROPICAL (SAVANNA) CLIMATE
	ARID (DESERT) CLIMATE	SUB-TROPICAL (STEPPE) CLIMATE
COOLER AREAS*	TEMPERATE (HUMID† TEMPERATE) CLIMATE	MEDITERRANEAN CLIMATE
	BOREAL (COLD TEMPERATE) CLIMATE	POLAR CLIMATE

** in the hotter areas the occurrence of rain is more common in the hotter ("summer") months, and in the cooler areas is more consistent in the cooler ("winter") months.*

† humid, or rain, temperate includes warm and cool.

For example, Anderson (1967, Anon. 1973) found the syndrome of *Ostertagia* infection in calves in part of southern Australia different from that in Scotland. The lesser known principal epidemiological features of trichostrongylid infection in the Mediterranean type climate are shown in Fig. 1, on which the original observations reported below in Section V are based.

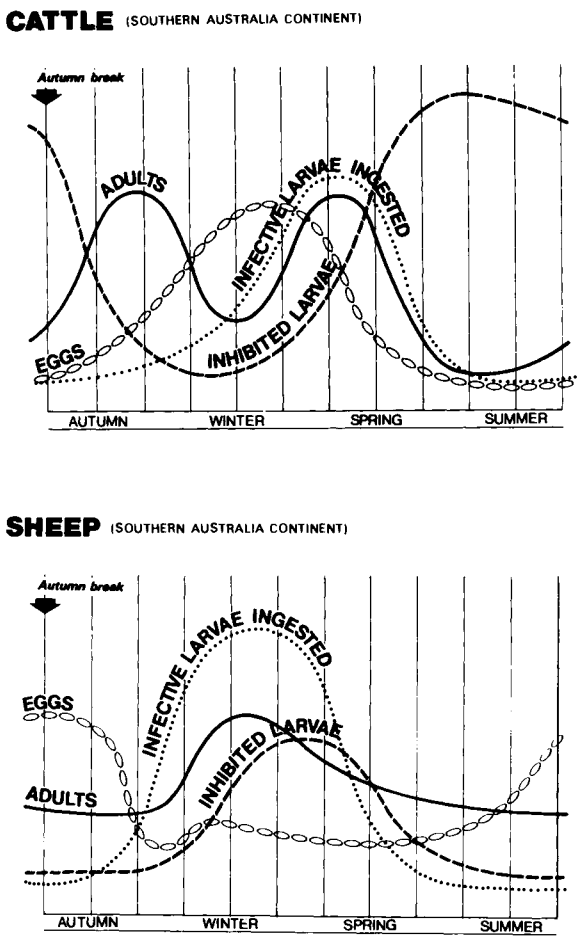


Fig. 1a and b. The epidemiology of the predominant trichostrongylid nematodes of ruminants (a, cattle; b, sheep) in a climate with hot dry summers and cool wet winters. The level of nematodes and the persistence of the larval or adult burden varies with the prior exposure and susceptibility of the host animal.

In this climate the temperature rises in spring, and the standing pasture dries out like hay in the fields over summer. The autumn break is the start of effective rainfall initiating growth - its timing may vary from year to year and place to place. The development of adult nematodes shown after the autume break represents type II *Ostertagia* infection in cattle, but not occurring in sheep.

The seasonal pattern of blood pepsinogen levels in cattle is shown in Fig. 2 for such a climate, where the highest levels are in autumn, associated with the type II origin of disease. These are miscellaneous samples from herds in which trichostrongylid gastritis occurs. It is seen that there is considerable variation in levels, and indeed many animals do not show clinical signs even when grazing together with severely affected contemporaries - a variation in pathophysiology that has yet to be investigated.

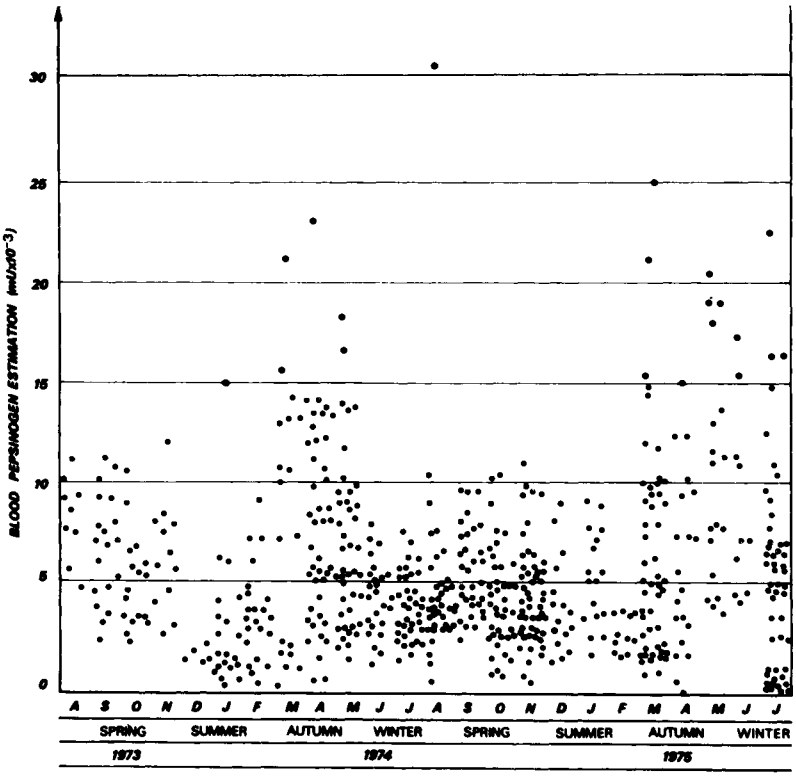


Fig. 2. Seasonal blood pepsinogen levels in grazing cattle in a climate with hot dry summers and cool wet winters.

CLINICAL SIGNIFICANCE OF ELEVATED PEPSINOGEN LEVELS

Diagnosis

Prior to an acceptance of the use of laboratory testing for elevated blood pepsinogen levels, it was not uncommon for field veterinarians to fail to diagnose gastric infection with trichostrongylid nematodes because there was no response to anthelmintic treatment and faecal egg counts were negligible. This occurs especially with type II *Ostertagia* infection in which larvae from the reservoir of the inhibited forms, which are refractory to therapy, resume development intermittently.

Interpretation of blood pepsinogen levels in ruminants may not be consistent between laboratories, even when the technique is based on or modified from that used at the Glasgow Veterinary School in which bovine serum albumin Cohn fraction V is recommended as the reaction substrate. With field infections in our environment (ref. Fig. 1), levels of less than 1000 mU are dismissed as not significant, and levels up to 5000 mU are not associated with clinically apparent disease. Blood pepsinogen levels of 5000 to 10000 mU are suggestive of *Ostertagia* infection in a differential diagnosis, and over 10000 mU confirm obvious disease which may be diagnosed clinically. Animals with more than 15000 in our Mediterranean climate usually die irrespective of treatment (see Fig. 2).

It is noticeable that blood pepsinogen levels tend to be lower in field infections reported from cool or warm temperate climatic zones (Anderson *et al.*, 1965; Armour, 1970; Mylrea and Hotson, 1969; Brunsdon, 1969, 1971, 1972), and it may be that type II disease is most spectacular following a hot dry summer.

Most of the fatal field cases observed in a Mediterranean climate arose from type II origin. However, in type I origin cases, in a temperate climate, it has been recorded (Brunsdon, 1969) that animals have died before blood pepsinogen levels exceed 5000 mU, and that there was no obvious difference in levels between these and the survivors. Such cases were preceded by high faecal egg counts. The importance of elevated blood pepsinogen levels in diagnosis is thus greatest when the egg counts are lowest (See also section C.).

Pathogenesis

It is commonly accepted that the pepsinogen level in the blood is a measure of damage to the gastric mucosa. In ruminants little is known of the mechanism, and differing suggestions have been made. That of Ross *et al.* (1969a), pro-

posing that elevated blood levels are a reflection of increased abomasal pepsin secretion, is not supported by the careful work of McLeavy *et al.* (1973) in which it was shown that secretion was suppressed in the cells of the infected abomasum. A positive correlation of rise in blood pepsinogen level and loss of gastric acidity is well established (Anderson *et al.*, 1966; Allen *et al.*, 1970). Also, Allen *et al.* (1970) reported blood pepsinogen activity correlated with raised serum glycoprotein concentration and was related to abomasal disfunction.

The proposal was made by workers at the Glasgow School that the elevated blood pepsinogen levels are due to leakage following an increase in mucosal permeability with *Ostertagia* infection (Jennings *et al.*, 1966; Armour, 1970), and supported by their studies of plasma albumin loss (Mulligan *et al.*, 1963), histological change (Ritchie *et al.*, 1966) and ultrastructure (Murray, 1969). The pathogenesis is probably more complex, for if this was a simple cause, the loss of gastric protein (pepsinogen) to the plasma might be expected to parallel the loss of plasma protein to the gut, which is not always the rule (compare data of Holmes and MacLean, 1971).

It has been observed that in grazing animals blood pepsinogen levels increase with age irrespective of current infection, at least up to 2 years old in cattle where levels reached 3000-4000 mU (Mylrea and Hotson, 1969). Further work on the pathophysiology of parasitic gastritis in animals of varying physiological and immune status is required and these may elucidate the marked individual variation that is seen in outbreaks of clinical disease.

Response to Anthelmintic Therapy

Brunsdon (1971, 1972) reported mean blood pepsinogen levels up to about 4000-5000 mU in grazing calves with type I development of trichostrongylids, compared to about 2000mU in calves treated weekly with excess thiabendazole or tetramisole. These groups were grazed separately, and the reduction of pepsinogen levels was most pronounced when treated calves were placed on clean pasture without further challenge. Mylrea and Hotson (1969) reported, however, that monthly thiabendazole treatment had no effect on blood pepsinogen levels.

Anderson (1972) was able to explain the failure of anthelmintic therapy to reduce blood pepsinogen levels by showing that with type I infections (direct development) in sheep, the level of pepsinogen was closely related to the level of infective larvae ingested, even in animals treated every two weeks with thiabendazole but grazing with the untreated group. The peak levels were 2000 to 3000 mU compared to the worm

free control level of less than 500 mU. When infective larvae were not available, pepsinogen did not persist in the blood of treated sheep.

Unpublished observations by Pullman *et al.* (1974) in South Australia on type II infection (delayed development) in cattle showed that repeated treatment with tetramisole (1875 mg laevamisole each) had no significant effect on the elevated blood pepsinogen levels compared with similar animals left untreated in the same experimental herd (Fig. 3).

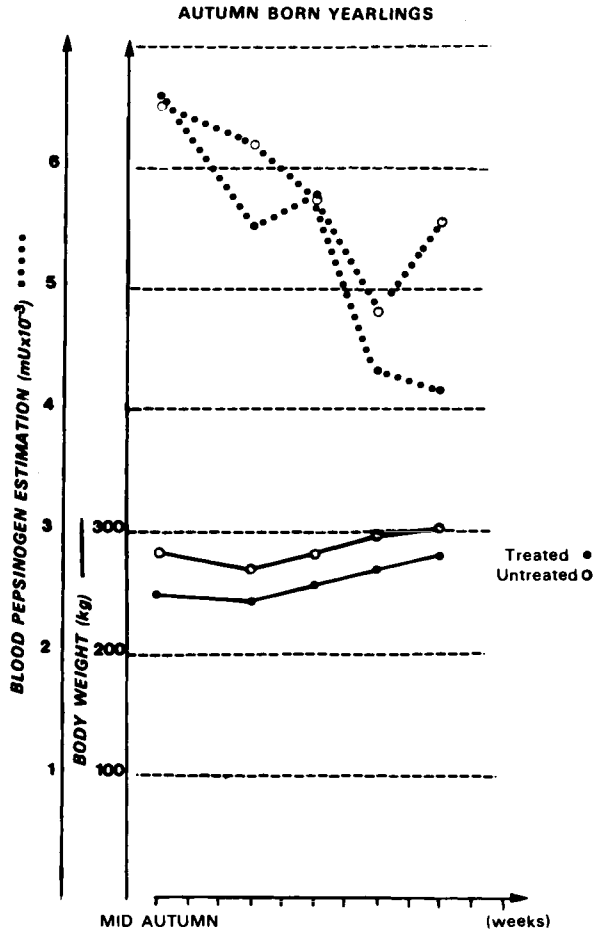


Fig. 3. Mean body weight and blood pepsinogen responses to anthelmintic therapy given at each sampling time. Heifers born autumn 1973.

The failure to obtain a more rapid response is attributed to the presence of residual inhibited larvae in the mucosa and the resumed development of some of them between treatments. This mimics the situation seen in commercial herds.

To predict the response to anthelmintic therapy it is important to sample for a faecal egg count and a blood pepsinogen estimation at the same time, because if both parameters are elevated, indicating type I infection, a response to therapy may be expected, but when the egg count is low response to a single treatment is unlikely.

PARASITOLOGICAL SIGNIFICANCE OF ELEVATED PEPSINOGEN LEVELS

Ostertagia spp. Infections

For a summary of recent findings on gastritis due to *Ostertagia ostertagi* see review and addendum by Armour (1970, 1974).

In a natural field infection, at first season's grazing of susceptible calves, a significant correlation between levels of blood pepsinogen and numbers of abomasal nematodes established has been shown (Brunsdon, 1971). This confirms the direct relationship established with experimental infections and first reported by Anderson *et al.* (1966). On the other hand, in animals sampled under a variety of field conditions there may be no correlation between blood pepsinogen level and nematode burden or faecal egg count (Mylrea and Hotson, 1969).

With sheathed third stage larvae (SL3) of given infectivity, Mylrea and Hotson (1969) established that elevation of blood pepsinogen in calves corresponded very well to the larval administration. The same relationship was discovered in grazing sheep by Anderson (1972) who measured availability of infective SL3 with "tracer" animals.

Studies of the effects of a daily dose of *Ostertagia* SL3 in calves by Allen *et al.* (1970) showed blood pepsinogen levels, at 3 months infection, of 3000-5000 mU for calves given 200-570 SL3/day, 6000mU for 950 SL3/day, and 7000-8000 mU for 1600 SL3/day. As the course of infection continued more larvae given became inhibited, with the number of adult nematodes and blood pepsinogen level both declining so that there was a significant correlation between them.

The mean peak blood pepsinogen levels reported in ruminants following single doses of SL3 vary from less than 2000 mU with low larval doses (Anderson *et al.*, 1966), to around 3000 to 4000 mU with moderate to high infections of 100 000 SL3 or more, for type I development (Anderson *et al.*, 1966, Armour *et al*, 1966), and to levels about 5000 mU for type II develop-

ment (Armour and Bruce, 1974). Higher levels of up to around 7000 mU in calves and 10 000 mU in sheep were recorded from animals in metabolism observations (Jennings *et al.*, 1973). For such experimental results to be referable to other situations, more attention is required to describe the physiological interactions both of the host and of the infective larvae (Ford, 1971; Armour and Bruce, 1974).

A dependence of blood pepsinogen elevation on development of larvae to adults is shown by the temporal relationship in experimental infections with direct (type I) development. In sheep there is a significant rise in blood pepsinogen around 1 to 2 weeks after the administration of SL3 (Armour *et al.*, 1966; Holmes and MacLean, 1971; McLeay *et al.*, 1973) when young adults emerge into the abomasal lumen (Armour *et al.*, 1966). In calves blood pepsinogen levels increase around 2 to 3 weeks (Jennings *et al.*, 1966), which is when young adults emerge from the gastric glands (Ritchie *et al.*, 1966).

Secondly, Armour and Bruce (1974) were able to show in calves that, when only larvae with development inhibited were present, blood pepsinogen levels remained low until 16 to 18 weeks after infection, when a rise in levels corresponded to resumed (type II) development as above.

The conclusion is inescapable that a blood pepsinogen elevation is caused by events at the emergence of larvae from the gastric mucosa, in both types of development. However, this cause is not exclusive. Thus, Jennings *et al.* (1966) reported that in a calf observed for 9 weeks after being given 300 000 SL3 of *O. ostertagi* blood pepsinogen level remained elevated with a residual nematode burden of 1200 adults. Similarly, in experimental *O. circumcincta* infections of sheep given 25000 SL3 of which 900-1600 persisted as adults for about a year, Ford and Dolling (1972) measured blood pepsinogen levels about 2000-3000 mU around 15 weeks infection after faecal egg counts had fallen.

Trichostrongylids other than Ostertagia

Working with calves experimentally infected with *Trichostrongylus axei*, Ross *et al.* (1967, 1968a) found imperceptible and inconsistent rises in blood pepsinogen up to about 1000 mU with low levels of infection, and rises up to about 2-00 mU with lethal infections in which there were obvious clinical signs. When *T. axei* infections were combined with those of *O. ostertagi* (Ross *et al.* 1968b, 1969a), then results consistent with infection by the latter nematode were obtained.

In lambs infected with *T. axei*, observed by Ross *et al.* (1969b), the blood pepsinogen showed a slight rise at week 17

only with low level infection and no clinical disease. With high level infections causing clinical disease and loss of weight, blood pepsinogen estimations rose 1 week after infection to 1000-2000 mU.

With massive doses of 1 million *Haemonchus contortus* SL3 in mixed sheep, Coop (1971) recorded a rise in mean blood pepsinogin levels to about 1000 mU at 5 days after infection. The elevated level persisted until 27 days after infection at which time only inhibited larvae were recovered. In an unpublished study by Judson and Ford (1973) in which groups of one year old sheep in metabolism cages were given 5000 or 50 000 SL3 of *H. contortus*, blood pepsinogen levels persisted around 3000-5000 mU or 5000-10 000 mU respectively, until 13 weeks when untreated animals had a mean residual burden of approximately 2500 nematodes (mixed adults and larvae). In animals treated with anthelmintic at this time, the blood pepsinogen levels fell to 700 mU or 1000 mU respectively 2-3 weeks later, these being similar to the levels observed in paired nematode free controls.

It thus appears that, while infections with *T. axei* may not contribute significantly, the establishment of *H. contortus* in the abomasum may be a factor contributing to a rise in blood pepsinogen. However, clinical disease due to *H. contortus* is minimal in a mediterranean climate, as the warm months of the year are dry.

PRODUCTION SIGNIFICANCE OF ELEVATED PEPSINOGEN ESTIMATIONS

Susceptible Juvenile Animals

Depression of body weight gain, and in severe cases loss of body weight, is a characteristic of the clinical disease associated with elevated blood pepsinogen levels (Anderson *et al.*, 1965; Jennings *et al.*, 1966; Allen *et al.*, 1970). The persistence of the adverse effect on weight gain is uncertain, as Ritchie *et al.*, (1966) reported that infected animals may grow at the same rate as before after a temporary interruption to body weight gain.

For grazing calves in a temperate climate Brunsdon (1971) showed a divergence of mean body weight and mean blood pepsinogen levels from the time of high egg counts at their first infection, so that the group with a higher mean pepsinogen had lower mean weight, but it is not recorded if this relationship was consistent in individual calves. Subsequently, Brunsdon (1972) showed that the highest mean blood pepsinogen levels were reached in the calves of lower weight after the mean rate of body weight gain had recovered to that of animals treated with anthelmintic. Thus an inverse relationship existed between mean blood pepsinogen levels and mean body

weight, but not body weight gain. Similarly Armour *et al.* (1966) reported the greatest mean depression of body weight gain in *Ostertagia* infected sheep at a different time to the greatest mean elevation of blood pepsinogen.

At a time of negligible larval intake following previous pasture infection, Anderson (1972) found higher body weight gains and greater wool growth in a treated group of sheep (which had negligible blood pepsinogen levels), than in non-treated members of the flock (in which the mean blood pepsinogen level remained elevated).

In a study by Ford *et al.* (1975), on an experimental herd in a Mediterranean climate with epidemiology as in Fig. 1, an attempt was made to show if the level of blood pepsinogen in individual animals was related to their production. Groups of cattle were held at different set stocking densities, and 24 calves sampled from 2 to 14 months of age to cover the important period of growth. Overall, there was no such relationship, although more than half of the month to month rises in blood pepsinogen levels were followed by decreased growth. However, these calves showed little evidence of type I infection, only about 8% of faecal samples having more than 100 eggs per gram of faeces.

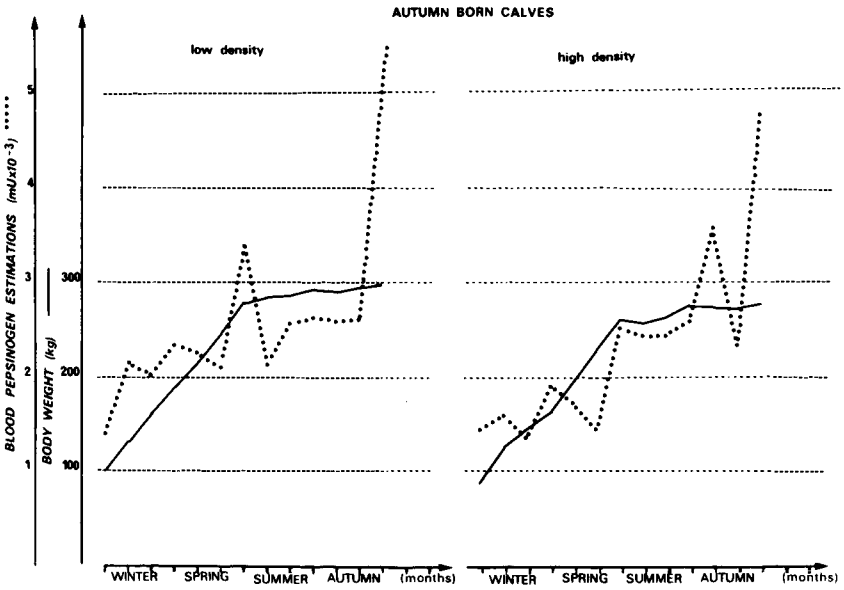
A comparison of means of blood pepsinogen and body weight for batches of calves is shown in Fig. 4. There is a marked depression of body weight gain associated with inadequate summer pastures, but not associated with a change in blood pepsinogen level. Poor nutrition may have masked effects due to subclinical infection. No calves reached blood pepsinogen levels in excess of 5000 mU prior to the increase of blood pepsinogen seen at late autumn in which every calf participated. At this stage, the demeanour of the animals and evidence of mild diarrhoea showed varying degrees of clinical disease attributed to type II infection with *Ostertagia* sp.

Thus there has been no evidence produced that blood pepsinogen levels can be used in the absence of clinical signs to determine if growth of young animals is affected, or to assess whether remedial action is warranted.

Immune Adult Animals

There are no known reports of attempts to monitor by pathophysiological means the effects of trichostrongylid infection on adult performance such as reproduction and lactation. In observations by Ford *et al.* (1975) on yearling beef heifers, a number showed a check in body weight gain during mid pregnancy, but there was no consistent relationship in individuals with rises or high levels of blood pepsinogen, although some samples had in excess of 10 000 mU. The variation between individual animals was pronounced. In this situation faecal

egg counts were low.



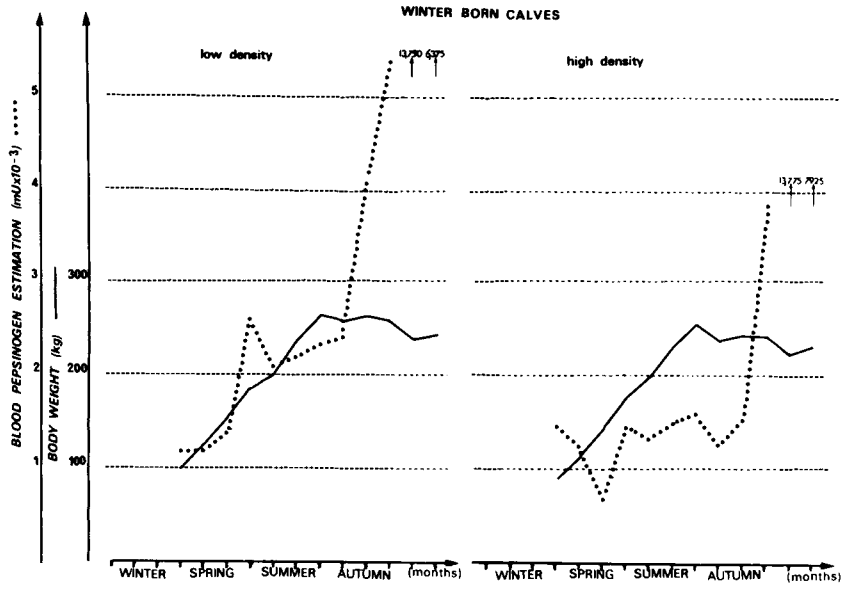


Fig. 4a and b. Mean body weight and blood pepsinogen levels from 2 months of age at 2 stocking rate densities. Calves born 1975.

Anderson (1973) reported that adult sheep with previous experience of infection reacted more to available infective larvae on pasture, with higher blood pepsinogen levels than weaner sheep, even when larval contamination was not high. He has found that production is depressed in the immune animals in such a situation and reported in grazing animals an additional type of parasitic gastritis which is related to the intake of infective larvae that are neither detected as adult nematodes nor produce type II infection (Anderson, 1968). This disease will explain some of the variation of the blood pepsinogen estimations shown in Fig. 2.

CONCLUSION

Published work on animal production (body weight, wool, and milk) in relation to blood pepsinogen tests is rare. Although there is a distinct relationship between infections by *Ostertagia* spp. causing clinical disease and elevation of blood pepsinogen, the test is confirmatory in animals requiring attention anyway. A clinical pathology test to monitor effects on production in animals with only subclinical infection is a long sought goal.

Nevertheless, it is considered that it is acceptable to interpret blood pepsinogen concentration as a better predictor of production loss than a parasitological measurement alone such as faecal egg count, at least for *Ostertagia* spp. infection. Efficient production will not be attained while there is any infection leading to elevation of blood pepsinogen.

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A MULTISTAGE MULTIPARASITE MODEL CONSISTING OF *OESOPHAGOSTOMUM* SPP, *HYOSTRONGYLUS RUBIDUS* AND *ASCARIS SUUM* IN YOUNG SWINE FOR PATHOLOGICAL AND ANTHELMINTIC STUDIES

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INTRODUCTION

In studies of anthelmintic compounds the results obtained by the classical "controlled test" do not always parallel those obtained in natural parasitism. This is particularly so in the case of adult animals in which the worm burden is high and the lesions produced by the infection are marked. To improve the reliability of anthelmintic tests in swine we have carried out trickle infection for each major parasite of swine which occurs in France. We have succeeded in establishing stable multistage populations with *Oesophagostomum spp*, *Hyostrongylus rubidus* or *Trichuris suis*. We define as stable a population in which the number of worms in the various organs and at the various stages of development remains constant for 1 or 2 weeks when the total number of parasites administered is the same.

In the course of such studies we have confirmed the findings of Oakley (1974) who reported that with *Ascaris suum* a continuous infection does not give rise to a stable adult infection. The moult from the 4th to the 5th larval stage in the bowel is responsible for the elimination of the already existing population of older parasites (Soulsby 1965). Such results were obtained with infection rates of 12,500 eggs per piglet twice per week. However, when the dose of eggs was greatly reduced to 100 eggs daily each day for 5 days a week for one or two weeks, satisfactory adult *A. suum* infections were obtained (Raynaud, 1975b; Raynaud, Brunault and Bouchet, 1975). These previous studies also demonstrated that for *Strongyloides ransomi* a trickle infection was inadequate to establish a stable infection. The present studies are a modification of Reinecke's (1966) "Sheep larval anthelmintic test" adapted for use in swine.

MATERIALS AND METHODS

Animals - Young 15 kg parasite free weaned piglets were obtained from clean farms, the pregnant sows having been de-

wormed under supervision.

Parasites. Eggs of *Ascaris suum* were incubated at room temperature for one month before use. The strain of parasite used was one which was assessed as pathogenic in swine and in mice (Raynaud, 1975a; Raynaud *et al.*, 1975).

Hyostrongylus rubidus larvae was obtained from parasites isolated from the stomach of pigs slaughtered at the local slaughter house (Raynaud, Bretheau and Graber, 1974).

Oesophagostomum spp larvae were obtained from eggs cultured from parasites isolated from swine slaughtered in a local slaughter house.

The relative proportion between the 3 species found in France, namely *quadrispinulatum, dentatum* and *granatensis* (Graber *et al.*, 1972) was not consistent and species identification was carried out on females only. At post mortem washings of the stomach, small intestine and the coecum and colon were collected. Pepsic digestion of the entire mucosa of the stomach and the small intestine was also carried out.

Design - Experiment A (See Table I)

In this *A. suum* eggs were given during the first and second weeks (100 eggs per day) as the first phase of infection and during the 5th and 6th week (100 eggs per day) during the second phase of infection. Larvae of *Oesophagostomum* spp. (300 L3 per day) were given during weeks 4, 5 and 6 and 500 L3 per day were given during weeks 7 and 8. Two hundred infective larvae of *Hyostrongylus rubidus* per day were given on weeks 5, 6 and 7 and 400 such infective larvae on weeks 8 and 9.

Animals were slaughtered on day 70 after the initial infection with *A. suum* eggs. At this time the age of the first phase *A. suum* infection ranged from 56-70 days, and 28-42 days for the second phase, while the age of the *Oesophagostomum* spp infection ranged from 14-49 days and the *Hyostrongylus* infection from 7-42 days.

Experiment B (See Table II)

Ascaris suum eggs (25 eggs/day) were given during week one to six, *Oesophagostomum* spp. larvae (300 L3 per day) were given during weeks 3, 4 and 5 and 100 L3 of such larvae per day were given during weeks 6 and 7. Two hundred infective larvae of *H. rubidus* were given per day during weeks 3, 4 and 5 and 400 such larvae were given during weeks 6 and 7 of the experiment.

Animals were slaughtered 57 days after the initial infection with *A. suum* at a time when the ascarid infections ranged from 18-57 days of age. The age of the *Oesophagostomum*

and *Hyostrongylus* spp infections ranged from 11-43 days.

RESULTS

The result of the two experiments are presented in Tables I and II.

TABLE I

Design and mean egg counts and worm counts of Experiment A consisting of swine infected with various doses of parasites.

PARASITES	ASCARIS (Strain 10P)	OESOPHAGOSTOMUM	HYOSTRONGYLUS
Design : infection			
Week 1	100 eggs/day		
Week 2	100 eggs/day		
Week 3			
Week 4		300 L3/day	
Week 5	100 eggs/day	300 L3/day	200 L3/day
Week 6	100 eggs/day	300 L3/day	200 L3/day
Week 7		500 L3/day	200 L3/day
Week 8		500 L3/day	400 L3/day
Week 9			400 L3/day
Total	2000 eggs	9500 L3	7000 L3
SLAUGHTER : DAY 70			
DAYS AFTER INFECTION			
after first infection	+ 70 days	+ 49 days	+ 42 days
after last infection	+ 28 days	+ 14 days	+ 7 days
number of infections	20	25	25
EGG COUNTS AT SLAUGHTER / g	2,820	2,000	
WORM COUNTS (average 5 animals)			
Immatures	5.2	4,534	1,686
	<0.5 cm = 2.6 0.5 to 2 cm = 2.6 >2 cm = 0	L4 mucosa = 1,824 L4 lumen = 1,480 L5 = 1,230	L4 = 854 L5 = 832
Adults	25.2	2,830	1,196
Females	18.8	1,600	694
Males	6.4	1,230	502
Total	30.4	7,364	2,882

TABLE II

Design and mean egg counts and worm counts of Experiment B consisting of swine infected with various doses of parasites.

PARASITES	ASCARIS (Strain 19MF)	OESOPHAGOSTOMUM	HYOSTRONGYLUS
Design : infection			
Week 1	25 eggs/day		
Week 2	25 eggs/day		
Week 3	25 eggs/day	300 L3/day	200 L3/day
Week 4	25 eggs/day	300 L3/day	200 L3/day
Week 5	25 eggs/day	300 L3/day	200 L3/day
Week 6	25 eggs/day	1,000 L3/day	400 L3/day
Week 7		1,000 L3/day	400 L3/day
Total	750 eggs	14,000 L3	7,000 L3
SLAUGHTER : DAY 57			
DAYS AFTER INFECTION			
after first infection	+ 57 days	+ 43 days	+ 43 days
after last infection	+ 18 days	+ 11 days	+ 11 days
number of infections	30	25	25
EGG COUNTS AT SLAUGHTER /g	29	20	
WORM COUNTS (average 5 animals)			
Immatures	14.4	10,806	923
	<0.5 cm = 6.2 0.5 to 1cm = 5.6 1 to 4 cm = 2.6 >4 cm = 0	L4 mucosa = 9,660 L4 lumen = 730 L5 = 416	L4 = 376 L5 = 548
Adults	0.4	366	1,081
Females	0.2	150	581
Males	0.2	2.6	500
Total	14.8	11,162	2,004

In Experiment A infection with *Ascaris suum* was done in two phases with the intent of having mature and immature parasites present at the same time. However, during weeks 7 and 8 after initial infection a large number of adult parasites and of larval stages over 4 cm in length were passed in the faeces. This confirms the statement of Soulsby (1965) that "the moult from the 4th to the 5th larval stage in the bowel provides an antigenic stimulus to produce a secondary response responsible for the elimination of the parasites". Consequently at slaughter the mean worm count was low (30.4)

and represented 1.5% of the dose of eggs given per animal.

The percentage development of *Oesophagostomum* spp. and *H. rubidus* was much higher, being 77.5% and 41.2% respectively. The three genera of *Oesophagostomum* were well represented, 28% being *O. quadrispinulatum*, 42% *O. dentatum* and 30% *O. granatensis*. The parasitic larval and adult of the *Oesophagostomum* spp. and *H. rubidus* were distributed in satisfactory proportions (Table III) and consequently this model can be considered as a useful one for the study both of the pathophysiology of parasitic infection of swine and the efficacy of anthelmintic medication.

TABLE III

Distribution of various developmental stages of Oesophagostomum *spp. and* H. rubidus *in groups of swine given "trickle" infections of infective larvae.*

	HYOSTRONGYLUS				OESOPHAGOSTOMUM spp.			
	L4	L5	Males	Females	L4 nodules	Females Quadri.	Granat.	Dentat.
Experiment A								
Swine n° 230	1170	1140	470	730	2780	1500	500	200
233	860	590	380	530	1920	200	450	800
237	890	740	250	330	1410	150	750	400
239	770	1030	800	980	1480	150	600	650
241	580	660	610	900	1530	250	100	1300
Average	854	832	502	694	1824	450	480	670
Experiment B								
Swine n° 769	425	285	450	585	9825	50	10	0
785	300	605	525	530	8350	200	30	30
807	435	720	375	370	10850	10	20	0
798	355	580	535	625	9800	60	80	20
805	365	550	615	795	9475	180	20	40
Average	376	548	500	581	9660	100	32	18

Quadri. = quadrispinulatum

Granat. = granatensis

Dentat. = dentatum

In Experiment B the egg counts were low for all three genera of parasites at the time of slaughter of the animals. Nevertheless the percentage recovery of worms, based on the dose of infective stages, was increased to 20% in the case of *A. suum*, it was similar to Experiment A in the case of *Oesophagostomum* spp. (77%) and reduced 28.6%) in the case of *H. rubidus*. The three species of *Oesophagostomum* were not so well represented as previously (Table III) and only 12% of them were *O. dentatum*. In this experiment immature worms predominated (e.g. with *A. suum* 97% were immature and with *Oesophagostomum* spp. 97% were immature).

DISCUSSION

These two experiments illustrate that it is possible to establish a predictable population of parasites with regard to the ratio of mature to immature worms and the ratio of mucosal to lumen dwelling stages. The use of "trickle" infections to achieve this follows the work in sheep by Reinecke (1966) and produces more satisfactory animal to animal homogeneity, in that the highest count of worms is not greater than twice the lowest count.

Such infections have been found useful for the study of pathophysiology of parasitism in swine and of the efficacy of anthelmintics.

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HAEMATOLOGICAL CHANGES DURING THE COURSE OF EXPERIMENTAL INFECTION WITH *FASCIOLA GIGANTICA* IN SHEEP

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INTRODUCTION

The pathogenicity of *Fasciola hepatica* in sheep has been studied by several investigators, and the literature has been reviewed by Sinclair (1964) and Roberts (1968). In spite of great economic importance of the diseased caused by *Fasciola gigantica* in the tropical and the subtropical countries, this parasite has not received the attention that it deserves. Some information has been provided by Tigin, Baysu and Guralp (1971) and Hammond (1973) who described the haematological changes occurring in sheep in mild chronic experimental infection.

The present study reports haematological data collected during the acute phase of experimental infection with *F. gigantica* in Awasi sheep in Iraq.

MATERIAL AND METHODS

Animals

Ten Awasi male lambs, age 3-4 months, average weight 15kg, were reared under worm free conditions. The lambs were divided at random into two groups of 4 and 6 animals each respectively which were housed and managed under similar conditions. The diet consisted of a concentrate mixture plus an excess quantity of green fodder and hay which with water was fed *ad libitum*.

Infection

Metacercariae were obtained from *Lymnaea lagotis euphratica* as described by Kadhim and Altaif (1970). The six lambs of the experimental group were each orally infected with 200, 7-day old, metacercariae given in a soft-gelatin capsule through a balling gun. Blood (5ml in EDTA) was collected from animals of experimental and control groups prior to the start of the experiment and every two weeks thereafter. Packed cell volumes and haemoglobin percentage were determined. Thin blood films were dried in air, fixed in absolute methyl alcohol and stained with Giemsa's solution. Differential leucocyte counts were made for each animal.

RESULTS

Of the six animals that were each given 200 metacercariae, one (no 7305) died at the end of the 12th week before patency of the infection. 77 parasites were recovered from this animal. The remaining five animals survived the duration of the experiment (i.e. 20 weeks). All animals were sacrificed 20 weeks after infection and 67, 34, 49, 46, and 53 parasites, respectively, were recovered from them. Thus approximately 27 per cent of the metacercariae had developed to adult parasites.

Haematocrit values

The mean PCV values for infected and control animals is illustrated in Fig. 1. In infected animals there was a progressive decline in haematocrit values which became marked at the end of the 12th week after infection. The lowest values occurred 14 weeks after infection after which the mean value levelled off to about 20 per cent of the original.

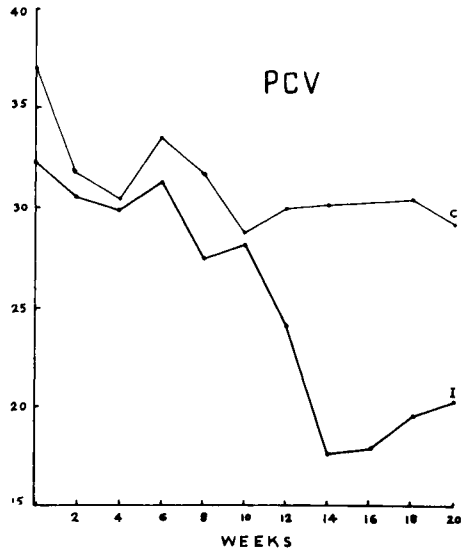

Fig. 1. Mean packed cell volume values for control (c) and infected (I) lambs. Ordinate; percent packed cell volume; Abscissa; weeks after infection.

Haemoglobin Values

The mean haemoglobin values for infected and control sheep are presented in Fig. 2. The changes were similar to those occurring in the PCV values. Thus in the infected animals, a sharp fall in haemoglobin values commenced around the end of the 14th week after infection when a mean value of 53.6 per cent was reached. Thereafter, the haemoglobin percentage rose to reach a mean level of 61.2 per cent on the end of the 18th week and 58.4 per cent on the end of the 20th week after infection.

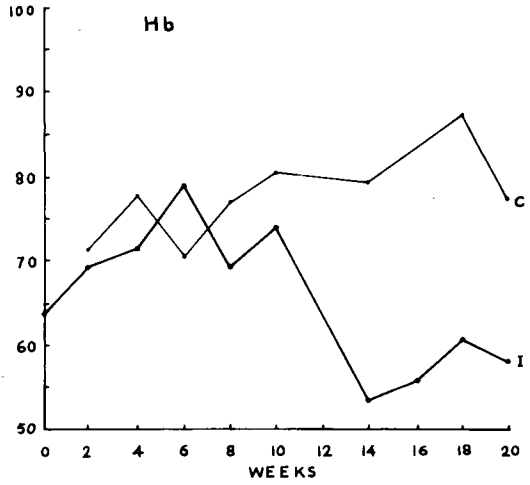

Fig. 2. Mean haemoglobin values for control (c) and infected (I) lambs. Ordinate; percent haemoglobulin; Abscissa; weeks after infection.

Erythrocyte Values

The mean erythrocyte counts of infected and control animals are presented in Fig. 3. In normal animals mean values varied from 8.25 X $10^6/mm^3$ to 10.7 X $10^6/mm^3$, and comparable mean values were seen in infected animals up to the end of the 6th week after infection. A progressive fall in values then occurred and this was most evident at the 14th week of infection. Values remained low and reached a mean of 5.2 X $10^6/mm^3$ at the 20th week.

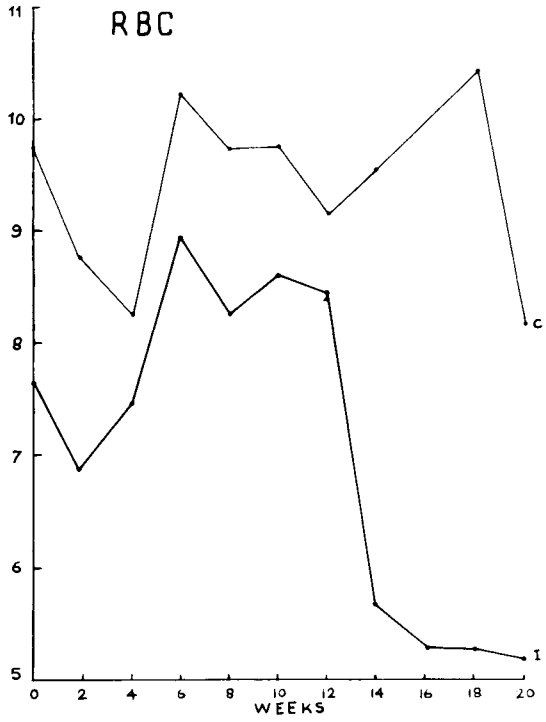

Fig. 3. Mean erythrocyte counts x $10^6/mm^3$ for control (c) and infected (I) lambs. Ordinate; RBC per $10^6/mm^3$; Abscissa; weeks after infection.

Eosinophil Counts

The mean values are presented in Fig. 4. In the infected animals, a sharp increase in the eosinophil count was observed as early as the end of the second week after infection, when the mean count reached 17.5 per cent and the end of the 4th week it had reached 20.16 per cent. Thereafter, it fluctuated but remained consistently higher than that of the control group.

The changes in the mean number of other white cells were not so well marked and showed no definite trends. Thus the group average ranged from 59.0 to 67.75 per cent in control animals, and 53.16 to 64.83 per cent in infected animals.

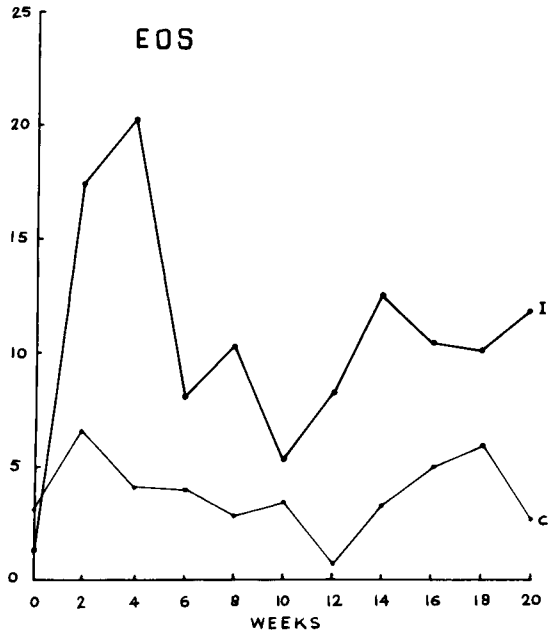

Fig. 4. Mean eosinophil percentages for control (c) and infected (I) lambs. Ordinate; percentage eosinophils: Abscissa; weeks after infection.

Other Values

Mean values for the Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobulin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) for infected and control animals are presented in Figs. 5, 6 and 7 respectively.

DISCUSSION

It has long been recognized that migrating young liver flukes induce haemorrage in the liver, and also may have deleterious effects on the production and life of the blood cells. As far as can be determined no systematic study of the blood picture of Awasi sheep has been made to date and, therefore, the data presented here for the control animals are not easy to interpret. In the present study somewhat wider variations than anticipated were seen in respect to haemoglobin percentage, erythrocyte and eosinophil of control animals. Comparison of the present results with those of previous workers is difficult since several variable factors,

such as age, breed of sheep used, species of the parasite (*F. hepatica* and *F. gigantica*), and the number of metacercariae given proportionate to the body weight of the animal. Therefore, it is considered appropriate to compare trends rather than absolute changes seen in the blood picture.

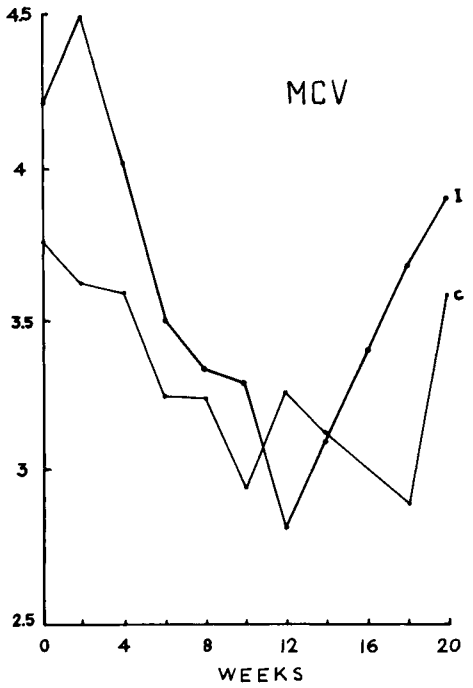

Fig. 5. Mean Corpuscular Volume Values for Control (C) and Infected (I) lambs.

In the present study, a fall in the PCV was observed in all the infected animals and this was well marked at the end of the 12th week after infection, reaching the lowest limit at the end of the 14th week. Sinclair (1962), working with *F. hepatica*, observed a similar trend and in sheep which died PCV values were 6.0 and 13.5 per cent, respectively. In the present study, the lowest count recorded for any infected sheep was 12 per cent. On the other hand, Furmaga and Gundlach (1967) reported a very gradual reduction of PCV values, these reaching 18 per cent 168 days after infection, whereas Roberts (1968) reported a sharp fall in the PCV occurring as early as the end of the third week after infection, when the

value was 60 per cent of the initial value. Tigin *et al.* (1971), working with *F. gigantica*, reported a sharp fall in PCV only at about the 10th week after infection and the values remained about 20 per cent thereafter. Hammond (1973) reported only a slight fall in PCV but he had used animals 14-15 months of age and had infected each animal with only 60 metacercariae. This illustrates that comparison between the results of workers, who used different regimens of infection, is not possible.

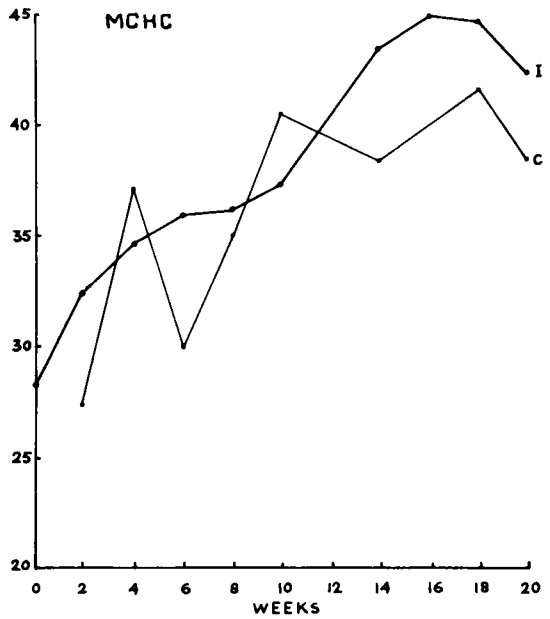

Fig. 6. Mean Corpuscular Haemoglobin Concentration Values for Control (C) and Infected (I) lambs.

The fall in haemoglobin was similar to that of the PCV, a sharp fall being observed at the end of the 14th week after infection. Similar results were obtained by other workers (above) and their haemoglobin values followed the PCV values.

In the present experiment, there was no significant change between the infected and the control animals in the number of erythrocytes in the early stages of infection. The change became apparent by the end of the 6th week after infection,

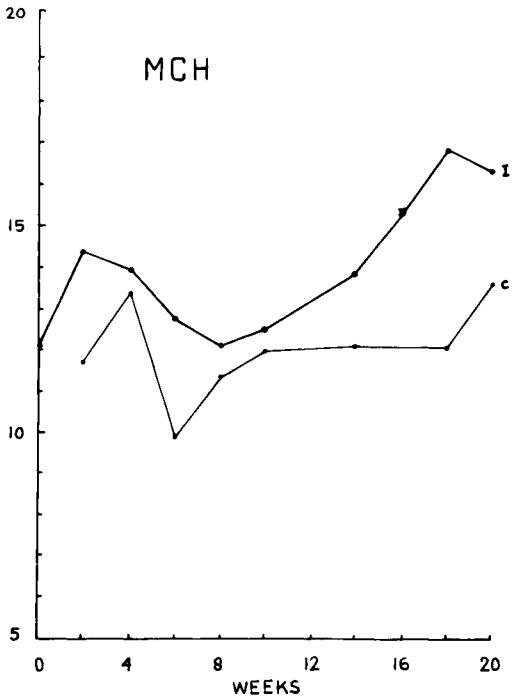

Fig. 7. Mean Corpuscular Haemoglobin Values for Control (C) and infected (I) lambs.

and the values declined steadily thereafter. Sinclair (1962) reported a decline in erythrocyte counts at the 9th week after infection, which continued until the 16th week after which the values levelled off. In one animal lower counts were recorded by Sinclair than in the present experiment, but this difference is attributed to the larger number (4 X 150) of metacercariae used by Sinclair to infect his animals. In a later paper, Sinclair (1964) had reported a fall in erythrocyte counts occurring at about the 60th day after infection and he concluded that "there was little difference in the rate of development and extent of the anaemia caused by a single dose of metacercariae and that which followed the administration of several doses of metacercariae". Furmaga and Gundlach (1967) had also observed a steady fall in the number of erythrocytes after the 8th week of infection and they recorded the lowest count of 5.2 X $10^6/mm^3$ at day 163 post-

infection. Roberts (1968) observed a similar progressive anaemia beginning weeks following infection.

The marked change in Hb, PCV, and RBC values which occur at the end of the 10th week and which last for about four weeks conincide with the four weeks when the juvenile parasites cause the greatest damage to the tissues of the liver with consequent loss of blood. The pathogenic effect is reduced once the parasites arrive in the bile ducts. In *F. gigantica* the prepatent period is 14-15 weeks.

The author (Kadhim, in press) has reported significant changes in serum protein level in sheep infected with *F. gigantica* and these occur at a comparable stage of infection to when the marked blood changes occur. Srivastava and Singh (1972a,b) have reported that the massive haemorrhages which leads to anaemia in animal infected with *F. gigantica* is closely associated with the feeding behaviors of the juvenile parasites.

A rise in the number of eosinophils is generally associated with the acute phase of an helminthic infection, or to the present of some other allogen. Eosinophil counts in the control animals, which were free of helminth infection, were higher than anticipated, but were much lower than those of the infected animals. A very sharp rise was evident as early as the second week after infection which reached a peak at the end of the 4th week. Sinclair (1962) has also observed a marked eosinophilia 14 days after infection with *F. hepatica* with a peak response 63 days after infection. Furmaga and Gundlach (1967) also reported high eosinophilia, which in individual animals occurred as early as 2 days after infection. A peak was reached at the end of the 4th after infection. Early comparable eosinophil responses following infection was reported by Roberts (1968) and Tigin *et al.* (1971).

SUMMARY

The haematological changes were studied in 6 sheep, age 3-4 months, each infected with 200 metacercariae of *Fasciola gigantica*. Subsequent to infection, there was a decrease in erythrocyte counts and in haematocrit values on the 6th and 10th weeks after infection respectively. Eosinophilia was observed at 2 to 4 weeks after infection.

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PATHOLOGY AND PATHOPHYSIOLOGY OF NEMATODE INFECTIONS OF THE LYMPHATIC SYSTEM AND BLOOD VESSELS

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"It is a remarkable fact that the dogs appear perfectly healthy" (Blackberg and Ashman, 1930).

INTRODUCTION

It might be expected that because of the great functional significance of the blood and lymphatics that parasites invading them would cause a considerable amount of damage to their hosts. However, with a few exceptions, this does not seem to be so. The quotation at the beginning of this review applies to heartworm *(Dirofilaria immitis)* infections in dogs but could well be used for many of the parasitic infections we shall be discussing. It is the purpose of our review to summarize the pathology of these infections in histological and anatomical terms and to attempt to correlate these changes with alterations in the functions of the parasitized organs and the effects of these changes on the efficiency of the host.

The parasites to be considered may be divided into 4 groups:

Those nematodes which inhabit the lymph nodes and lymphatics. Such as *Wuchereria bancrofti* and *Brugia* spp.

Those nematodes which live in the bloodstream, i.e. the lumens of the blood vessels. Examples are *Dirofilaria immitis* adults, *Angiostrongylus* spp. and many species of microfilariae.

Those nematodes which inhabit the walls of the blood vessels. Such as *Onchocerca armillata* and *Strongylus vulgaris*.

Those nematodes which as part of their normal migratory cycle pass through the lymphatic system and the blood vascular system, such as *Dictyocaulus* spp., *Ascaris* spp. and *Trichinella spiralis*.

Because of our special interest in the medically important, lymphatic-dwelling parasites we shall deal with these first. We shall not be able to mention all the nematodes involved with our field especially as with many of them little or nothing is known.

NEMATODES PARASITIC IN THE LYMPHATIC SYSTEM

Wuchereria bancrofti, *Brugia malayi*, *Brugia patei* and *Brugia pahangi* live in the lymph nodes and lymphatics of man and various other mammals. They enter their mammalian hosts through the small puncture wounds left by the mouthparts of their mosquito intermediate hosts. Within a few hours of penetrating through the skin they invade the lymphatics and migrate towards the heart until they reach a lymph node. They remain in the node for 14-21 days, before migrating back down into the afferent lymphatics where they produce microfilariae.

The whole question of host-parasite relationships in lymphatic filariasis is complicated and this is especially so when one considers the pathophysiology. Many people who are infected with *W. bancrofti*, and who have microfilariae in their blood, are comparatively little affected by the disease. Infection rates as high as 40% may exist with little evidence of morbidity - except for hydrocoels and occasional lymphangitis. But a small proportion of the affected individuals will develop a response which removes microfilariae from their blood; at the same time lymphoedema and elephantiasis develop. The same seems to be true in cats infected with *B. pahangi*. Most of the animals with circulating microfilariae are remarkably little affected clinically even though on dissection their lymphatics may be grossly abnormal. The infection in cats is very long lived (Denham *et al.*, 1972a, Wilson and Ramachandran, 1971). However, if cats are repeatedly infected they eventually produce a response which prevents further reinfection, clears the blood of microfilariae and kills adult worms. It is only at this time that lymphoedema is seen (Denham *et al.*, 1972b).

The antibody response to these worms is also rather odd. In both human patients and cats in which microfilariae circulate in the blood there is no detectable anti-microfilarial antibody (Danaraj and Schacher, 1959, Wong and Guest (1969), Ponnudurai *et al.*, 1974). Antibodies which react with antigens associated with infective larvae, fourth stage worms and adult worms may be found in such patients and animals. In those patients and cats who have lymphoedema or elephantiasis and are amicrofilaraemic antibodies are found which react with microfilariae. This observation is of considerable practical importance as it represents a way in which elephantiasis caused by filarial infection can be differentiated from non-filarial elephantiasis which is widespread in some tropical areas, especially Ethiopia and Kenya.

Two distinct types of immunological response occur in lymph nodes stimulated by antigens and other agents (Weiser *et al.*, 1969, Anderson and McKeating, 1970, Cottier and Turk, 1971). The cell-mediated-type immunological (CMI) response is characterized by proliferation of mononuclear thymus-dependent cells in the paracortical region of the lymph node, and the antibody-type (AB) response by proliferation of lymphocytes in rapidly enlarging germinal centres and the production of large numbers of plasma cells in the cortico-medullary junction and medullary cords of the nodes. Nodes from germ-free animals show virtually no immunological activity but those taken from normal animals are always to a certain extent active.

As these parasites are in the lymphatics any antigens which they release will travel to the adjacent lymph nodes and stimulate a response. The lymph node, immediately above the site being parasitized, responds actively to antigens derived from the worms (Schacher and Sahyoun, 1967, Rogers *et al.*, 1975). Within two days of infection with *B. pahangi* the lymph nodes of cats show a strong CMI type response but the beginnings of an AB response are not apparent until at least 4 days; even then the CMI response is dominant and it is not until 14 days after infection that the AB response is fully developed. The CMI response drops off after a few months but strong AB reactions are seen for as long as the cats remain infected. Repeated infection strengthens the AB type response but has little or no effect on the CMI reaction in the body (Rogers *et al.*, 1975). During the course of infection there are extensive fibrotic changes in the nodes. Schacher and Sahyoun (1967) described granuloma like reactions around eggs and microfilariae in lymph nodes but Rogers *et al.* (1975) could not find these.

Schacher and Sahyoun (1967) believed that many of the changes in lymph nodes could be associated with specific changes in the life-cycle of the parasite, such as moulting and the achievement of sexual maturity, whereas Rogers *et al.* (1975) suggest that they are linked with the sequential development of an immune response in the host, although the changes in the lymphatics themselves are almost certainly due to the migration down the lymphatics and the huge increase in size of the worms.

The changes in lymphatics of cats infected with *B. pahangi* are much more dramatic than those in lymph nodes but special techniques are required to see these changes during life. Normal feline lymphatics are virtually invisible but if Evans Blue is injected subcutaneously they can be seen as bright blue strands about 1 mm in diameter. This method is usually

employed to visualize the lymphatics at operations or before autopsy; it is of little value for observing sequential changes. To overcome this lymphangiography has been used to study changes in human lymphatics to distinguish filarial from non-filarial elephantiasis (Cohen *et al.*, 1961). It has been successfully adapted to the study of animal infections with *Brugia* spp. (Gooneratne *et al.*, 1971, Ewert *et al.*, 1972, Schacher *et al.*, 1973). In lymphangiography iodized oil (LIPIODOL ULTRAFLUID - May and Baker) is pumped under pressure directly into one of the small afferent lymphatics and conventional X-ray pictures are taken. This produces excellent pictures but the method has the following disadvantages: Lipiodol is a heavy, viscous substance, which requires pressure to force it through the lymphatics, and this may distort them and damage the lymph nodes; it is an iodine-based compound, which has been fatally toxic in some individuals; the technique requires a delicate operation to insert the cannula and great care when injecting Lipiodol. The process cannot be repeated frequently and, thus, has disadvantages for a chronological study of lymphatic pathology.

Rogers *et al.* (1974) have adapted the technique of xeroradiography for studying lymphatics. Xeroradiography enables soft tissues to be radiographically visualized (Boag, 1973). The X-ray intensity transmitted by an object is recorded as a change density pattern on the surface of a semi-conducting selenium plate. "Edge-contrast" patterns are yielded by the Xerox powder development method. For examining the lymphatics of the hind leg, the cat is anaesthetized, Hypaque is injected subcutaneously into each hind foot and the limb positioned over a Xerox cassette. One minute later a xeroradiogram is taken using a Siemens "Mammomat" X-ray unit with a Molybdenum anode in association with a Xerox 125 system (Gillbe, 1973). The xeroradiograms are produced in 90 seconds and the whole procedure easily completed within 5 minutes. This technique was used to study the sequential development of the gross changes seen in lymphatics parasitized by *B. pahangi* (Rogers, personal communication). She has shown that in the lymphatics of the leg of a cat infected with a single infection there is a rapid increase in the size of the afferent lymphatics from 35-60 days after infection but usually from this time on there is no further increase and there is a gradual improvement in the lymphatics from about 120 days onwards. However, if further infective larvae are inoculated into the cat the lymphatics again become markedly larger. Continuous, repeated infection gives rise to a progressive extension of the damaged area and more enlargement of already affected lymphatics. Apart from the sequential study of the

development of pathology, Denham and Rogers (1975) used this technique to study the lymphatics of infected cats after chemotherapy. It has often been suggested that the main pathology in *W. bancrofti* is associated with dead worms (von Lichtenberg, 1957, Warren, 1971) but histological examination of lymphatics some weeks after treatment with diethylcarbamazine did not confirm this (Rogers and Denham, 1974). It was found that soon after treatment there is a further hugh enlargement of the infected lymphatic but this reduces spontaneously and 7 weeks after treatment the lymphatic is the same size as that in the uninfected contra-lateral limb (Denham and Rogers, 1975). This observation probably accounts for the discrepancy between those who believe that dead worms cause pathology, and those that believe it to be due to living worms. Our work suggests that elephantiasis results from a host response which also kills the parasite. The presence of dead worms in the lymphatics is not the cause of the elephantiasis but the consequence of the immunological changes that have led to the death of the parasite. Treatment at an early stage of the infection - before the development of this immunological response - will prevent rather than precipitate elephantiasis.

The histological changes in the lymphatics have been described by Schacher and Sahyoun (1967) and Rogers and Denham (1975).

There have been comparatively few studies on the function of lymphatics as a drainage system. One of the problems is how to measure the rate at which lymph is conducted away from the tissues. Sage and Gozun (1958) and Sage *et al.* (1964) used radio-active colloidal gold to measure lymph flow and functional patterns of lymphatics and lymph nodes in the extremities of human patients. Rogers and Denham (1975) used this technique to study lymph flow patterns in cats infected with *B. pahangi*. The suspension of colloidal gold was injected into the dorsum of the feet of cats infected in this region with *B. pahangi* and the amount of radio-active gold at the site of inoculation at different times after its injection was determined. The clearance of the gold and, therefore, the activity of the lymphatic system was calculated from the data collected.

It was originally expected that lymph flow in cats infected with *B. pahangi* would be decreased without induction of visible lymphoedema. It was postulated that as the cat lymphatic system is highly efficient, normal maintenance of tissue drainage would be possible even when the lymphatics were damaged and only partially functional.

In uninfected cats the rate of disappearance of colloidal

gold from both feet was usually similar in a particular animal but the percentage disappearance of gold varied considerably between the various animals. This may be due to individual physiological differences or to the degree of activity of the cats during the intervals between measuring the radioactive counts. In cats infected in one leg with *B. pahangi*, only 7 of 19 cats had slower lymph flow from the uninfected hind leg suggesting that there is no significant difference between infected and uninfected hind limbs in their lymph drainage capacity. Lymph flow studies were also made on cats which had been repeatedly infected. These cats received a primary infection into one leg and were then repeatedly challenged into the same leg at approximately 10 day intervals for from 6 to 37 months (Denham *et al.*, 1972b). Six of the cats studied became microfilaria negative at different times before study. There was great variation in the rate of disappearance of gold in the feet of these cats. Lymph flow studies on cats reinfected for less than 2 years showed no significant decrease in gold disappearance rate although different cats showed great variations in the results obtained. Of the 7 cats studied after 2-3 years of repeated infection, 5 showed less gold removal from the infected limb than from the normal limb.

Only one cat was studied when it had externally visible signs of filariasis. This cat had an intermittently, oedematous, fibrous left foot. When first studied 19 months after infection the cat had been microfilaria negative for 2 months, but at the time of the test the foot was normal in appearance. The gold disappearance rate from the infected leg was only slightly less than from the uninfected leg. However, 24 months after infection virtually no gold disappeared from the foot of the oedematous infected leg. The cat was killed soon after this when the old, infected, lymphatics were non-functional fibrotic cords with the lumen occluded by the remains of dead worms enclosed in lymphoid tissue and new small lymphatics were developing. Perhaps the most significant finding from this study was that the highly varicosed lymphatics seem with heavy infections of *B. pahangi* still function as well as do normal lymphatics which means that the original hypothesis - that damaged lymphatics would carry less lymph - is not valid. This may also be true in human patients with large numbers of circulating microfilariae.

PARASITES LIVING IN THE BLOODSTREAM

Microfilariae

Microfilariae have exploited the bloodstream as a habitat

to make contact with blood sucking arthropods. However, microfilariae do not grow whilst in the bloodstream, are relatively small (c.f. *Schistosomes* and adults of *Dirofilaria*), usually sparse in numbers (c.f. *Trypanosomes*) and they do not produce much damage in a normal host. There is, for example, no analogous situation to the blocking of capillaries seen in *Plasmodium falciparum* infections of the brain.

The death of microfilariae either from natural causes - such as old age or the onset of an immune response by the host or successful chemotherapy, may cause either local lesions or more generalized symptoms. Mandrils infected with *Loa loa papionis* eventually develop an immune response which kills microfilariae. The spleen in these mandrils becomes very enlarged and has nodular growths throughout its substance. Microfilariae are still being born at this stage of the infection and may be recovered from the lungs (Duke, 1960a) but are seldom, if ever, found in peripheral blood. It is suggested that microfilariae of *Loa* migrate from the subcutaneous regions, where they are born, through the lymphatic system, into the blood vessels and then to the lungs where they remain for some time to mature. When they re-enter the normal blood circulation they are trapped by the spleen and their death gives rise to massive granulomata (Duke, 1960b). The treatment of people with high densities of *Loa* microfilariae can lead to serious neurological damage and encephalitis due to microfilariae dying in the brain.

The syndrome of human tropical pulmonary eosinophilia (TPE) is believed to be caused by microfilariae. In this condition there is a very high eosinophilia, extraordinarily elevated levels of IgE and a persistent hacking cough. Antibodies reacting with filarial antigen are detectable (Danaraj and Schacher, 1950). Patients with this syndrome appear to be hypersensitive to filarial worms but the controversy is - to which filarial worms? Microfilariae are infrequently isolated from the blood or lungs of patients with TPE. Buckley (1958) developed severe TPE after a self-administered *B. pahangi* infection and recovered when treated with diethylcarbamazine (which kills microfilariae). Buckley believed that the syndrome was caused by an exaggerated host response to an animal filaria but his ideas have not been generally accepted. The present view is that most cases of TPE are due to an exaggerated host response to infections with *W. bancrofti*. Exposure to filariae which normally develop in animals may precipitate an attack (Nelson, 1973).

The death of microfilariae consequent on successful chemotherapy may well produce pathological changes. These are obvious and extreme in the case of the skin dwelling microfi-

lariae of *Onchocerca volvulus* where the reaction to treatment with diethylcarbamazine forms and basis for the Mazzotti diagnostic test. In heavily infected patients the reaction can be fatal, especially in malnourished individuals (Fuglsang and Anderson, 1974).

Dogs infected with *D. immitis* develop muscular cramps after treatment with microfilaricides and Wallace and Screws (1972) have shown that this is caused by the death and disintegration of microfilariae in the capillaries of the muscle beds. They also showed that microfilariae died in other capillaries; those of special significance being the capillaries of the heart and kidneys. It is not known where the microfilariae of most human parasites are destroyed after diethylcarbamazine treatment although the microfilariae of *Loa loa* are phagocytosed in the liver (Woodruff, 1951, as are those of *Litomosoides carinii* in cotton rats (Hawking, *et al.*, 1950). Patients infected with *B. malayi* are frequently ill after treatment but this is thought to be mainly due to death of the adult worms.

Adult nematodes in the lumen of blood vessels

D. immitis is undoubtedly one of the most thoroughly investigated parasites in the group under consideration. These relatively enormous worms, up to 30 cm in length, normally live in the right ventricles and pulmonary arteries of dogs although in heavy infections they can be found in the left ventricles, the atria, venae cavae, aortas and throughout the arterial system (Liu *et al.*, 1966). Much of the earlier literature on heartworm must be treated with caution because of the failure to realize that dogs may be infected with a wide variety of filarial worms. The unsheathed microfilariae of *Dipetalonema reconditum* and *D. dracumuloides* and *Dirofilaria repens* are easily mistaken for those of *D. immitis*. Any future clinical or physiological studies must be accompanied by a positive identification of the adult worms.

Dogs infected with *D. immitis* have a pronounced lack of stamina and a chronic cough and, if encouraged to undertake violent exercise, often collapse with congestive heart failure. Part of this syndrome is undoubtedly due to mechanical interference with the correct functioning of the mitral and tricuspid valves by the adult worms. Anoxia is a strong contributing factor as is pulmonary arterial hypertension (Knight, 1968, Yarns and Tashjian, 1967).

The right side of the heart of infected dogs is dilated and hypertrophied (Knight, 1968). The pulmonary arteries in infected dogs are grossly dilated and lose the smooth taper and arborization seen in normal dogs. These changes are well

illustrated by angiocardiography either during life or post mortem (Liu *et al.*, 1966, 1969, Knight, 1968, Tashjian *et al.*, 1970, Bisgard and Lewis, 1972). The diaphragmatic lobar arteries are much more severely affected than the apical, cardiac or intermediate lobar arteries (Liu *et al.*, 1969). The right diaphragmatic artery is more seriously affected than the left diaphragmatic artery (Liu, 1971). Bisgard and Lewis (1972) used the technique of lobar pulmonary arteriography in which the catheter is placed in a single lobar artery. Clearer pictures are obtained by this method than by cannulating the pulmonary artery.

In normal dogs the lungs receive blood from both the left and right sides of the heart. The pulmonary artery carries deoxygenated blood from the right ventricle to the distal regions of the lung tissue for oxygenation. The distal bronchiae, the hilar lymph nodes and the *vasa vasora* of the pulmonary arteries receive blood from the bronchial artery direct from the left side of the heart. In dogs in which the gross changes described above have developed there are anastomoses between the bronchial and pulmonary systems which originate in a breakdown of the walls of the *vasa vasorum* of the pulmonary lobar arteries (Liu *et al.*, 1969, Liu, 1971). The changes in the pulmonary arteries are slow and progressive (Tashjian *el al.*, 1970) and the development of the collateral blood supplies may help the dog to overcome part of the disability due to chronic heartworm disease. In the acute disease occasioned by a very heavy primary infection pulmonary infarction may occur (Liu, 1971).

The histopathological lesions in the pulmonary arteries are caused by living and dead *D. immitis* adults. Adcock (1961) has given an excellent description of the histopathology. He found that the distal and proximal arteries are affected in different ways. The presence of adult worms in the larger pulmonary arteries gives rise to rugose and villous endarterial fibrosis. The proliferating tissue of the artery appears to mould itself around the living worms in the lumen of the artery. The walls of the arteries become much thickened and this probably causes a loss of elasticity (Knight, 1968). Adcock (1961) described "severe obstruction of the arterial lumen". The smaller, normally elastic branches of the lobar arteries showed proliferating endothelial cells with small growths into the lumen and inflammatory infiltration of the adventitia. Later the walls become very fibrosed and large protruberances encroach into the lumen (Adcock, 1961). The villous and nodular lesions contain gamma globulin (Casey *et al.*, 1972). Dead and decaying worms cause thrombotic lesions and granulomas which cause permanent

damage to the artery or lung when they become organized and fibroblastic. Calcified remains of dead worms can be found during autopsy, by histological examination and by X-ray examination during life.

Electrocardiograms have been performed on dogs with *D. immitis* infection (Blackberg and Ashman, 1930, Knight, 1968). In resting dogs there was no difference in the rate of heartbeat, the width of QRS wave complex or the height of the T wave. After exercise there were striking dissimilarities between infected and normal dogs. After exercise in normal dogs the QRS period is unchanged or even shortened - indicating a more rapid transfer of the beat impulse within the ventricle, but in dogs with heartworm, the QRS period becomes longer. There is also a decrease in the height of the R wave. These two features suggest a moderate depression of the intraventricular conduction rate. Knight (1968) suggested that the changes in right ventricular weight, pulmonary blood pressure during exercise and the electrocardiogram alterations are caused by the loss of elasticity of the terminal branches of the pulmonary arteries. Despite the high pulmonary blood pressure the lung is inefficient in oxygenating the blood and during exercise the heart overworks, under anoxic conditions, and congestive heart failure occurs.

Occasional zoonotic infections with *D. immitis* occur in man where the usual pre-operative diagnosis has been carcinoma of the lung and the patients have been subjected to unnecessary surgery (see Pierson, 1972 for references).

Several other species of filarial worm are found in the heart and major blood vessels.

Dipetalonema evansi occurs in the right side of the heart and the pulmonary arteries and the spermatic arteries and lymph spaces of camels. Heavy infections cause arteriosclerosis and heart insufficiency and parasitic orchitis. It would be interesting to study this parasite is more detail to determine whether there are really two different species as the two sites of infection are so different but so far the vector is unknown although it is suggested that it may be a Hippoboscid fly.

Some adult filariae of the genus *Elaeophora* live in the arteries of ruminants and horses, for example *E. schneideri*, which is up to 12 cm in length, lives in the arteries of the thorax of sheep. It lies free in the lumen and does not invade the wall of the arteries. There appears to be no pathology associated with the adult worms.

Angiostrongylus cantonensis has an unusual migration within its mammalian host the rat. The larvae migrate from the intestine to the brain before migrating to the arteries of

the lung. Little has been reported of its effect on the lung of the rat but it is one of those unfortunate zoonoses in which severe signs are found in infected humans as the larvae fail to complete their normal cycle and stay in the brain giving rise to parasitic eosinophilic meningo-encephalitis. This disease occurs in several Pacific islands (Rosen *et al.*, 1962). Another member of this genus *Angiostrongylus costaricensis* has a fascinating life cycle which involves lymphatics and arteries. This parasite of rodents leaves the intestine near the ileo-caecal junction and penetrates the lymphatics. Here it moults twice before migrating through the walls of the lymphatic directly into the mesenteric arteries (Morera, 1973). *Angiostrongylus vasorum* infection in the dog is much more pathogenic than the other members of the genus, especially in hunting dogs due to their excessive activity. The adult is up to 25 mm in length and lives in the pulmonary artery and right side of the heart. Eggs are carried to the terminal arterioles and capillaries and first stage larvae cross into the bronchioles.

The clinical features of the disease include dypsnoea, coughing and expectoration of mucus and blood. The heart shows signs of abnormal tricuspid valve activity, with a jugular pulse and marked tachycardia. These signs are progressive and the condition of the dog deteriorates rapidly. Large numbers of granulomas are found around eggs in the lung which becomes more and more inefficient as fibrosis develops resulting in anoxia. The heart is dilated with chronic myocarditis and infarcts are distributed throughout the myocardium. Within the pulmonary arteries there is endoarteritis especially towards the terminal ends of the arteries, which become calcified. An acute form of the disease occurs in younger dogs. This presents as pneumonia and broncho-pneumonia. This is associated with extensive granulomatous reactions, congested capillaries and pus-filled dilated bronchioles.

It is clear that the pathogenesis of the disease caused by *A. vasorum* is different from that of *D. immitis*. The granulomas and their eventual fibrosis in the actual gas exchange regions of the lung are the cause of the anoxia in *A. vasorum* infections whereas in *D. immitis* infection there is, at least, a partial blockage of the pulmonary arteries by the adult worms. Whilst, to some extent, the dog can compensate for the damage to its pulmonary arterial system caused by *D. immitis* by using the bronchiolar arterial system, once the lung tissue itself is destroyed by the progressive development of fibrotic lesions accommodation is impossible (Cuille and Darraspen, 1934, Darraspen *et al.*, 1953). The quotation

by Blackberg and Ashman (1930) at the head of this review certainly does not apply to *A. vasorum* infections.

NEMATODES WHICH PARASITIZE THE WALLS OF BLOOD VESSELS

The filarial worm *Elaeophora poeli* lives in the aorta of Zebu cattle, straight-backed cattle and buffalo in South East Asia. The adults, which are up to 30 cm long, invade the wall of the arteries, especially the wall of the aorta in the thoracic region. They form nodules ½-1 cm in diameter and the endothelium of the artery becomes roughened and thickened. No abnormality of behaviour or clinical signs have been reported as a result of *E. poeli* infection. *Elaeophora böhmi* lives in nodules in the walls of the arteries and veins of the extremities of the limbs of horses. In heavy infections there is thickening of the walls, the vessels lose their elasticity and lameness may occur. But in lighter infections there are no signs or symptoms of infection.

Onchocerca armillata parasitizes the wall of the aorta of cattle in Africa and Asia. The pathology of this infection has been studies by Chodnik (1958) in Ghana, Mostafa *et al.* (1966) in the Sudan and Patniak (1962) in India. The area of the aorta parasitized varies according to the age of the animal infected. In young animals the area around the aortic arch is affected but in older animals there is an extension of the lesions towards the posterior parts of the aorta, presumably because of continual reinfection. In heavily parasitized animals lesions extend posteriorly to include the thoracic aorta and the brachiocephalic artery but no worms are found in the abdominal aorta. The walls of parasitized arteries are thickened, grossly corrugated, deeply corroded internally and sclerosed. In very advanced cases atrophy is marked and there are occasional sacular aneurisms. There are also nodular formations under both the intima and adventitia of the arteries. Early in an infection zig-zag tortuous channels containing female worms are raised just above the surface. Later, empty tunnels and the sinuous tunneling caused by the worms in the wall of the aorta are seen. The nodules contain the male worm and the anterior part of the female, the posterior part of the female extends into the labyrinth of sinuous tunnels beneath the intima and in the media. When the nodules are small and soft the adult worms can be easily recovered but, as the nodules become bigger, they become fibrosed and it is almost impossible to dissect the worms out. The deeper into the wall of the aorta the nodules are found the smaller they are.

In some parts of Africa *O. armillata* may infect a high

proportion of the cattle but is not usually regarded as a serious pathogen. The blindness described by Patniak (1962) in India has not been found by other workers but insufficient attention has been given to this parasite. Experimental infection must await the discovery of the vector.

In many parts of the world almost 100% of horses are affected by verminous arteritis mainly involving the cranial mesenteric artery. The causative organism of this condition is *Strongylus vulgaris*, one of the numerous strongyloid parasites of the horse's intestinal tract. This is about the only uncontested statement one can make about verminous arteritis in horses. There have been almost as many theories to explain the presence of these arterial lesions as there have been investigators studying the problem. The main controversy centres around how the larvae get to their site in the arterial walls. This problem is plagued because nearly all observations have been made on naturally infected horses. Olt (1932), Farrelly (1954) and Poynter (1960) basically believe that larvae leave the gut after ingestion, migrate in the blood to the heart and lungs. Olt (1932) believed that they then migrate up the trachea and back down the oesophagus in the same way as do many other strongyles and ascarids. He suggests that those seen in the arterial aneurisms of the cranial mesenteric artery are aberrant larvae which left the intestine directly into the peritoneal cavity from where they penetrate the cranial mesenteric artery. Farrelly (1954) accepted that the normal life-cycle was as described by Olt (1932) but that some larvae pass through the lungs and heart and attach to the inside of the walls of the cranial mesenteric artery. Poynter (1960) was in general agreement with this hypothesis and maintained that the initial lesions were in the aorta, not in the mesenteric arteries.

The opposing view of Wetzel and Enigk (1952 and earlier), Ershov (1956) and Mahaffey and Adams (1963) is that the larvae do not migrate through the heart and lungs but that the invasion of the cranial mesenteric artery is primary. The main difference between the views held by these authors is that Ershov (1956) believed that larvae penetrate into the arteries and migrate along the inner surface, whereas Wetzel and Enigk, and Mahaffey and Adams claim that they penetrate and migrate through the intima of the arteries.

It is impossible on the present evidence to decide which of these theories is correct. Whilst this controversy might be held to be of interest only to pure parasitologists it is also important to the pathologist because however the larvae arrive at the site of the lesion they could either invade the wall of the artery from the inside through the endothelium,

from the outside through the muscle layers or from the intima. Unfortunately the interpretation of the lesions is as controversial as the way in which the worms get to the site of the lesion. Those who believe the worm attacks from the lumen of the artery describe the primary lesion as damage to the endothelium with a deposit of fibrin around the worm which is later incorporated into the intima. The opposing school of thought maintains that the lesion originates deeper in the wall of the artery and that the endothelial component is an attempt by the host to prevent the penetration of the larvae into the lumen of the arteries.

The details of the various types of arteritis have been well described by Macaffey and Adams (1963).

NEMATODES WHICH MIGRATE THROUGH THE LYMPH AND BLOOD SYSTEMS

Many nematodes as part of their normal life-cycle are ingested and have to undergo a complicated migration which involves the lymphatic and circulatory systems. This is true of many of the nematodes that eventually develop to maturity in the gut (e.g. hookworms and *Ascaris* spp.). There are three likely ways in which migration from the gut to another site could take place. One route involves the larvae burrowing through the wall of the intestine into the peritoneal cavity and then migrating directly to the muscles, probably by burrowing along the connective tissue fascias. The other two migratory routes are through the vascular system. One is by way of the blood vascular system and involves the larvae leaving the intestine in the hepatic portal system and then travelling via the liver and venous system to the heart. In the other vascular route the larvae enter the lymphatic drain of the intestine and travel to the jugular vein in the chyle duct.

Leuckart (1876) and Fiedler (1864) were both of the opinion that larvae of *Trichinella spiralis* burrowed through the connective tissue to get to the muscles but from about 1900 until 1965 it was generally accepted that migration was by way of the lymphatic system. Berntzen (1965) reopened this question by suggesting that larvae migrate through the connective tissue with only a few stragglers finding their way to the lymphatic and venous system. This question seems to have been settled beyond reasonable doubt by Harley and Gallicchio (1971) who placed a cannula into the thoracic lymph duct. Over 70% of the number of larvae found in the muscles of control animals were recovered from the lymph of the cannulated animals.

Despite the migration of the young *T. spiralis* larvae

through the mesenteric lymph nodes, any damage caused must be of very minor significance in the pathology of *T. spiralis* infection. Graham (1897) found that migrating larvae of *T. spiralis* invading the muscle fibres from capillaries appeared to cause some stasis in the capillaries. This observation has been confirmed by the work of Humes and Akers (1952) who showed a similar phenomenon in the capillaries of hamster cheek pouches.

Larvae of *Dictyocaulus filaria* migrate from the intestine to the lungs through the lymphatics of the bowel, the mesenteric lymph nodes and the venous system to the heart (Hobmaier, 1929). *Dictyocaulus viviparus* migrates in a similar fashion in cattle. Michel (1956) demonstrated that whilst in normal mice, rabbits or lambs the larvae migrate rapidly through the mesenteric lymph nodes, in animals which had been immunized, with a previous infection migration to the lungs was considerably delayed and many larvae failed to leave the mesenteric lymph nodes. He also showed that if the mesenteric lymph nodes were by-passed, by injecting larvae intravenously, similar numbers of larvae could be recovered from the lungs of normal or immunized mice. Whilst histological studies have demonstrated that changes do occur in the mesenteric lymph nodes of cattle infected with *D. viviparus* (Jarrett and Sharp, 1963, Poynter *et al.*, 1960) these probably have no ill effect on the host and may be regarded as a normal part of the defence system of the host.

CONCLUSIONS

It can be seen that the lymphatic and blood systems have been extensively exploited by nematodes. Much is known about the histological changes caused by these parasites but much less about the physiological problems.

Many of the parasites whilst present in large numbers seem quite, or nearly innocuous and it is unlikely that detailed physiological studies will be made with these infections.

In the serious diseases with which we have dealt, especially *W. bancrofti* in man and *D. immitis* and *A. vasorum* infections of dogs, there is still much which can profitably be studied to enable us to understand the full complexity of the infections.

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THE PATHOLOGY OF EXPERIMENTAL *DRACUNCULUS* INFECTION AND ITS RELEVANCE TO CHEMOTHERAPY

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INTRODUCTION

Dracunculus medinensis, the guinea worm is a parasite of man in rural communities in India and Pakistan, the Middle East and Africa. While human infection is rarely fatal it causes much incapacity and can be of real economic importance as infection is particularly common in the 14 to 40 year age group (the working population) during the planting season.

Infection has been reported from a wide range of mammals including primates, carnivores and herbivores (Muller, 1971). It is probable that infection in carnivores in particular is very much more common in many areas than the sporadic reports would indicate. For instance, Crichton (1971) found a 28% infection rate in mink and 13.6% in raccoons on dissection in southern Ontario while there had been only one previous report from Canada and parasites are not uncommonly found on dissection of dogs and cats in the formerly endemic area of Kasachstan (U.S.S.R.) although not noticed in the live animals (Ghensis, 1972; Chun-Sun, 1958).

There have been no reports on the pathology associated with natural infections in animals. The parasite appears to cause little discomfort to carnivores and one reason why infection is so rarely observed is probably because these animals extract worms with their teeth. The species position within the genus is so confused that it is usually not possible to ascertain whether a parasite occurring in an animal is the same species as that which occurs in man.

The classic account of the mechanism of pathology in man is that of Fairley and Liston (1924) and their conclusions have remained virtually unchallenged to the present day, providing the basis for the account in most standard textbooks. Recent reports of the efficacy of various chemotherapeutic agents which have no action against other nematode infections, such as niridazole (Raffier, 1969a; Lucus *et al.*, 1969) and metronidazole (Padonu, 1973, suggested that the pathogenesis needed further investigation.

MATERIALS AND METHODS

The strain of *D. medinensis* originated from a human case in western Nigeria but has been maintained in the laboratory for 7 laboratory cycles in rhesus monkeys (*Macacca mulatta)* and in *Cyclops vernalis americanus*. The techniques for laboratory maintenance have been described by Muller (1972). Although other laboratory animals can be infected, rhesus monkeys were the most susceptible and most suitable for studying the pathology. Thirty three monkeys have been infected and a total of 87 mature female worms developed in them; 14 animals were used for pathology and chemotherapy investigations.

Biopsy specimens were fixed in neutral formol-saline or in Dubosq-Brasil or Carnoy's fixative and paraffin sections strained with Harris' haematoxylin for general histology or with Mawhinney's (unpublished) modified methyl green and pyronin technique for plasma cells. In the latter method, metanil yellow in deionised water is used for differentiation instead of n-butyl alcohol and formol-saline fixation can be used instead of Carnoy's fixative. Eosinophils were stained by carbol chromotrope 2R (Lendrum, 1944).

Seven anthelmintic compounds were tested against preemergent worms in rhesus monkeys. These were cambendazole (Merck, Sharpe and Dohme Ltd.), diethylcarbamazine citrate (*Banocide;* Burroughs Wellcome and Co. Ltd.), methyridine (*Promintic;* Imperial Chemical Industries Ltd.) metriphonate (Bayer Chemicals Ltd.), metronidazole (*Flagyl;* May and Baker Ltd.) and thiabendazole (*Mintezol;* Merck, Sharpe and Dohme Ltd.). Dosage schedules are shown in Table I. All compounds were given by stomach tube except methyridine which was given by intraperitoneal injection. Monkeys were immobilized with 3 mg/kg of phencyclidene hydrochloride (*Sernylan*; Parke Davis and Co.). The outline of one or more pre-emergent female worms could be seen in all the treated animals and worms were surgically removed at, and one week after, the end of treatment. Larvae were obtained from all worms and their ability to develop in *Cyclops* ascertained. Sections of worms and tissues were removed for histological study. The monkeys were then kept under observation to see whether other worms would emerge normally.

Three compounds were tested against very immature parasites of both sexes in cats. The young worms were recovered by dissection 37 to 85 days after infection as shown in Table II.

TABLE I

Chemotherapeutic trials in rhesus monkeys with pre-emergent female guinea worms started 10 months after infection 1

Compound	No. of animals treated	Dosage schedule	Total no. of worms removed or emerged	Effect on worms or larvae
None	4	-	13	-
Cambendazole	1	100 mg/kg for 4 days	4	Nil
Diethylcarbamazine	1	10 mg/kg for 25 days	23	Nil
Methyridine	1	10 mg/kg single dose (ID)	9	Nil
Metriphonate	21	25 mg/kg for 4 days	8	Probably effective
Metronidazole	2	50 mg/kg for 10 days	4	Nil
Niridazole	4	60 mg/kg for 34 days	12	Nil
Thiabendazole	2	200 mg/kg for 4 days	6	Nil

TABLE II

Trial of chemotherapeutic agents on young stages of Dracunculus *in cats (each animal given 50 infective larvae).*

Compound	No. of animals	Dosage regimen	Total duration of infection	No. of worms recovered
None	3	-	40	15, 13, 2
Metronidazole	2	100 mg/kg for 29 days	56	5, 2
Thiabendazole	2	100 mg/kg for 19 days	37	9, 2
Diethylcarbamazine	1	12.5 mg/kg for 25 days	85	1 (dead)

TABLE III

Effect of chemotherapeutic agents on the activity of first stage larvae of Dracunculus *in distilled water at 22°C (key: +++ = very active; ++ = active; + = just moving; 0 = dead).*

Drug	Concn.(%)	Treatment time (hours)				
		1	4	18	24	96
Diethylcarbamazine	Saturated	+++	+++	+++	+	0
	0.1%	+++	+++	+++	+++	+
Methyridine	0.1	+	+	0		
	0.01	+	+	+	0	
Metriphonate	0.1	+++	+++	0		
	0.01	+++	+++	0		
Metronidazole	Saturated	+++	+++	+++	+++	+
	0.01	+++	+++	+++	+++	++
Niridazole	Saturated	+++	+++	+++	+++	0
	0.001	+++	+++	+++	+++	+
Piperazine	Saturated	+++	+++	+++	+++	+
	0.01	+++	+++	+++	+++	++
Tetramisole	0.1	+	+	+	+	0
	0.01	+	+	+	+	0
Thiabendazole	Saturated	+++	+++	+++	+++	0
	0.01	+++	+++	+++	+++	0

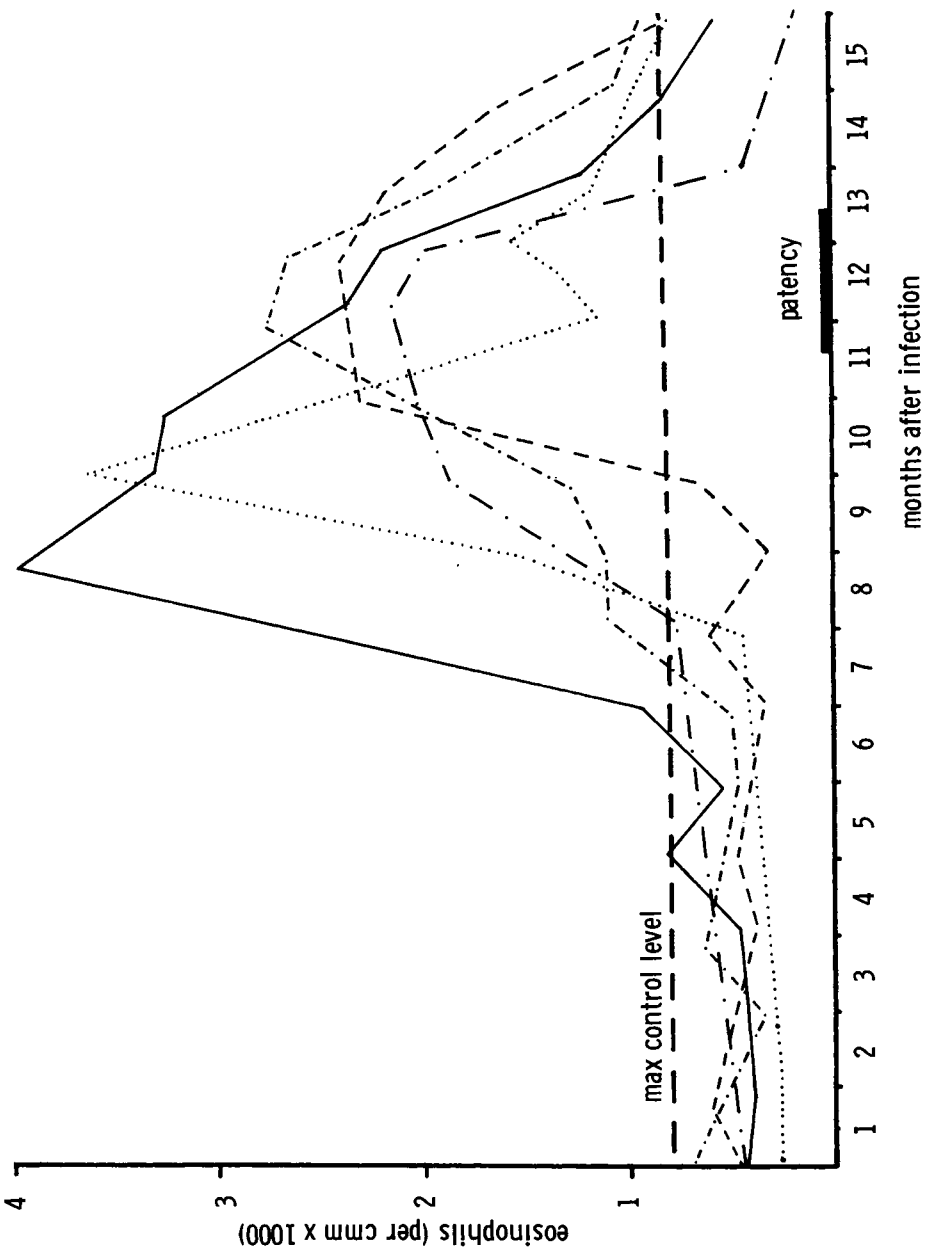

Fig. 1. Absolute eosinophil levels in 5 infected rhesus monkeys. The period of patency (when one or more female worms were emerging) is an average.

High concentrations of eight compounds were tested in distilled water against first-stage larvae (Table III) The activity of the larvae in petri Dishes was observed 1, 4, 18, 24 and 96 hours later.

RESULTS

Pathology

There was some eosinophilia which usually appeared about 3 months before patency but eosinophils were rarely higher than 15% of the total white cell count (Fig. 1).

Female worms normally emerged from beneath the skin of the monkeys 329-360 days after infection. Because of the relatively small amount of fat and subcutaneous tissues in these hosts, developing female worms could be seen under the skin 2-3 months before they emerged (Fig. 2). At this stage they were about half their final size and often in the subcutaneous tissues of the thoracic and abdominal regions. Almost all worms had moved down to the extremities by 10 months after infection before they emerged.

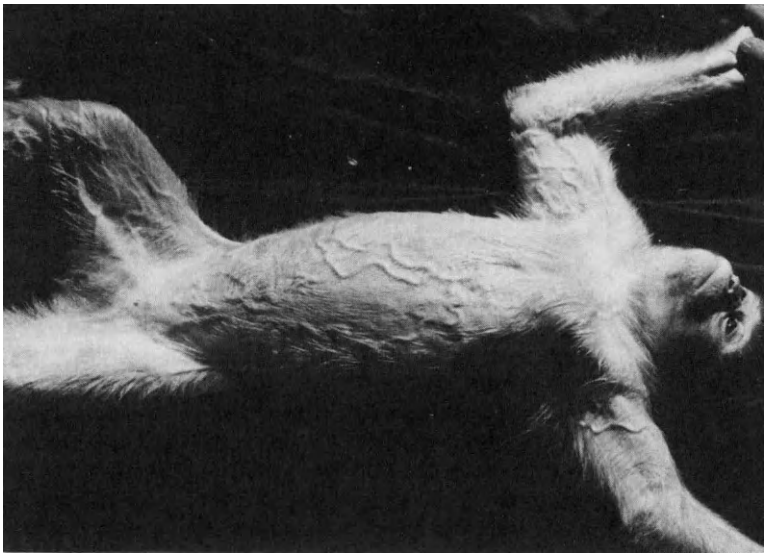

Fig. 2. Outlines of pre-emergent worms on rhesus monkey 9 months after infection.

The clinical features associated with the emergence of the female worms is similar to those described for man (Carayon *et al.*, 1961; Fairley, 1924) with the formation of a small blister which grows to become a few centimetres in diameter

before bursting in 3-5 days. The blister fluid was bacteriologically sterile and in the first two days contained principally polymorphs then increasing proportions of macrophages, lymphocytes and eosinophils. Larvae were always present and usually had white cells adhering to them; principally polymorphs (Fig. 3). Once a blister had burst the uterus of the worm was extruded through the ulcer, the edges of which consisted of pinkish granulation tissue.

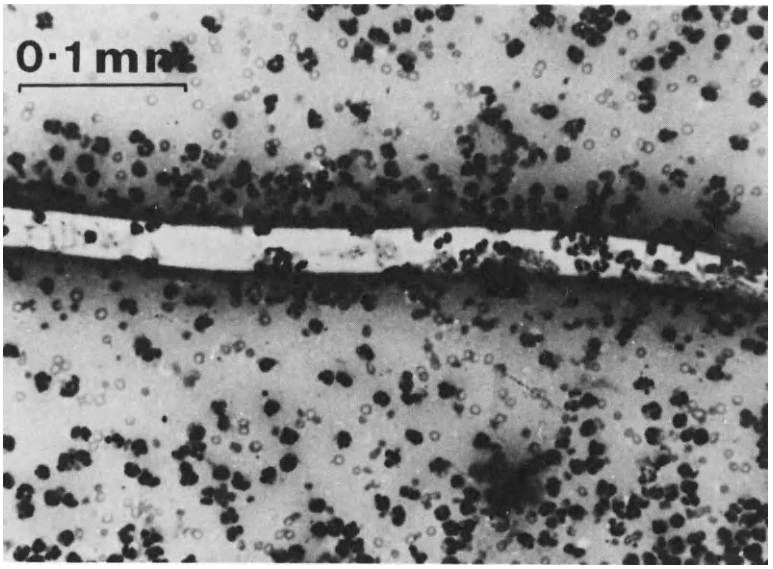

Fig. 3. Larva and adherent leucocytes (principally polymorphs) from blister fluid before bursting.

Histological sections of biopsy specimens containing portions of worm and surrounding tissues showed a striking change in the host reaction once the blister had commerced to form. Sections of specimens taken just before blister formation contained a worm with its uterus filled with larvae, surrounded by a thin, loose, connective tissue sheath, containing fibroplastic tissue with a few eosinophils, within which the worm could move freely. At this stage an entire worm measuring about 30 cm could be easily extracted if a small (1 cm) incision was made in the skin. However, once the blister formed there was intense tissue reaction all along the length of the worm and not just at the anterior end (Fig. 4). Host tissues adhered closely to the cuticle of the worm and extraction of an entire worm was no longer possible.

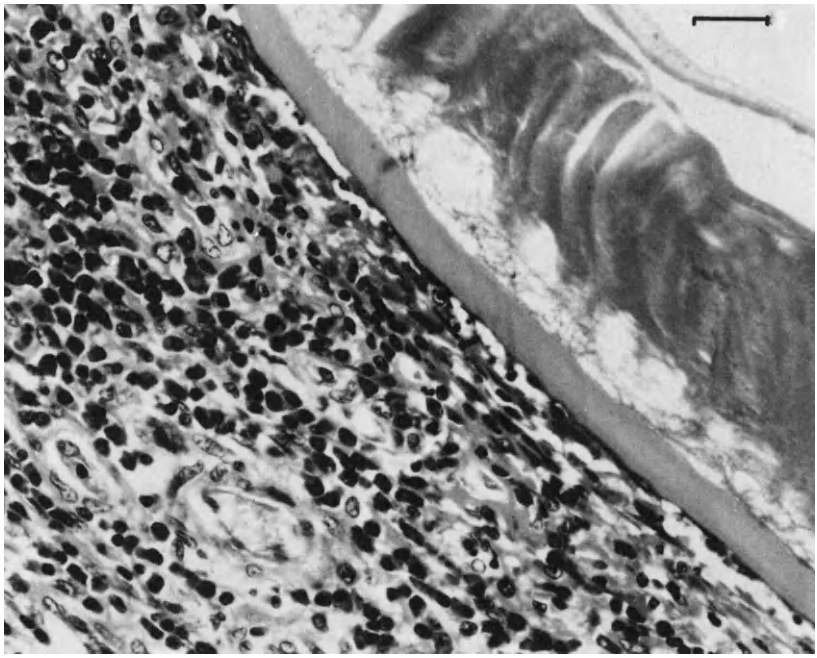

Fig. 4. Section of adult worm with intense inflammatory tissue reaction 4 days after bursting of blister. (Scale equals 0.2 mm).

After a few days many cell types were present including degenerating polymorphs, eosinophils, macrophages, multinucleate foreign body giant cells, epithelioid cells and fibroblasts. A chronic type of inflammatory response with some necrosis and vasculitis persisted while a portion of the worm was present but quickly resolved once the worm was completely expelled.

Secondary bacterial invasion of ulcers and the track of the worm with *Streptococcus pyogenes* and *Staphylococcus aureus* often occurred but did not appear to be an important factor in pathogenesis. There was no increase in time observed for infected lesions to resolve and even a very septic channel healed rapidly once the worm was removed. The intramuscular injection of penicillin (*Triplopen*) had little effect on the course of the disease.

Breaking of a worm in the tissues on the other hand caused a very severe cellulitis and the formation of abscesses filled with pus along the track of the worm; a similar effect to that found in human cases.

The bursting of the anterior end of the worm releasing many thousands of larvae (a process which occurred particularly often after immersion of the affected limb in water) resulted in a few centimetres of worm becoming completely flaccid and flattened and this portion was then extruded from the ulcer (Fig. 5). In a proportion of human cases the female worm bursts in the tissues, releasing up to one million larvae into the tissues, and this process is followed by the formation of a large bacteriologically sterile abscess (Dejou, 1951). A similar effect was common in monkey infections and occurred in all animals which had more than 10 worms; the bursting of worms was presumably from mechanical damage caused by the confinement of monkeys in small cages and the superficial situation of the worms in these hosts. Similar abscesses followed the subcutaneous infection of larvae into previously infected monkeys.

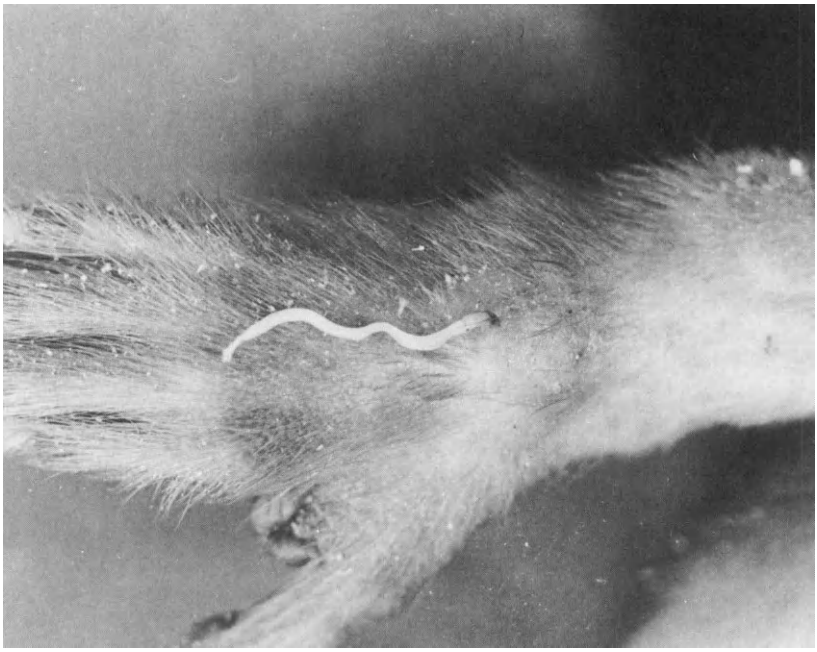

Fig. 5. Extrusion of anterior end of adult worm from ulcer.

Male worms died and became encapsulated in the subcutaneous tissues 3 to 7 months after infection. Apart from an area of fibrosis around each worm they caused little pathology.

A proportion of females failed to emerge and eventually calcified. There was no adhesion of the host tissues to the

cuticle of the worm and little reaction apart from some fibrosis. Calcification was confined entirely to the worm and did not involve any of the surrounding tissues. The appearance was very similar to descriptions of the condition in man (Reddy *et al.*, 1968; Dönges, 1966; Carayon and Camain, 1961).

Chemotherapy

None of the compounds tested except metriphonate appeared to have any effect on the adult worms or the enclosed larvae (Table I). The worms were active when removed and appeared normal at the histological level when sectioned. Larvae were capable of developing to the third-stage when ingested by *Cyclops*. However, worms removed from the one monkey given metriphonate were immobile and the contained larvae were incapable of infecting *Cyclops* although still moving slowly. At the histological level the nuclei of the musculo-cutaneous cells of the parasite were pyknotic and the muscle fibres of the cells appeared unusual. The monkey died on the fourth day of treatment with symptoms resembling anaphylactic shock, but it could not be determined whether death was due to a host reaction to the dying worms or to toxicity of the drug.

Worms which had not been removed surgically emerged normally about two months after the commencement of treatment from all monkeys given other compounds, except those given diethylcarbamazine and metronidazole in which all worms had already been removed. It was noticeable that the host tissue reaction to worms in monkeys given niridazole and thiabendazole was markedly less than in controls and worms were expelled more rapidly. The clinical course of the infection resembled closely reports of the effect of these compounds in man (Lucas *et al.*, 1969; Raffier, 1969a,b). The diminution in host response after treatment was also evident in histological sections of biopsy speciment.

Three of these compounds were also tested against young worms of both sexes in cats and again had no apparent effect with the possible exception of diethylcarbamazine (Table II). None of the compounds reported to be effective in man had any effect on first-stage larvae in distilled water, even at high concentrations (Table III).

DISCUSSION

The pathology of guinea worm infection in man was attributed to three main causes by Fairley and Liston (1924). They stated: *"The female guinea-worm is the most important factor in the causation of pathological lesions. By the secretion of some toxic substance it is directly responsible*

for the generalized symptoms including .....

The embryos are less harmful. Their escape into the tissues may occasionally culminate in the formation of subacute sterile abscesses.

Secondary bacterial invaders are directly responsible for all the severe septic complications, chief amongst which may be mentioned acute abscess, cellulitis and synovitis."

Although these statements are repeated in most texts on tropical diseases, only the first sentence reflects the situation in monkeys. It is unnecessary to postulate that the mature female worm secretes a toxic substance in order to reach the exterior; the release of larvae into the tissues can account both for the formation of the blister and for the majority of complications. The blister, which is typical of a 'normal' infection is a manifestation on a smaller scale of the sterile abscess which occurs when a worm bursts in the tissues and releases many thousands of larvae. The minor importance of bacterial infection on the course of the acute phase in man has been remarked in some recent clinical studies (Kothari *et al.*, 1969; Reddy *et al.*, 1970), although in the author's experience bacterial complications do increase the degree of disability experienced at the chronic stage.

It is concluded that, in order to reach the surface of the skin, the mature female worm in the tissues ruptures the body wall and uterus slightly at the anterior end, releasing a few hundred larvae (Fig. 6). The adult at this stage is extremely turgid and is filled with over 1 million larvae. This ejection of larvae from a point near the mouth has been observed in mature female worms *in vitro*. The release of the larvae is followed by an intense host reaction leading to the formation of the typical guinea worm blister. This is probably an immunological reaction as in man the blister is often preceded by a generalized urticaria and occasionally by dyspnoea (Fairley and Liston, 1924). Also, in previously infected monkeys, an anaphylactic type shock follows the intravenous injection of a soluble extract of larvae (Muller, unpublished). The course of events with the rapid infiltration of polymorphonuclear neutrophils is very suggestive of an Arthus reaction, probably followed by a delayed hypersensitivity response.

The site of the blister and severity of reaction appear to be determined by the number of larvae released, as well as by host factors. Following the formation of the blister, there is an intense inflammatory reaction along the entire length of the worm with cells closely adhering to the cuticle of the worm. A chronic inflammatory reaction lasts until the remains of the worm are expelled. The serious complications,

such as abscess, arthritis, synovitis and ankylosis, are caused by release of large numbers of larvae into the deeper tissues such as the synovial cavities.

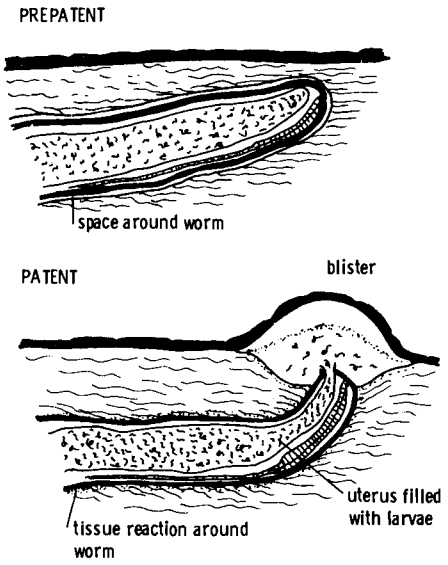

Fig. 6. Diagram illustrating postulated method of formation of blister.

It might be thought remarkable that, of the chemotherapeutic agents said to have striking effects in aiding the emergence of female worms in man (metronidazole, niridazole and thiabendazole), none were shown to have any direct anti-parasitic effect on the adult worms or on their contained larvae in monkeys. However, on further reflection this is not so strange. Although most reports on tests in man describe the death of worms following drug treatment (Lucas *et al.*, 1969; Raffier, 1969a,b), an emerging worm has already lost its anterior end and it is not possible to judge from its gross appearance whether it is dead or alive. It would not be surprising if reports were mistaken on this point and the action of the drugs was to act as an anti-inflammatory agent. Striebel (personal communication) could find no changes in the histological appearance of emerged worms brought about by treatment with niridazole. It is possible that the mode of action differs in the two hosts but this is unlikely in view of the efficacy of these compounds in reducing the host tissue reaction to emerging worms in monkeys. On recent trial with thiabendazole and metronidazole in

human cases did in fact find that they had no direct action on the worms (Belcher *et al.*, 1975).

The anti-inflammatory action of thiabendazole has been demonstrated by Campbell (1971), who believes that this may explain in part its mode of action in trichinosis. Niridazole has also been shown to have anti-inflammatory activity (Reisterer *et al.*, 1971) and because of the preliminary results reported here Mahmoud and Warren (1974) investigated its action on the growth of schistosome egg granulomas in mice. They found that it had a marked suppressive effect, as did tartar emetic. This is of interest because Tournier in 1922 reported that tartar emetic facilitated the extraction of guinea worm and reduced the host reaction to it, although Fairley (1924) demonstrated that it had no action on either the adult worms or their larvae and concluded that it was therefore of no value in treatment.

However, niridazole appears to have no effect on an IgE type of anaphylaxis. 50 mg/kg given orally to rats for 7 days did not affect the PCA reaction (Ogilvie, unpublished), nor did the same dosage given to sensitized guinea pigs prevent anaphylactic shock following intravenous injection of egg albumin.

The death of the monkey given metriphonate, the one compound which had some direct antiparasitic action, suggests that it might not be advisable to kill such larvae worms in the tissues. However, anti-inflammatory compounds may be of benefit in an infection where the host reaction is responsible for most of the serious pathological effects associated with the disease.

SUMMARY

The pathological processes associated with the emergence of female *Dracunculus medinensis* has been investigated in experimental infections in rhesus monkeys *(Macaca mulatta)*.

It is concluded that the formation of the blister, preceding emergence of the worm, is a immunologically based host reaction to the release of larvae by the mature female worm and constitutes the normal process by which the worm reaches the exterior in order to complete its life cycle. Most of the serious complications, such as cellulitis and abscess formation, are the result of this process and not because of secondary bacterial invasion.

Three compounds, said to have effect against infection in man (metronidazole, niridazole and thiabendazole), had no effect against pre-emergent worms in monkeys but markedly reduced the host tissue reaction against the emerging worms and thus allowed their easy extraction.

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HOMOCYTOTROPIC ANTIBODIES IN *MASTOMYS NATALENSIS* INFECTED WITH THE FILARIAL PARASITE *LITOMOSOIDES CARINII*

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INTRODUCTION

Helminth infections in man and animals are usually accompanied by increased levels of homocytotropic antibodies. However, little is known of the pattern of production of these antibodies during the course of infection and the relationship of the response to different developmental stages. Limited study of these antibodies has been made in filarial infections (Sadun, 1972).

Previous studies in *Brugia pahangi* infected *Mastomys natalensis* (multimammate rat) have demonstrated relatively high titers of homocytotropic antibodies which showed reagin-like characteristics (Benjamin and Soulsby, 1974). Recent investigations in the same host infected with *Litomosoides carinii* also revealed homocytotropic antibodies analogous to IgE of the rat and man. Skin sensitizing antibody with a short term latent period (IgG_1 type) was not detected in spite of the ability of the multimammate rat to produce this latter type of antibody in other, non parasitological, systems (Soulsby and Zahner, Unpublished data).

In the present experiments the pattern of the IgE-like antibody response to *Litomosoides carinii* infection in the multimammate rat was followed in a time sequence after infection using passive cutaneous anaphylaxis and intradermal tests to demonstrate active cutaneous anaphylaxis.

MATERIALS AND METHODS

Animals: Conventionally bred and reared *Mastomys natalensis*, strain "GRA Giessen" were used at 4-6 weeks of age for infection. Passive cutaneous anaphylaxis was performed in animals of at least 4 months of age and over 50 gms. bodyweight. The animals were maintained in groups in polycarbonate cages and fed on a diet developed for Syrian hamsters (Schuster, *et al.* 1973). Water was available *ad libitum*.

Infection: Mites, *Ornithonyssus bacoti*, were infected with *L. carinii* by being fed on cotton rats with a high microfilaraemia. The infected mites were then used to infect *M. natalensis* with *L. carinii*.

In all, twelve groups, each of 5 to 10 animals were used in the experiments. Active cutaneous anaphylaxis intradermal tests were performed on 11 groups with infections which ranged from 30 to 420 days after infection (Table I). Serum for passive cutaneous anaphylaxis studies was obtained from six of the above groups, representing a period of infection of 300 days (Table I). For PCA studies each group was bled at 2-3 week intervals over a period of 50-112 days. Since a low level of microfilaraemia occurred in group 6, sera were collected from another group of 5 animals with higher microfilariae densities (group 6a) at 1 week intervals from the 11th to the 18th week after infection.

Blood and serum samples: Counting of microfilariae:

Blood was collected from the retro-orbital plexus by puncture using a Pasteur pipette. The density of microfilariae per cubic millimeter of peripheral blood was determined by the counting chamber method of Raether and Meyerhöfer (1967). Serum was stored at -40C until used.

Autopsy. Groups of a minimum of 2 animals were killed after intradermal tests with antigen had been performed. The number of adult parasites and larvae present in the pleural cavity were counted.

Antigen. An aqueous extract of adult *L. carinii* was used (Lämmler, Enders and Zahner, 1969). Freeze dried worms were homogenized in distilled water for 2 minutes at 20,000 revs/min at 4C, sonicated in a N_2 - atmosphere at 4°C for 2 minutes and extracted with stirring at a concentration of 1 gm dry matter to 100 ml distilled water. The homogenate was centrifuged at 25,000 g for 30 minutes at 4°C and the supernatant lyophylized.

Passive cutaneous anaphylaxis (PCA): Skin sensitizing antibodies in sera were demonstrated in normal homologous recipients. Intradermal injections were done under anaesthesia (50 mg/kg Pentobarbital Sodium, Deutsche Abbot GmbH, Ingelheim/Rh. sc.) and not more than 6 injections of test sera were performed on the back of each animal. Recipient rats were sensitized in duplicate with 0.05 ml of undiluted and two fold serial dilutions of sera in phosphate buffered saline (PBS) starting from a 1:5 dilution of serum. Appro-

priate controls were included. Recipients were challenged 72 hours later by intravenous injection of 1 mg of antigen in 0.2 ml of a 0.6% solution of Evans Blue in saline given into the retro-orbital plexus. Animals were killed 30 minutes later with chloroform, skinned and the reactions on the internal skin surface were graded on a 0 to 4 plus scale. The end point of a serum titration was taken as a "one plus" reaction.

Intradermal (Active Cutaneous Anaphylaxis) Tests.

Infected animals were anaesthetized and injected intradermally with 0.05 ml quantities of antigen diluted in PBS, injections comprised 0.1 mg, 0.05 mg, 0.01, 0.005 mg or 0.0001 mg protein respectively (determined by the Biuret method); 0.05 ml of PBS was injected as a control. Approximately 5 minutes later 0.3 ml of a 0.6% solution of Evans Blue in saline was injected intravenously. The animals were killed with chloroform 30 minutes later and the reactions were graded by size on the internal surface of the skin.

RESULTS

Parasite Burdens. The mean number and range of *L. carinii* per animal group are given in Table I. The number of worms per group and in some animals necropsied later than 290 days after infection only encapsulated worm fragments enclosed in fibrinous masses were present. This table also indicates the animals which were used for PCA and active cutaneous anaphylaxis studies.

Passive cutaneous anaphylaxis (PCA) Studies. Sera from non infected multimammate rats never elicited a PCA reaction with the *L. carinii* antigen. Sera from infected animals was first examined 15 days after infection (Figure 1). Three of 8 sera did not react and only low titers occurred with the rest, the highest titer being 1:5. There was no correlation between serum titers and the eventual adult worm burdens.

In general, the mean serum titers showed a marked initial increase and then tended to level off about 45 days after infection, although some animals showed a drop of antibody titers during this period (group 6). Maximum titers of PCA antibody occurred with the onset, and during the early phase, of microfilaraemia (groups 5, 6, 6a) and high PCA titers were accompanied by high microfilaraemia. In the subsequent period of infection when microfilaraemia was maintained or increased (80 to 120 days) mean PCA titers decreased (Groups 6 and 6a). After 120 days of infection, when microfilaraemia was decreasing mean titers of approximately 1:10 were evident (Groups 6, 7). By 180 days of infection and afterwards, when microfilarial numbers in the blood continued to decline, titers

TABLE I

Number of *Litomosoides carinii* (mean and range) per group of *Mastomys natalensis*, time of autopsy after infection and the groups used for active (ACA) and passive cutaneous anaphylaxis (PCA) studies.

Group No.	No. of Animals	Mean No. of Parasites	Range of Parasites	Necropsy days post infection	PCA conducted over days p.i.	Group used for ACA
1	10	6.3	3 - 21	30		+
2	10	22.9	1 - 72	45		+
3	8	51.3	4 - 80	60		+
4	8	97.5	8 -208	80		+
5	7	168.4	77 -252	120	0 - 70	+
6	8	29.9	2 - 79	180	45 - 155	+
6a	5	46.2	24 - 69	125	77 - 112	-
7	10	34.0	4 - 50	240	120 - 212	+
8	8	14.3+	4 - 38	290	190 - 250	+
9	10	10.8+	1 - 44	360	240 - 300	+
10	8	14.9+	0 - 91	400		+
11	8	7.3+	0 - 23	420		+

+ dead *Litomosoides carinii* found in the pleural cavity

increased temporarily in a few animals (Group 7 and 8), but thereafter PCA titers fell to low levels as microfilariae disappeared from the circulating blood (Group 9).

Statistical analysis (Table II) of the data presented in Fig. 1 revealed a significant correlation between the numbers of microfilariae/mm^3 in the blood and the PCA titers during the period of from 120 to 300 days after infection (Groups 7, 8 and 9). During the early phase of patency (Groups 6, 6a) no significant relationship between the two was evident (Table II).

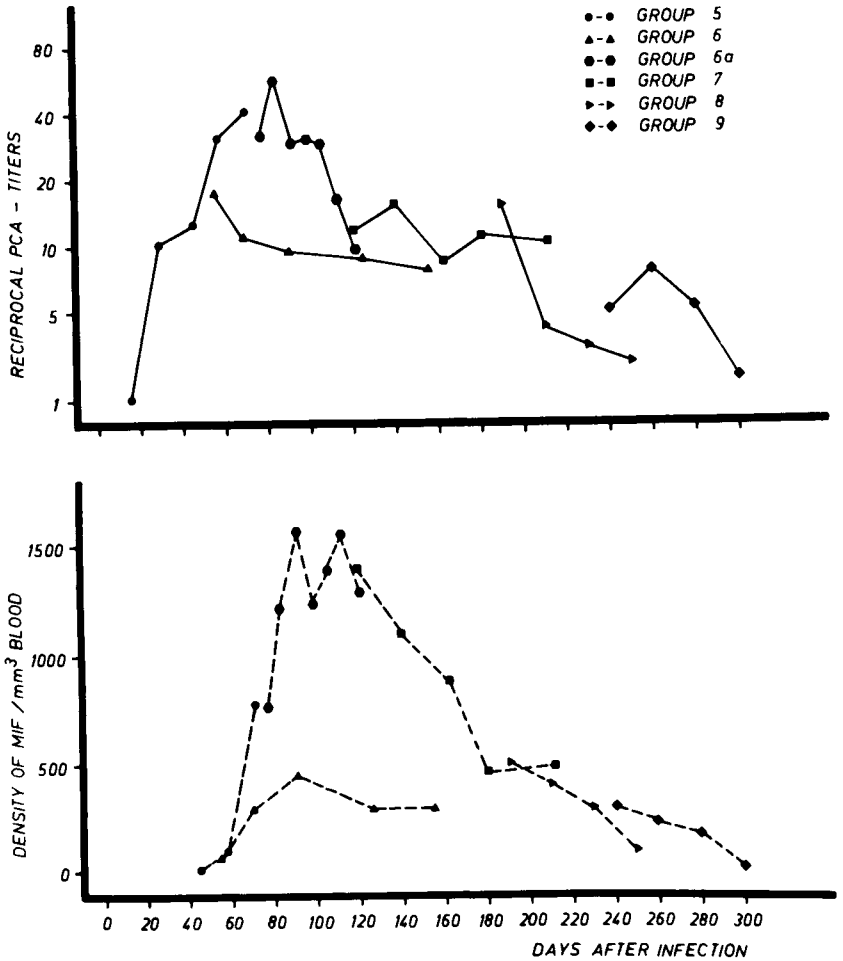

Fig. 1. Mean PCA-titers (upper graph) and mean microfilariae counts (lower graph) of various groups of multimammate rats infected with Litomosoides carinii.

TABLE II

Correlations between the mean microfilariae density and mean PCA-titers in groups of multimammate rats infected with *Litomosoides carinii*. Data taken from results presented in Fig. 1.

Animal Group	Number of determinations	$\underline{r}_s$	P<
6	33	0.04	-
6a	35	-0.09	-
7	41	0.38	0.05
8	32	0.47	0.01
9	33	0.51	0.01

Active cutaneous anaphylaxis (ACA) Studies. Intradermal injections of *L. carinii* antigen into normal multimammate rats never resulted in positive skin reactions. Intradermal injections of antigen into infected animals resulted in clear positive reactions around the injection site.

A marked reaction was observed with the higher concentrations of antigen as early as 30 days after infection; and 45 days after infection the intensity of the skin reaction varied with the amount of antigen injected. Thus more intense skin reactions were produced with the lower protein concentrations at 45 days as compared to 30 days after infection (Table III).

TABLE III

Mean size of intradermal reactions in square millimeters following the injection of different concentrations of *Litomosoides carinii* antigen into multimammate rats infected with *L. carinii* 30 and 45 days previously (10 animals per infection group).

Days after Infection	mg Protein injected				
	0.1	0.05	0.01	0.005	0.001
30	106.4	90.8	62.4	18.8	1.5
45	97.0	90.7	78.6	52.5	39.6

Throughout the remainder of the infection, though the size of the reaction varied according to the age of the infection, it was proportional to the amount of antigen injected. Skin reaction of animals in the various groups indicated in Table I, representing animals infected for 420 days are presented in Fig. 2 for antigen concentrations of 0.1 mg protein and

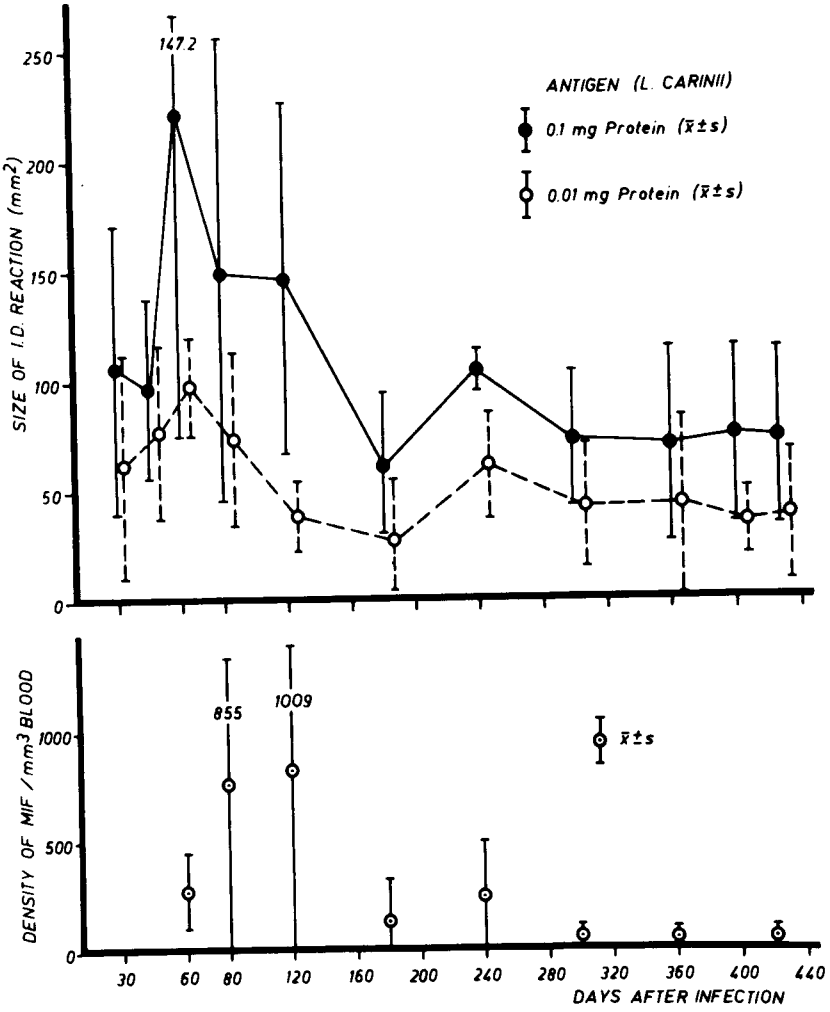

Fig. 2. Mean size of intradermal reactions (in mm²) of multimammate rats, infected with Litomosoides carinii, *30 minutes after injection of 0.1 mg or 0.01 mg protein (upper graph) over a period of 420 days of infection. Mean number of microfilariae in the blood at the time of the intradermal test is given in the lower graph.*

0.01 mg protein. Results with protein concentrations of less than 0.01 mg are not included since later in the infection this protein concentration often failed to elicit a reaction.

Fig. 2 indicates that a curve of reaction was obtained for the levels of PCA antibodies during infection. The most marked skin reaction occurred 60 days after infection, which was just after patency of the adult worms. This was followed by a decrease in the size of the skin reactions with increasing levels of microfilaraemia and the levels of reaction then remained nearly constant from approximately 180 days after infection until the end of the experiment, 420 days after infection. In the later stages of the infection (250 days and later) the injection of 0.1 mg protein still elicited a positive reaction in all the animals, regardless of whether adult filariae or their remnants or microfilariae were present or not.

The reduced intensity of active cutaneous anaphylaxis with increasing microfilaraemia in the early phase of patency was statistically significant. Eighty days after infection there was a significant ($p<0.05$) inverse correlation between the size of the intradermal reactions and the density of microfilariae/mm^3 blood (Fig. 3).

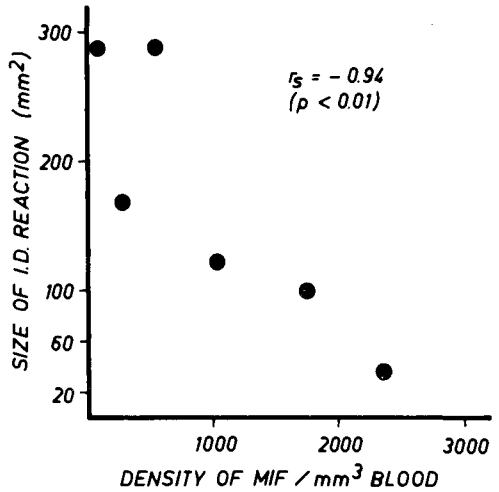

Fig. 3. Size of intradermal reactions 30 min. after injection of Litomosoides carinii *antigen (0.1 mg protein) in relation to microfilaria density in multimammate rats infected with* Litomosoides carinii *80 days previously.*

DISCUSSION

These experiments indicate that strong allergic stimuli occur during an infection of multimammate rats with *Litomosoides carinii* resulting in a marked production of homocytotropic antibodies. The chemical, physical and immunological characteristics of these antibodies are analogous to those of IgE of the rat and man (Soulsby, Zahner and Geyer, In Preparation).

The production of these homocytotropic antibodies appears to be related to the development of the filarial parasite in the host. Marked active cutaneous anaphylaxis (ACA) reactions were evident in the prepatent period and maximum ACA reactions and passive cutaneous anaphylaxis (PCA) antibody levels occurred at the beginning of patency. This was followed by a decrease in the PCA titers and ACA reactions paralleling an increasing microfilaraemia. Later a stage of equilibrium existed between the level of microfilaraemia and the presence of skin sensitizating antibodies until during the last phase of infection when though PCA-activity of the sera gradually disappeared, the intradermal tests remained positive.

The homocytotropic antibody response during the prepatent period seems is similar to that observed in *Brugia pahangi* infections of *Mastomys natalensis* (Benjamin and Soulsby, 1974). However, these authors reported a gradual waning of homocytotropic antibody through 33 weeks of infection at a time when the microfilaraemia was still rising. In the experiments with *B. pahangi* a heterologous antigen, from *Dirofilaria immitis*, was used and it is possible this may be associated with the difference in levels of homocytotropic antibodies during the microfilaraemic stage of the two infections.

The present investigations demonstrated that the homocytotropic antibody response was not related to the sequence of development of other antibodies detected by immunoprecipitation (Zahner *et al.*, 1970) haemagglutination or complement fixation tests (Zahner, 1974). The homocytotropic antibody response also developed independantly of changes in the IgM and IgG levels (Zahner *et al.*, 1972). This is similar to the independant response of homocytotropic antibodies in several other helminth systems (Sadun, 1972).

An analysis of the development of the homocytotropic antibody response during *L. carinii* infection indicates that several developmental stages probably are capable of inducing the response. Homocytotropic antibody reactivity of sera as early as 15 days after infection must be attributed, if not to the metacyclic larvae, then to the early 4th stage larvae

or to the corresponding moulting stages which occur at this time (Scott *et al.*, 1951). Subsequent developmental stages clearly are concerned in the response and antigens produced at early patency seem particularly important. Indeed, the complex development of metazoon parasites during the prepatent and early patent periods with the potential for the liberation of variable amounts of different antigens may result in antibodies with different specificities. This has been demonstrated during the course of *L. carinii* infection using precipitation techniques (Zahner *et al.*, 1970) and a similar situation may occur with the homocytotropic antibodies. The difference in ACA reactions at 30 and 45 days to various amounts of antigen may be a reflection of this, in that the later response may represent a broader sprectum of antigens which was more readily detected by the adult antigen preparation than the earlier response.

Following patency, the course of the homocytotropic antibody response appeared to be related to the density of microfilariae in the peripheral blood, but the relationship is not simple. The production of microfilariae seems associated with PCA antibodies since high levels of this antibody occur in early patency. Nevertheless, at a later stage of the infection there is a correlation between the level of microfilariae and PCA antibodies. In addition, increasing microfilaraemia is associated with decreasing PCA titers. Thus, at 80 days after infection an inverse correlation existed between the microfilariae density and the size of the immediate type hypersensitivity reactions. This might suggest that preformed antibodies are absorbed by parasite antigens, or that the excessive production of antigen during the period of maximum fertility of worms induces a temporary state of tolerance resulting in an overall decrease of antibody levels.

The origin of the antigens concerned in the stimulation of the immune response has yet to be determined. Ensheathed microfilariae may be poor immunogens and when intact, at least, do not appear to be antigenic (Mantovani and Salzer, 1967). On the other hand, important antigenic components may be present in the fluids of the uteri which may be liberated with the microfilariae. The uterine fluids of *Dipetalonema viteae* have been shown to be antigenic (Diesfeld *et al.*, 1973).

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VASOACTIVE AMINES AND PEPTIDES: THEIR ROLE IN THE PATHOGENESIS OF PROTOZOAL INFECTIONS

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INTRODUCTION

Protozoal infections cause inflammatory reactions in the host. They probably release metabolites, enzymes or toxins; the parasites contain foreign proteins that act as antigens and give rise to allergic reactions.

All that is needed to trigger off the acute inflammatory response in the host is the injury of body cells so that the normal intracellular constituents are released into their surroundings. Direct reaction with tissue fluid liberates histamine and 5-hydroxytryptamine and these agents cause an immediate increase in vascular permeability and the leakage of plasma. Noradrenaline is destroyed, causing a change in local vascular tone. Proteolytic enzymes from the injured cells increase the leakage by their direct action on the vascular endothelium and activate kallikrein, the enzyme that releases the vaso-active peptides bradykinin and kallidin. Proteins in the tissue spaces, together with their peptide breakdown products, attract circulating granulocytes, which accumulate at the site of injury and proceed to devour each other. Complement is almost certainly involved, being fixed by aggregated, leaked plasma protein, and activation of C3 and the trimolecular complex C567 gives rise to chemotactic fragments that encourage further leucocyte emigration (Hurley, 1972; Ward, 1968).

Released proteases digest the inhibitors of collagenolysis and the destruction of fibrillar tissue begins. Connective tissue releases an enzyme that converts fibrinogen to fibrin, and the aggregation of platelets at the site of vascular injury causes thrombosis. Blockage of the microcirculation causes ischaemia, anoxia, acidosis and further cell injury.

It is clear that such a chain reaction can end in massive necrosis and circulatory collapse - and it sometimes does. The mechanism seems to be equipped to run in one direction only - "towards the piling-up of degranulating phagocytic cells in a self-perpetuating process that appears to invest tissue with the capacity to devour itself" (Ramsey and Grant, 1974).

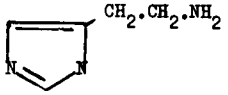
In a thought-provoking essay included in a recent review of the inflammatory process, Thomas (1974) suggests that although "all the stages of the inflammatory response can be made to sound wholly useful and good, the net outcome of this combined operation is most conspicuously the destruction of tissue rather than defense, and most of the subsequent processes of repair seen to be necessary to undo the damage done by the inflammatory process itself rather than primary damage done by the agent". The real pathogens, if one is looking for culprits, are the host's own cells - macrophages that process antigen, lymphocytes that make antibody and polymorphs that inflict the injury on the vessel walls. Thomas suggests that single reactants become lethal when called into exaggerated play, or that the simultaneous overengagement of several participants can cause "the overkill that leads to disease".

Parasitic protozoa, because of the quantities of foreign protein they manufacture within the body of the host and because of their propensity to vary antigenically, thus repeatedly challenging the host's defence mechanisms, usually give rise to chronic inflammatory reactions. The condition is characterised by a tissue infiltration of mononuclear leucocytes, fibroblasts and, especially, macrophages - the basic architectural units of chronic inflammation. The reaction is maintained when the irritant agent persists in the tissues, either in the form of living organisms, or dead ones that have escaped degradation by macrophages, and it disappears when the irritant is removed (Spector, *et al.*, 1968). Controls must exist to make it possible for the inflammatory process to come to an end; perhaps chemical agents take over to switch off or neutralize protein-denaturing enzymes, thus shutting the system down and allowing reconstruction to take place. In protozoal infections the removal of the parasite by effective chemotherapy often results in tissue repair of astonishing speed; the switch must be a very effective one. Boreham and Wright (1976) have recently reviewed the release of pharmacologically active substances in parasitic infections.

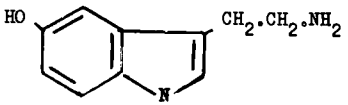
CHEMICAL MEDIATORS OF INFLAMMATION

Amines

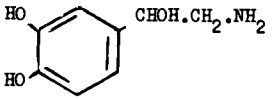
Since the original studies of Lewis (1927), histamine has been recognised as a substance associated with the early stages of acute inflammation. It is present at the site of tissue injury and can be shown to reproduce the vascular changes it is supposed to mediate. Antihistamine drugs partially suppress the inflammation caused by thermal injury (Spector and Willoughby, 1959).



Histamine



5-Hydroxytryptamine



Noradrenaline

Arg-Phe-Pro-Ser-Phe-Gly-Pro-Pro-Arg		Bradykinin
"	-Lys	Kallidin
"	-Lys-Met	Kinin III (bovine plasma)
	↑	
	(N-terminal end)	

Fig. 1. Substances that affect vascular permeability.

Histamine was found by Richards (1965) to be present during the course of *Trypanosoma brucei* infections in mice, but Yates (1970) could detect no significant changes in histamine or the enzyme (histidine decarboxylase) that releases it, in the tissues of infected rats or mice.

Associated with the release of histamine is the appearance of 5-hydroxytryptamine (5-HT), a potent inflammatory agent in some species of animals (Erspamer, 1954). It is present in platelets and mast cells, and is released when these cells disintegrate. Few observations have been made on the part played by 5-HT in protozoal infections but van den Ingh and his colleagues in Utrecht (personal communication) have recently shown that plasma 5-HT levels in *T. vivax* infections in goats change with parasitaemia and with the extent of platelet aggregation. Davis *et al.* (1974) believe that *T. rhodesiense* trypanosomes liberate a substance with a direct toxic effect on platelets, causing them to aggregate. They would then be expected to liberate the considerable amounts of 5-HT they contain. Like histamine, the action of 5-HT appears to be exerted during the acute stages of the inflammatory response.

Adrenaline and noradrenaline maintain vascular tone and probably also decrease vascular permeability. As long as 1929, Krogh suggested that there might be an "antipermeability hormone" that restored the normal degree of vascular permeability during recovery after injury. The most likely substances to act as antiinflammatory hormones are adrenaline and noradrenaline; if these catecholamines are protected from destruction by giving a drug that inhibits the enzyme, amine oxidase, the oedema and increased capillary permeability caused by thermal or chemical injury is suppressed (Spector and Willoughby, 1964). Acute protozoal infections usually end in cardiovascular collapse, and Maegraith *et al.* (1956) showed that a drastic temporary recovery occurs when a monkey infected with *Plasmodium knowlesi* is given a dose of noradrenaline. A similar effect is produced in puppies in a state of severe shock caused by *Babesia canis* infection (Maegraith *et al.*, 1957). The recovery is shortlived, but there is no doubt that catecholamines counteract the vasodilatation and probably also the outflow of fluid, protein and cells from leaking vessels.

Paradoxically, localized vasoconstriction can play a damaging role in malarial shock, limiting the blood flow to the liver by portal vein constriction (Skirrow *et al.*, 1964) and causing renal failure by reducing cortical blood flow. Phenoxybenzamine, which blocks the vasoconstrictor action of catecholamines improves the hepatic and renal circulation in

the malarious monkey (Migasena and Maegraith, 1969) and has been used with advantage in the treatment of severe human malaria infections (Maegraith and Fletcher, 1972).

Rabbits infected with *T. brucei* show arterial constriction in the ears and elsewhere (Goodwin and Hook, 1968) and they sometimes die in renal failure (Goodwin, 1970; 1971; Goodwin and Guy, 1973). Electronmicrography of kidney biopsies has shown this to be due to vascular changes (Goodwin, Hutt and Davies, unpublished). The renal failure that sometimes occurs after several months of infection by *T. brucei* in vervet monkeys springs from a different cause - the deposition of antigen:antibody complex in the glomeruli (see Houba, V. this volume).

Peptides

In 1949, Rocha e Silva *et al.* described a substance released from serum globulin by the action of snake venom *(Bothrops jararica)*, or of trypsin. They called it bradykinin because it caused a slow contraction of smooth muscle; it also decreased the blood pressure and increased vascular permeability. A similar agent, called kallidin, was found by Frey *et al.* (1968) to be liberated from blood by the action of the enzyme kallikrein (kininogenase), which was found in blood in an inactive form (kallikreinogen). Kallikrein, a protein of small molecular size, was originally isolated from urine and was shown to have a powerful vasodepressor action of its own (Frey *et al.*, 1950). Like kallidin, it also dilates vessels and increases permeability and plays a part in the pathogenesis of septic and traumatic shock (Attar *et al.*, 1971).

There is now an extensive literature on the kinins - bradykinin, kallidin and 'kinin III' - polypeptides containing 9, 10 and 11 amino acid residues respectively. They are released as a result of enzyme activation and cleavage from blood or tissue substrates, much in the same way as the clotting process is initiated. Activated Hageman factor promotes the change of plasma kallikreinogen to kallikrein, which then activates kallidinogen to kallidin; this may, in turn, be converted by a plasma aminopeptidase to bradykinin. Once formed, kinins are rapidly destroyed by the action of endogenous peptidases and this makes their detection and demonstration very difficult (Fig. 2). A further permeability factor (PF) formed when plasma is diluted with saline in contact with glass, was described by Miles and Wilhelm (1955); it is probably a stage in the pathway for the formation of kallikrein.

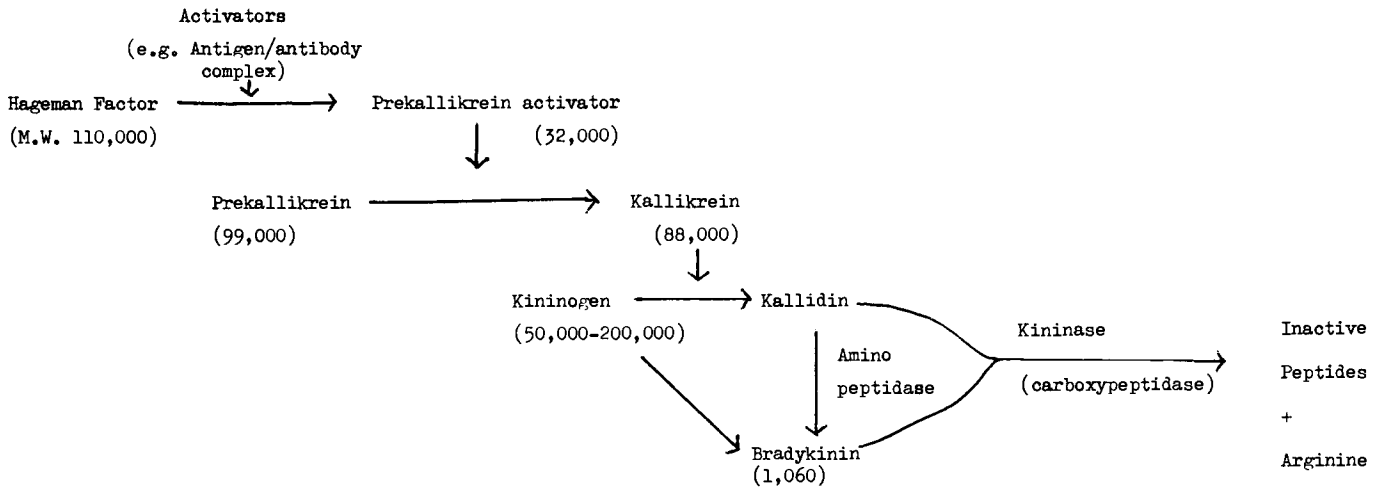


Fig. 2. Simplified summary of the formation and destruction of Kallikrein and Kinins (Adapted from Wilhelm, 1973).

The work done in recent years to determine the role of kinins in inflammation has been summarized by Willoughby (1973). The difficulties that result from the extreme lability of kinins, the absence of any effective method of preventing or delaying their destruction by tissue enzymes or of blocking their effects, and the ease with which artefacts are generated by experimental techniques, have rendered the results of much of the work inconclusive. Willoughby considers kinins, like histamine and 5-HT, to be transiently involved in the acute inflammatory response.

Nevertheless, there is reason to believe that the part played by active peptides and by kallikrein in protozoal diseases is not transient and that these substances make a considerable contribution to vascular pathology. Goodwin and Richards (1960) found pharmacologically active peptides in the urine of mice infected with *B. rodhaini*, *T. brucei*, *P. berghei* and other organisms, and Richards (1965) demonstrated the presence of kinins in the blood of mice with *T. brucei* infections. Tella and Maegraith (1962) had meanwhile shown that the shock in monkeys infected with *P. knowlesi* was associated with the liberation of kinin. Boreham (1968a) showed that in cattle given an infection with *T. brucei*, an impressive release of kinin occurred just after the second parasitaemic peak, coincidentally with the appearance of anti-trypanosome precipitins and fluorescent antibodies. As the infection progressed, the plasma kinin level fell, but after the trypanosomes were killed by chemotherapy the kininogen level leapt to a figure about twice that of the normal value. These experiments suggest that antigen:antibody reactions bring about kinin release and that continued infection is accompanied by a massive turnover of kininogen and kinin. A similar picture is seen in rabbits infected with *T. brucei*, and kinin release has also been detected in human trypanosomiasis (Boreham, 1970).

The trypanosomes probably activate the precursor of the kininogenase that releases kinin from plasma α-globulin (Boreham, 1968b), and follows the absorption of Hageman Factor onto the surface of antigen:antibody complex (Boreham and Goodwin, 1969). Antigen:antibody complexes form every time the host's defences react to the emergence of a new parasite variant, and the release of kinin in such an infection must therefore be regarded as a recurring phenomenon.

In monkeys infected with *P. knowlesi*, Onabanjo and Maegraith (1969; 1970 a,b,c,d), showed that a significant increase in the enzyme kallikrein appeared in the blood; this is itself capable of causing increased vascular permeability and dilatation, and so also is the kallidin that it liberates.

More recently, Wright (1972; 1973) and Wright and Mahoney (1974), in a study of the pathological effects of *B. argentina* showed that the plasma kallikrein activity in infected calves increases on the 3rd day of infection, probably activated by a substance released by the parasites. Splenectomized animals die in 11 - 12 days in a state of shock; the plasma kallikrein levels are very high.

There seems little doubt that peptides and the enzymes that release them contribute to the pathogenesis of protozoal infections. Released in the small vessels and at the tissue face in the presence of antigen:antibody combination, they may exert profound local effects that find no quantitative reflexion in the levels detected in the circulation.

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IMMUNOGLOBULN M IN TRYPANOSOMIASIS

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IgM in man

The most striking change in the serum protein pattern of people infected with African trypanosomes is a great increase in a globulin fraction, which was first described by Mattern and his colleagues as β2 macroglobulin (Mattern *et al.*, 1961 Mattern, 1962) and subsequently named Immunoglobulin M (IgM). This work was carried out on clinical cases of trypanosomiasis caused by *Trypanosoma gambiense*, which is responsible for the typical West African chronic sleeping sickness. The serum IgM levels increased to 8 to 16 times the normal concentration and the increase commenced as soon as 2 weeks after infection. Mattern (1964) examined the serum of 234 confirmed cases of trypanosomiasis of which 226 showed greatly increased IgM. He found that only 90 of 1425 patients with other diseases had equally high levels and concluded that the measurement of serum IgM was a reliable and useful method for diagnosis in field surveys of trypanosomiasis. Mattern (1962, 1964) further showed that the IgM concentration of the cerebro-spinal fluid increased in the later stages of the disease and could be used to indicate the stage of infection. When specific treatment was given the serum IgM level fell to normal levels in a few months, provided there was no central nervous involvement, where the fall to normal levels of both serum and CSF was much slower.

Lumsden (1965) showed that similar increases in serum IgM occurred in infection of man with *T. rhodesiense* and his work was extended by Cunningham *et al.* (1967) who used blood obtained by finger puncture dried onto filter paper. They concluded that the test could be used as a screening method in the diagnosis of sleeping sickness. Cornille and Hornung (1968) found IgM levels of 10 times normal in 42 of 45 confirmed cases of *T. rhodesiense* infection. The IgM level had only fallen slightly or not at all 1-2 months after treatment.

Onyango *et al.* (1972) examined the effect of specific anti-trypanosome treatment on the serum immunoglobulin levels of 60 patients, 15 of whom were considered to have *T. gambiense* and the remainder *T. rhodesiense*.

There was no significant difference between the raised IgM

concentration of *T. gambiense* and *T. rhodesiense* patients. IgM levels fell by one month after treatment but the fall varied considerably from patient to patient.

Binz (1972) and Binz and Watson (1972) examined the results of IgM determinations critically, both in cases where parasites were seen but IgM levels were normal and in cases where no parasites could be found but the IgM levels were high. In the former case they concluded that if parasitological diagnosis was made early in infection there may not have been sufficient time for IgM production. Binz (1972) also showed that IgM levels may take 2 to 3 years to fall to normal, especially in patients with the more chronic form of the disease.

Binz and Watson (1972) did a survey in various areas of Kenya and found 286 people with increased IgM who did not appear to have had trypanosomiasis. They examined the possibility that other diseases such as malaria, schistosomiasis, leprosy and helminthiasis could be responsible but could find no correlation between these diseases and high IgM concentration. Increased IgM levels were much more common in areas where tsetse and trypanosomiasis occurred than in an area which was tsetse free and they suggested that some cases were associated with inapparent infections with human or animal trypanosomes.

It is clear from these studies that increased serum IgM is a useful, but not infallible, indication of African trypanosomiasis in man and that a normal IgM level virtually excludes trypanosomiasis. It would be possible for an individual to have increased IgM months or years after successful treatment and the value of the test, therefore, lies in the screening of large numbers of people in field surveys.

Studies have also been made on domesticated, wild and laboratory animals and experimental infections in these species enable a more careful examination to be made of the mechanisms which may cause the increased IgM.

IgM in other primates

Houba *et al.* (1969) infected rhesus monkeys *(Macaca mulatta)* with different strains of *T. brucei*, *T. gambiense* and *T. rhodesiense* and measured immunoglobulin levels and serum titres of non-sensitized sheep red cell agglutinins and M-antiglobulins (rheumatoid factors). IgM rose to about 10 times normal and remained high for months. The IgM remained high in monkeys which relapsed after treatment but fell slowly in one animal in which treatment successfully removed the parasites. Antibody levels to non-sensitized sheep cells rose at the same time as the IgM increase but

fell more rapidly than the IgM level.

Baker and Taylor (1971) infected 4 chimpanzees *(Pan troglodytes)* with *T. brucei* and *T. rhodesiense*. All showed a rise in IgM to about 10 times the preinfection value within a month of infection and then remained practically unaltered till death, in one case some 7 months after infection.

IgM in laboratory animals

Seed *et al.* (1969) infected rabbits with *T. gambiense*, separated the immunoglobulins by chromatography, and examined the fractions for agglutinating antibody. IgM increased rapidly after infection and agglutination antibody first appeared in the IgM fraction. IgM remained high but later antibody was found in both IgM and IgG.

Klein *et al.* (1970) extended their work on *T. equiperdum* in rabbits (Mattern *et al.*, 1963) and showed that the increased IgM level was associated with the appearance of rheumatoid factors (M-globulins) but stated that these did not appear in infected mice. Frommel *et al.* (1970), also used *T. equiperdum* in rabbits and found an increase in IgM to over 10 times normal level within 3 weeks of infection and also showed that low molecular weight (7S) IgM was produced in 4 of 5 infected rabbits.

Takayanagi and Enriquez (1973) working with *T. gambiense* in mice did not measure the serum immunoglobulins but did fractionate serum into IgM and IgG. They then compared the ability of these fractions to passively protect mice, to agglutinate trypanosomes and to cause the formation of variant trypanosome populations. Whilst both immunoglobulins were active in each system IgM was particularly effective in the formation of variant serotypes and in protection whilst IgG was effective in the agglutination reaction. Capbern *et al.* (1974) infected mice with *T. gambiense* and *T. cruzi*. The IgM concentration of the *T. gambiense* infected mice rose to 20 times the preinfection level by 1 month after infection and then remained high for 2 months, when the mice died. Whilst some increase in IgM occurred in mice infected with *T. cruzi*, the increase in IgG was proportionately greater.

Clarkson (1975) infected LAC/A strain mice with *T. brucei* or *T. congolense* and followed the alterations in immunoglobulin levels. There was a rapid sustained rise in both infections followed by a fall. There was a further rise in the mice infected with *T. brucei* but the mice infected with *T. congolense* died when the IgM was relatively low (Fig. 1). These studies indicate that the mouse is a convenient animal for the study of the mechanism of IgM production in trypanosomiasis.

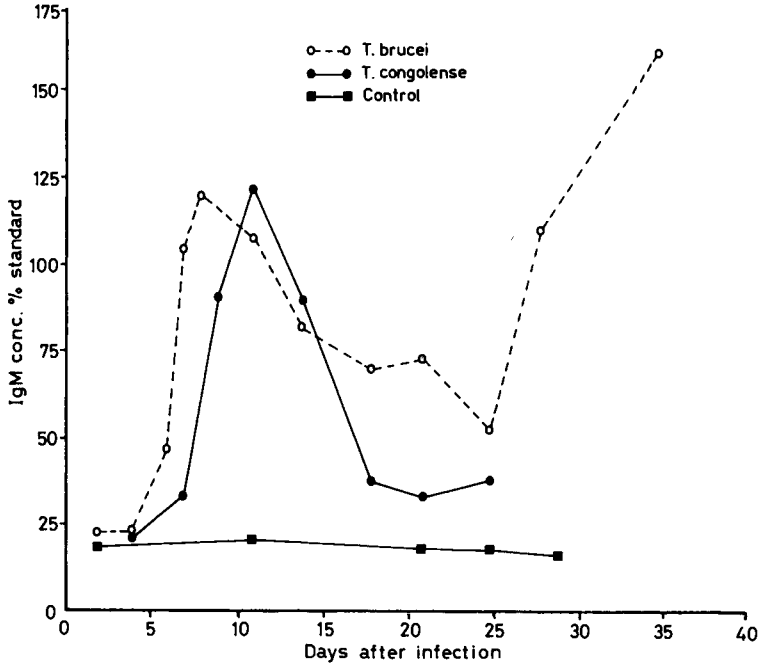

Fig. 1 Effect of T. brucei *on serum IgM concentrations of LACA mice.*

IgM in cattle and wild animals

Relatively few studies have been made on the important trypanosomes of cattle which belong to the subgenera *Duttonella* and *Nannamonas*.

Luckins (1972) examined the serum IgM levels of Zebu cattle living in an endemic area of trypanosomiasis and compared them with the levels of Zebu in a tsetse free area. The cattle became infected with *T. vivax*, *T. congolense* and *T. brucei* and were treated periodically for trypanosomiasis. IgM increased to 2-9 times the preinfection levels. These animals were repeatedly infected with all 3 species and probably with *Babesia*, *Theileria* and *Anaplasma* species but the results strongly suggested that cattle infected with trypanosomes of the subgenera *Duttonella* and *Nannomonas* showed a similar response to other hosts infected with members of the subgenus *Tryponozoon*.

Clarkson and Penhale (1973) and Clarkson *et al.* (1975) described the effect of experimental infection of British calves with different strains of *T. vivax*. The response

varied depending on the course of infection and the pathogenicity of the strain. In animals which died of trypanosomiasis within a month of infection IgM concentration increased to 2 to 3 times normal but in animals which lived longer greater increases occurred and fluctuations occurred, even occasionally falling to normal levels. Changes in IgM of different animals are shown in Fig. 2.

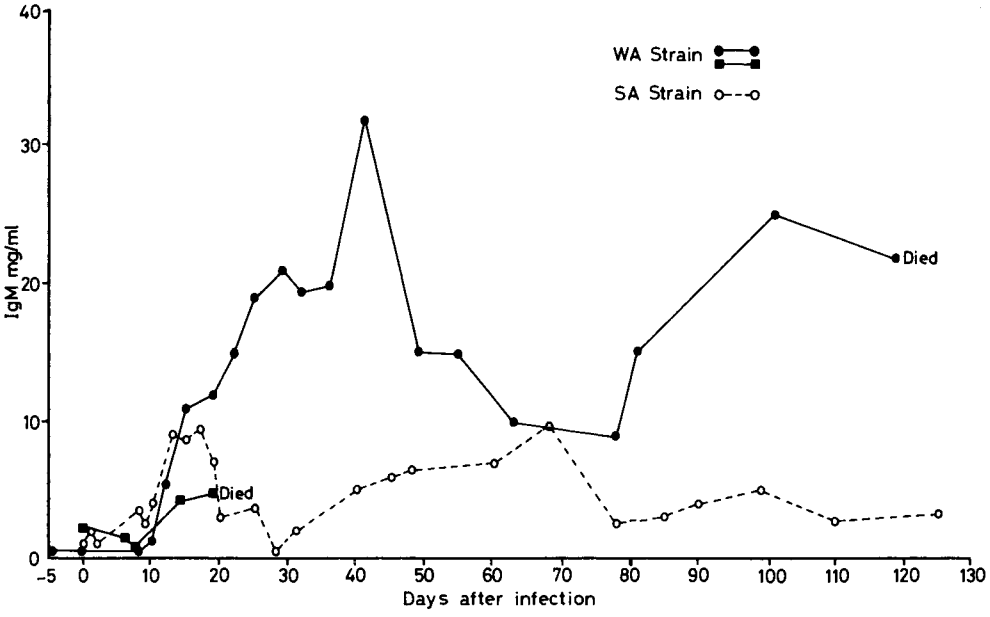

Fig. 2 Effect of T. vivax *on serum IgM concentrations of 3 calves, illustrating differences in response. The WA strain originated in Nigeria, the SA strain in Columbia.*

There was never a significant alteration in IgG concentration until 2 to 3 months after infection and then only to twice the normal levels. IgM levels fell after treatment but remained high if relapses occurred (Fig. 3).

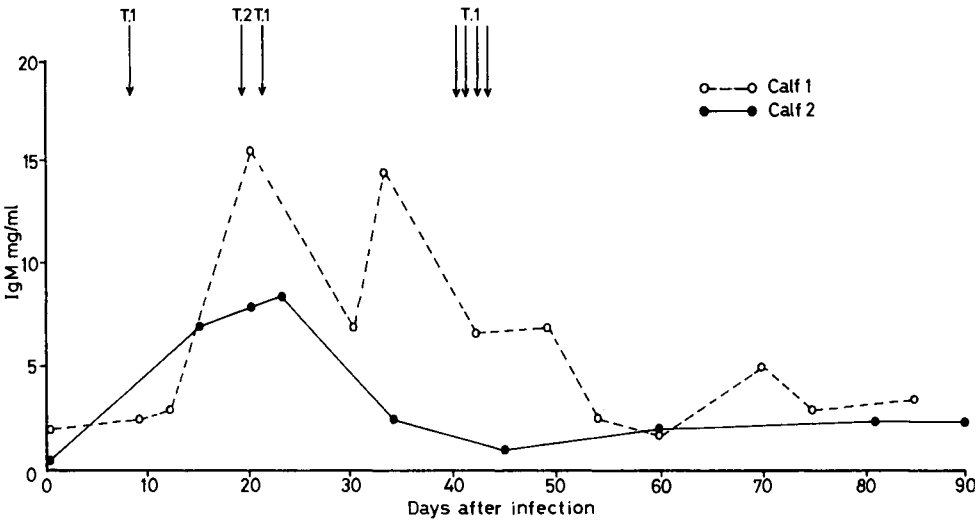

Fig. 3 Effect of treatment on serum IgM concentrations of 2 calves infected with T. vivax, *WA strain. Calf 1 was unsuccessfully treated resulting in relapses; Calf 2 was successfully treated and was free of parasites from Day 20 onwards.*

These results show that the usual response occurs in calves infected with trypanosomes of the subgenus *Duttonella* and

suggest that raised levels could be used as a screening diagnostic test but individual infected animals might be missed.

Luckins (1974, 1976) carried out similar studies on African Zebu cattle which were experimentally infected with *T. congolense* and *T. vivax* and treated 50 days after infection. The increased IgM fell after treatment but were still higher than normal 50 days later. He also compared the neutralizing ability of whole serum and IgG and IgM fractions from one Zebu infected with *T. congolense*. The IgM fraction was much more effective than IgG but not as active as the unfractionated serum.

Luckins (1975) compared the immunoglobulin levels of bushbuck naturally infected with *T. vivax, T. congolense* or *T. brucei* with bushbuck which did not show trypanosomes in their blood but were serologically positive and others which were serologically negative. The infected bushbuck had a much higher IgM and IgG level than serologically negative bushbuck or Zebu cattle with trypanosomiasis. Whilst Luckins (1975) indicates the problem of obtaining 'normal' values in wild animals, the increased IgG and IgM may be significant in that bushbuck are generally regarded as being comparatively resistant to trypanosomiasis. Further studies on IgM and IgG levels and their function in cattle and game animals would be valuable.

IgM and pathogenesis of trypanosomiasis

Since very high IgM concentrations are almost universally seen in man and animals infected with salivarian trypanosomes it is worth considering whether these proteins play any part in the pathogenesis of the disease. The amount of circulating IgM is even higher than is indicated by the IgM concentration since, in addition, trypanosomiasis causes an increase in plasma volume. Clarkson (1968) found that sheep infected with *T. vivax* had an increase in plasma volume of 18% over control sheep and Naylor (1971) found increases of about 40% by 4 weeks after infection of cattle with *T. congolense* with a rapid increase in the 2nd week. This coincides with the increase in IgM concentration and Clarkson (1968) suggested that increased osmotic pressure was responsible for the increase in plasma volume. Whatever the mechanism, it is clear that the amount of circulating IgM is even greater than indicated by the increase in IgM concentration. It is known that IgM is largely restricted to the vascular compartment of normal animals and, though infections with trypanosomes of the subgenus Trypanozoon cause increased vascular permeability, and therefore leakage of IgM into the extravascular compartment (Goodwin and Guy, 1973), there is

no evidence that this occurs in infections with trypanosomes of the subgenera *Duttonella* or *Nannomonas*.

Naylor (1971) indicated that the mean plasma volume of 6 infected cattle rose from 6500 ml before infection to 9000 ml 4 weeks after infection. If we assume that the preinfection IgM concentration was 2 mg/ml and it rose 4 weeks later to 20 mg/ml (10 fold) the circulating IgM will be about 13g before infection, rising to 180g after, a rise in total IgM of almost 14 fold. IgM has a short half life of no more than 5 days and a high fractional turnover rate of 18% in man and it is likely that cattle IgM is similar. This would mean that a normal bovine would synthesize about 9mg/kg/day and an infected animal 130 mg/kg/day, provided the catabolic rate is unaltered in trypanosomiasis. This gives an indication of the enormous increase in IgM synthesis which occurs in infected animals.

It is still not clear if this increased IgM is involved in the pathogenesis of trypanosomiasis and further studies are needed. However, it would seem extremely likely that such a high level would adversely affect the host and Mackenzie and Boreham (1974) have suggested that this is likely to lead to an increased erythrocyte sedimentation rate and increased viscosity of the serum, causing circulatory embarrassment. They also suggest that immune complexes may form leading to the release of pharmacologically active substances. The formation of such complexes could explain many of the pathological features of trypanosomiasis, particularly as seen in *T. brucei* in the rabbit. This Type III hypersensitivity causes platelet aggregation, and complement activation, leading to the release of histamine and kinin forming enzymes (Roitt, 1974) which are known to occur in trypanosomiasis (Boreham, 1968). Nagle *et al.* (1974) demonstrated that rhesus monkeys infected with *T. rhodesiense* developed a glomerulonephritis associated with deposits of C3, properdin and IgM in the glomeruli. Serum complement levels fell which is what would be expected in a type III hypersensitivity reaction in antigen excess. In infected cattle, the pathological changes are not so clear cut and may largely be attributed to the profound anaemia which develops, which may also be immunological in origin.

It is also generally believed that the high IgM production is linked with the marked immunosuppression to other antigens which occurs in trypanosomiasis (Goodwin *et al.*, 1972; Terry *et al.*, 1973).

Mechanism of increased IgM

The cause of the increased IgM is probably due to a combi-

nation of factors and has led to considerable discussion (CIBA, 1974). When an animal is injected with antigen it is normal to get the production of 'early' IgM antibody followed by 'late' IgG antibody after a rapid switch from one to the other.

Trypanosomes are very antigenic and change their antigenic makeup very frequently. Jones and Clarkson (1974) isolated 9 variants over a period of 53 days from one calf but, since Jones and Clarkson (1975) showed that antigenic variants of *T. vivax* appeared in calves every 2 to 4 days, it is probable that at least twice this number of variants had appeared. This means that the calf was being stimulated by a rapid succession of distinct but related antigens which gives rise to a succession of waves of antibody production, much of which is probably originally IgM. Seed *et al.* (1969) and Seed (1972) suggested that the raised IgM was caused, at least in part, by the continuous synthesis of new specific antibody to each antigenic relapse. Whilst several workers have failed to absorb the IgM with trypanosome antigens and have concluded that most of the IgM is not specific trypanosome antibody (Houba *et al.*, 1969) it should be remembered that it would require absorption with all the antigenic variants to conclusively demonstrate its specificity, which has not yet been attempted. *T. cruzi* does not undergo antigenic variation and great increases in IgM also do not occur.

Many workers have shown that man and animals infected with trypanosomes produce antibody to a wide range of antigenic determinants including those of many host tissues (Houba and Allison, 1966; Mackenzie and Boreham, 1974) and it is also known that low molecular weight (7S) IgM and even free light chains are produced (Frommel *et al.*, 1972; Greenwood and Whittle, 1975). Arising out of these observations Urquhart *et al.* (1973) and Greenwood (1974) suggested that trypanosomes contain a B-cell mitogen which results in a 'polyclonal' stimulation of cells, thus giving rise to antibodies of many specificities. Bacterial endotoxin produces a similar effect but no one has been able to detect such a toxin in the salivarian trypanosomes, neither are there any reports on lymphocyte transformation *in vitro* by trypanosome extracts. Clarkson (unpublished) has shown that athymic nude mice do not show the typical IgM response when infected with *T. brucei*. Since these mice are regarded as having a normal B-cell system, it seems likely that T-cells are involved. Terry *et al.* (1973) have suggested that trypanosome infections interfere with T-cell control of B-cells and have developed this theory to include an explanation of immunosuppression. However, Clarkson (unpublished) has shown that

immunosuppression to sheep red blood cells can occur in mice infected with *T. brucei* when there is no increase in serum IgM and it is clear that further work is needed on the mechanism both of IgM production and immunosuppression in trypanosomiasis.

The observations of Mattern and his colleagues of abnormal immunoelectrophoresis patterns in patients with Gambian trypanosomiasis has thus stimulated studies not only on the diagnosis and pathogenesis of trypanosomiasis but also on the nature of T and B cell interactions, which are likely to prove of fundamental interest.

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A COMPARATIVE STUDY OF THE HEMATOLOGICAL ASPECTS OF THE DISEASES CAUSED BY *TRYPANOSOMA VIVAX* AND *TRYPANOSOMA CONGOLENSE* IN CATTLE

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INTRODUCTION

Although anemia is a well recognized finding in bovine infections with either *T. vivax* (Vohradsky, 1971) or *T. congolense* (Fiennes, 1950, 1954; French and Hornby, 1934; Holmes *et al.*, 1974; Hornby, 1929; Mamo and Holmes, 1975; Naylor, 1971), the cause, or causes, of the anemia have not been definitively established. In order to clarify the significance of the anemia in the morbidity and mortality experienced in bovine trypanosomiasis, to elucidate the cause(s) of the anemia, and to further investigate the immunopathology of trypanosomiasis, a comprehensive experiment has been conducted. Preliminary results of this experiment are presented in this paper.

MATERIALS AND METHODS

Animals, Conditioning, Housing

A group of Holstein-Friesian steers 14 to 30 months old and 175 to 305 kgs in weight were obtained from a tsetse fly free area of Kenya. Prior to beginning the experiment, they were immunized against foot and mouth disease (FMD: SAT 1, SAT 2, bivalent A/O. The Wellcome Foundation Ltd. Nairobi, Kenya), blackleg and anthrax (Blanthrax. Cooper Kenya Ltd., Nairobi, Kenya) and contagious bovine pleuropneumonia (T_1 broth culture vaccine, EAVRO, Kikuyu, Kenya). All animals were dipped or sprayed with acaricide (Pyespray, Pfizer Laboratories Ltd., Nairobi, Kenya; Coopertox, Wellcome Foundation Ltd., Nairobi, Kenya) once a week throughout the experiment. All animals were checked for the presence of *Trypanosoma theileri* by blood culture (Sollod and Soulsby, 1968). An anthelmintic (thiabendazole and rafoxanide; Ranizole, Merck, Sharp, and Dohme, B. K. Haarlem, Netherlands) was administered orally to each animal twice prior to the experiment. The steers were housed in a large fly-proof barn in three randomly assigned groups of 25 and all were in close contact throughout the experiment. All animals were weighed

at weekly intervals. The control group was fed the same quantity of grass hay plus ranch cube supplement (Maida Ltd. Nairobi) as was consumed by the *T. congolense* group on the previous day. Mineral salt lick (KNZ mineral block, Maida Ltd. Nairobi) and water were available free choice. To prevent the complication of iron deficiency anemia, each animal received intramuscularly 0.5g of iron dextran (Pharmeta N/V, Rotterdam) one month prior to infection. The calves were housed in the barn for four weeks prior to trypanosome infection.

Trypanosome stabilates

Twenty-five calves were each infected intravenously with approximately 100,000 *T. congolense* contained in one ml of blood from a calf which had been infected with a cryopreserved stabilate (EATRO 1800). The blood was drawn from the donor calf during the first rising parasitemia. Similarly, another twenty-five calves were each intravenously infected with approximately 100,000 *T. vivax* obtained from a calf which had been infected with EATRO stabilate 1721, a West African *T. vivax*. The steers in the control group each received 1.0ml of a 1:1 mixture of EDTA anticoagulated blood from the two trypanosome donor calves obtained prior to their infection.

Sample collection

Every animal was bled of the same volume of blood every morning, except Sunday, before 9 a.m. Blood for hematology was collected into 7 ml Bijou bottles containing 0.05 ml of 20% dipotassium ethylene diamine tetra-acetate and thoroughly mixed. Blood for serum was collected in dry sterile Universal bottles. Sternal bone marrow aspirations were made weekly from each animal and six air-dried smears were made of the aspirated marrow.

Hematology

The following parameters were measured daily in each blood sample: packed cell volume (PCV), white blood cell count (WBC), and hemoglobin (Hb). Red blood cells (RBC) were counted twice weekly in the infected groups and once weekly in the control group. The following red cell indices were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Schalm, 1965). Platelets were diluted and counted in hemacytometers twice weekly from the infected groups and once weekly from the controls. Differential cell counts were determined on 200 leukocytes per Giemsa stained peripheral blood smear, and on 1000 nucleated cells per bone marrow sample.

Direct Coombs' Test

Blood was drawn from the jugular vein into Alsever's solution. The red blood cells were washed six times in about 10 times their volume of phosphate buffered saline (PBS), pH 7.2. The antiserum employed was prepared against bovine IgG, and it had activity against IgG only as judged by immunoelectrophoresis. The antiserum was titrated in doubling dilutions, the lowest dilution used was 1:4. One drop of 2% red cell suspension was added to one drop of antiserum dilution in plastic microtiter plates and incubated for one hour at 37 C. The plates were examined for hemagglutination macroscopically as well as at low magnification under the microscope.

Trypanosome counting

Trypanosomes and leukocytes were counted in 100 or 400 oil immersion fields of a Giemsa stained thin smear of each peripheral blood sample. The trypanosome: leukocyte ratio was then multiplied by the respective total leukocyte count to obtain an estimate of the trypanosome concentration in the peripheral blood.

Postmortem examination

All animals were necropsied immediately after death. Animals which were recumbent and unable to rise for more than 12 hours were transported to the post mortem room and electrocuted. Several control animals were killed at the time at which infected animals were dying.

Statistics

The term "mean" refers to arithmetic mean. The standard deviation and standard error of the mean were calculated by accepted statistical methods. The significance of the difference between means was determined by the student's t test, either paired or unpaired as was appropriate. A p value of <0.05 was considered to be a statistically significant difference throughout.

RESULTS

Trypanosomes were first detected in both infected groups on day 5, reached peak concentrations on days 7 and 8, and thereafter fluctuated with considerable inter-animal variation. Preliminary indications are that the concentration of this strain of *T. vivax* reaches much greater peaks in the peripheral blood than does *T. congolense*, possibly because of the capillary localization of *T. congolense* (which we have observed in histopathological sections). Fluctuations in the numbers of *T. vivax* became less marked with time, and trypanosomes were often undetectable in thin smears of jugular

venous blood. The *T. congolense* concentration was low and trypanosomes were usually detectable constantly throughout the course of the disease.

Trypanosoma theileri were detected by culture in about 10% of the cattle prior to infection with pathogenic trypanosomes, and by the microhematocrit technique were occasionally observed in the blood of various animals in all groups throughout the experiment.

The numbers of animals remaining in each experimental group are presented in Table I. Some of the trypanosome-infected animals were severely affected by secondary bacterial infections, e.g. salmonellosis, possibly as a result of immunosuppression (Holmes *et al.*, 1974). All deaths were therefore not due to uncomplicated trypanosomiasis. None of the control animals developed similar infections although they were housed with the trypanosome-infected animals under identical conditions.

TABLE I

Numbers of animals surviving in each experimental group.

Group	Days post infection												
	0	7	14	21	28	35	42	49	56	63	70	77	84
1	25	25	25	25	22	19	14	13	10	8	7	6	0
2	25	25	25	25	25	25	24	21	16	11	3	0	
3	25	25	25	25	25	25	25	25	24	21	20	19	19

Group 1: T. vivax *infected; Group 2:* T. congolense *infected; Group 3: Control.*

Anemia developed rapidly in both groups of calves following infection with trypanosomes (Fig. 1). The mean PCV in the *T. vivax* group declined to its minimum value of 20.0% on day 36. The mean PCV was significantly less than the initial value and the control values after day 12. The mean PCV in the *T. congolense* group decreased to a minimum value of 18.2% on day 34, and was significantly less than the initial value and control values after day 13. In the control group, the

mean PCV significantly increased over the course of the experiment.

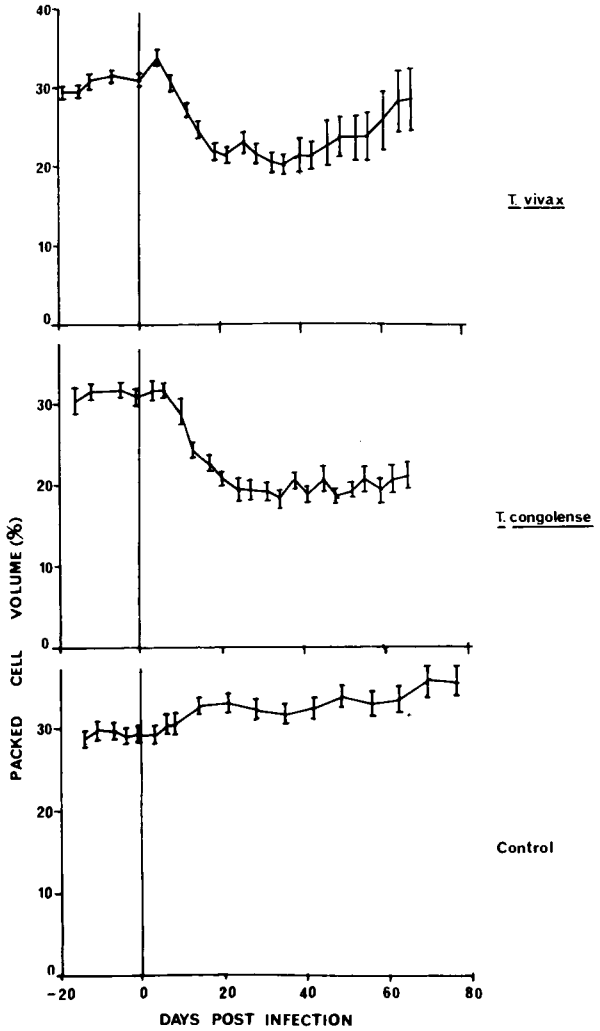

Fig. 1. Packed cell volumes (PCV) of trypanosome-infected and control groups of calves. Means ± 2 standard errors of the mean are plotted.

Changes in mean hemoglobin concentration (Fig. 2) and mean red blood cell concentration (Fig. 3) closely parellelled changes in mean PCV. Mean Hb and RBC concentrations were significantly below initial and control values after day 8 in the *T. vivax* group and after day 10 in the *T. congolense* group.

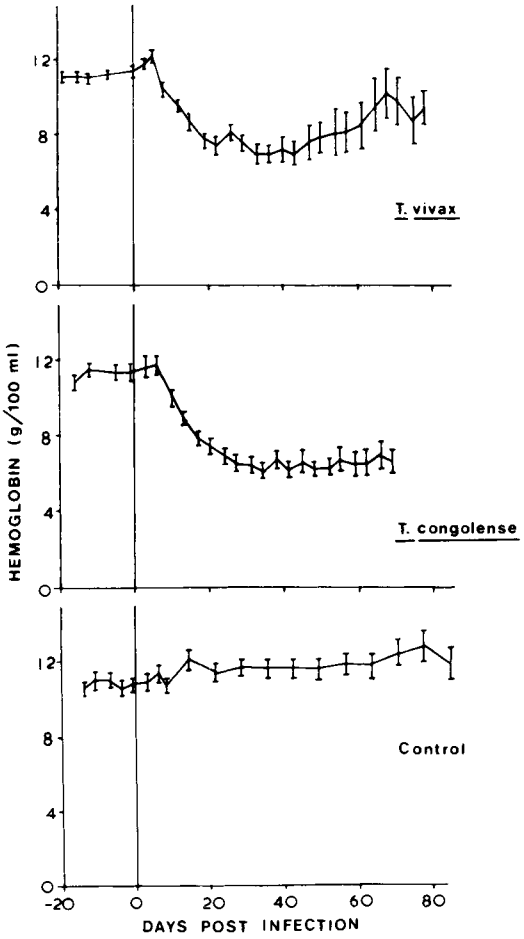

Fig. 2. Hemoglobin concentrations of trypanosome-infected and control groups of calves. Means ± 2 standard errors of the mean are plotted.

The mean MCV in the *T. vivax* group rose slowly to a peak value of 52.8μ^3 on day 57 (Fig. 4). This value was significantly greater than initial and control values. The mean MCV in the *T. congolense* group rose to a peak of 63.7μ^3 on day 45, which was significantly greater than initial, control, and *T. vivax* values. The mean MCV of the control group also rose slightly but significantly above baseline values to a peak of 45.6μ^3 on day 63. The anemia in both infected groups was therefore originally normocytic and became macrocytic after about day 40 in the *T. vivax* group and after about day 27 in the *T. congolense* group.

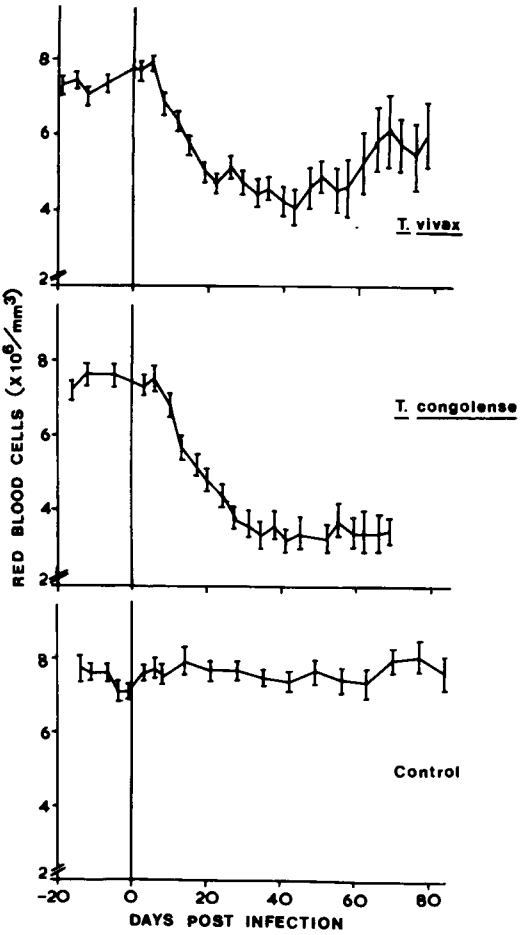

Fig. 3. Red blood cell concentrations of trypanosome-infected and control groups of calves. Means ± 2 standard errors of the mean are plotted.

The mean MCH for the *T. vivax* group was not significantly elevated above baseline or control values except on day 57 (Fig. 5). The mean MCH for the *T. congolense* group was significantly greater than baseline and control values on day 27 and thereafter. The control mean MCH did not vary significantly over the course of the experiment.

The mean MCHC in all three groups declined somewhat over the course of the experiment (Fig. 6), but there were not significant differences among the groups, i.e. the red cells remained normochromic in all groups.

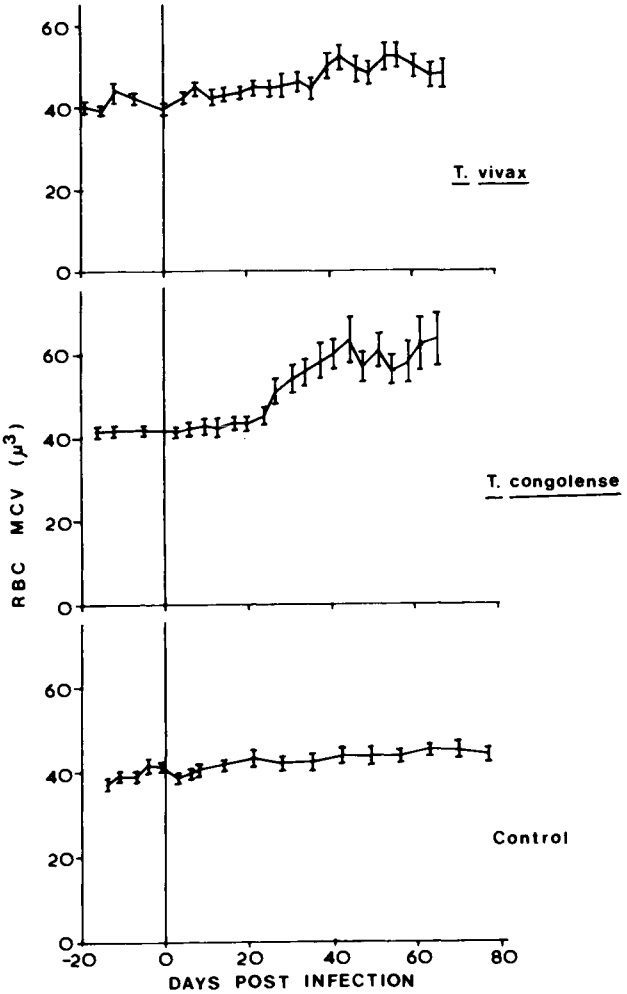

Fig. 4. Red blood cell mean corpuscular volumes of trypanosome-infected and control groups of calves. Means ± 2 standard errors of the mean are plotted.

Preliminary studies of bone marrow differential count indicate that the erythroid response was much more marked in the *T. congolense* group than in the *T. vivax* group.

Results for the direct Coombs' test were obtained from the 40th to the 74th day post infection. Within this period the test was carried out on 23 days on 10 control animals, 10 animals infected with *T. congolense*, and 7 animals infected with *T. vivax*.

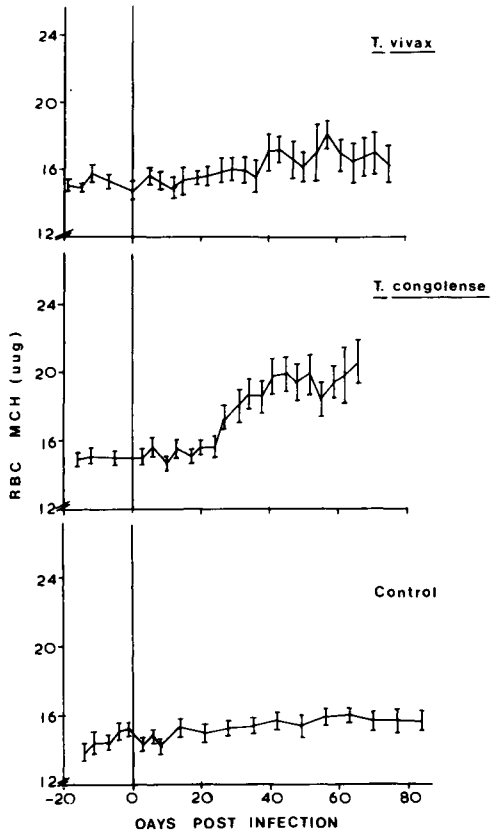

Fig. 5. Red blood cell mean corpuscular hemoglobins (MCH) of trypanosome-infected and control groups of calves. Means ± 2 standard errors of the mean are plotted.

No hemagglutination was observed with the red cells obtained from the control animals or the *T. congolense* infected animals throughout this period. The *T. vivax* infected animals showed positive direct Coombs' tests to varying degrees, the titers ranging from 1:4 to 1:64 (Table II). It is noteworthy that the tests were not consistently positive. Animal no.1 was negative throughout while animal no.10 was positive most of the time. The Coombs' titers were poorly correlated with the degree of anemia.

TABLE II

Titers of direct Coombs' test in cattle infected with Trypanosoma vivax.

Animal No.	Days post infection																					
	40	41	42	43	44	45	47	48	49	50	52	53	54	55	57	58	60	61	65	67	69	74
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	64	8	4	-	4	-	-	-	-	16	8	8	4	8	-	32	32	32	-	-	32	32
4	-	-	*																			
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
7	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	32	-	-	-	-	-
9	64	8	4	4	4	-	4	8	-	8	8	4	4	8	32	32	32	64	16	*		
10	4	4	4	4	-	4	-	8	8	8	8	8	8	16	32	32	64	64	16	64	64	32

- Titer < 4

* Animal died

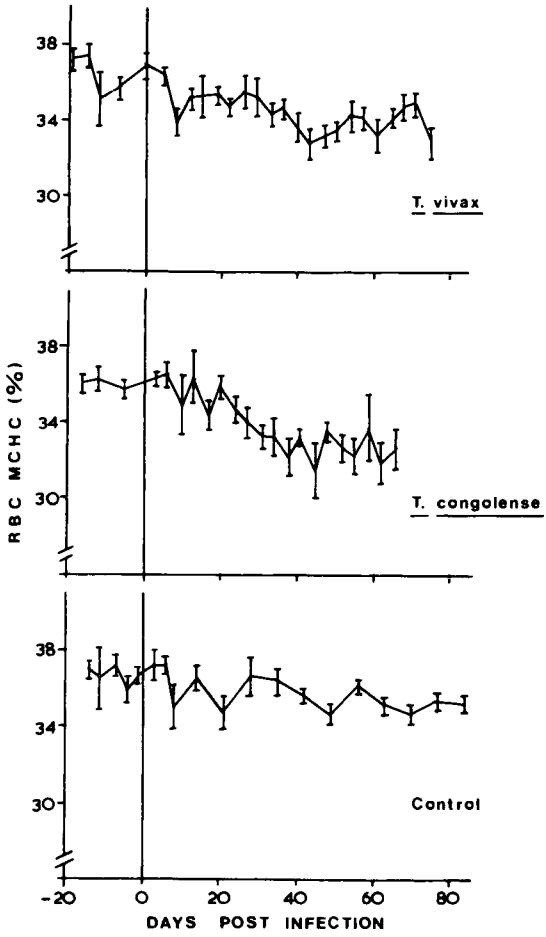

Fig. 6. Red blood cell mean corpuscular hemoglobin concentrations (MCHC) of trypanosome-infected and control groups of calves. Means ± 2 standard errors of the mean are plotted.

The mean total leukocyte count in the *T. vivax* animals decreased to its lowest level of 5900/mm^3 on day 9 post infection (Fig. 7). The WBC count then returned toward normal in a week but thereafter fluctuated widely. The WBC count was significantly less than initial and control values only on days 8 and 9. In the *T. congolense* group, the WBC count decreased to 5800/mm^3 on day 10 and remained at a low level

until about day 50. The WBC concentrations were significantly less than initial and control values from day 10 to day 50. Differential leukocyte counts are not yet complete. Initial indications are that the leukopenia in both groups was due to concomitant neutropenia and lymphopenia.

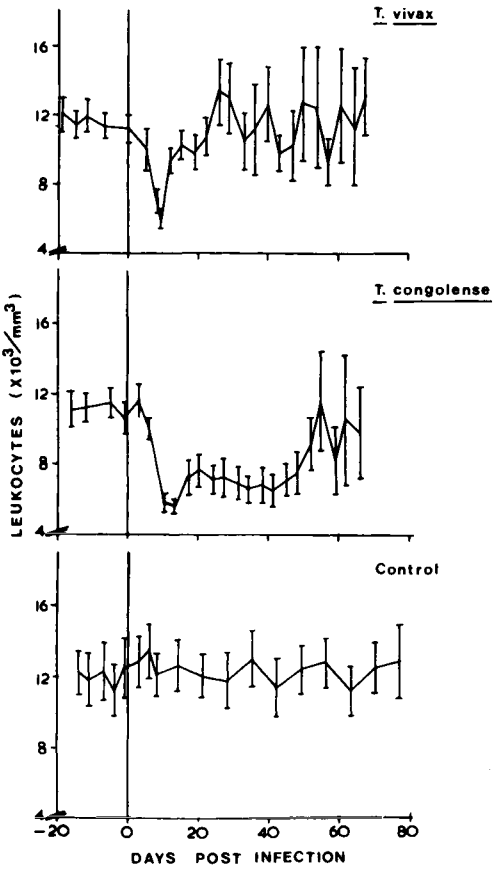

Fig. 7. White blood cell concentrations of trypanosome-infected and control groups of calves. Means ± 2 standard errors of the mean are plotted.

The mean platelet concentration in the *T. vivax* group rapidly decreased to a minimum value of 138,000/mm^3 on day 12 and was significantly below baseline and control values from day 8 post infection (Fig. 8). Similarly, the mean platelet count in the *T. congolense* group decreased to 166,000/mm^3 by day 13 and was significantly decreased from day 10 until the

termination of the experiment. The mean platelet count of the control group did not change significantly over the experiment.

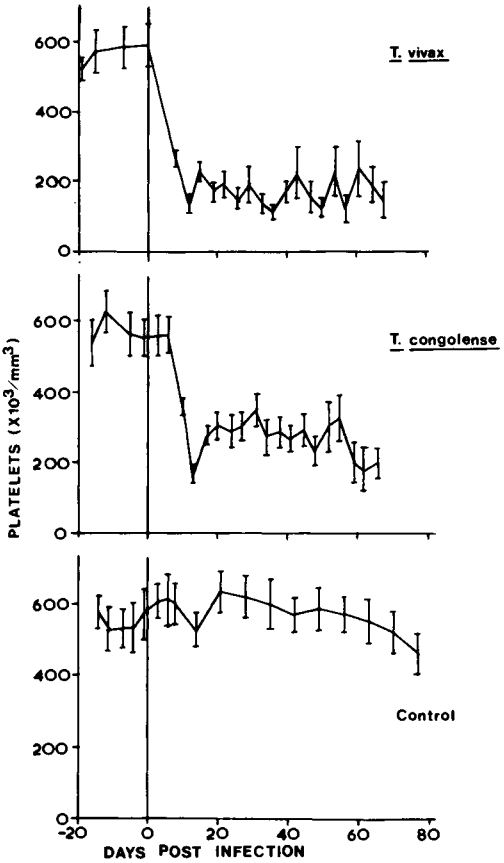

Fig. 8. Platelet concentrations of trypanosome-infected and control groups of calves. Means ± 2 standard errors of the mean are plotted.

DISCUSSION

The development of macrocytic normochromic anemia in cattle following infection with either *T. vivax* or *T. congolense* is generally in agreement with previous work. Vohradsky (1971) found microcytic, hypochromic anemia in *T. vivax* infected cattle, but this may have been due to concomitant iron deficiency. Fiennes (1950, 1954) and Naylor (1971) have

reported that the anemia in *T. congolense* infections is macrocytic and normochromic in the acute stages of the disease and later becomes normocytic and normochromic. Naylor (1971) stated that the anemia resulted from hemodilution, extravascular hemolysis, and toxic dyshemopoiesis. Mamo and Holmes (1975) reported that *T. congolense* caused a severe normocytic, normochromic anemia which was primarily due to loss of RBC's from circulation and did not result from depression of erythropoiesis.

There appear to be differences between the responses of our two infected groups as an equally severe anemia was produced by both infections, but an apparently lesser response by the bone marrow was able to retunr the erythron in the *T. vivax* group to normal while a greater marrow response in the *T. congolense* group was only able to maintain the erythron at its subnormal level. This major difference may be related to differing mechanisms of pathogenesis in the two infections.

The fact that the direct Coombs' test failed to demonstrate the presence of immunoglobulins on the red blood cells of *T. congolense*-infected cattle would indicate that the anemia in *T. congolense* infection is not caused by an immunological mechanism. On the other hand, the observation of positive direct Coombs' tests with erythrocytes of *T. vivax* infected-cattle indicated coating of red blood cells by immunoglobulins. This in turn suggests that antigen-antibody complexes may adhere to red cells in cattle with *T. vivax* infections and that adhered immune complexes either cause hemolysis or enhance phagocytosis *in vivo*.

The peripheral blood leukocyte response to the two trypanosome infections differed in that *T. congolense* animals remained leukopenic as shown previously (Naylor, 1971; Wellde *et al.*, 1974) while the *T. vivax* animals' mean WBC count increased after transient leukopenia, also as observed previously (Vohradsky, 1971). The cause of this difference is unknown but implies differences in the pathogenesis of the diseases. Leukocytes may be attracted to trypanosomes localized in the microcirculation in the *T. congolense* animals and thus cease to circulate. Also bone marrow erythropoiesis may be so intensive in the *T. congolense* animals that the stem cell population is primarily devoted to erythroid cell production, leaving only a small proportion available for granulopoiesis. These suggestions may be clarified on completion of analyses in progress and by further experimentation.

Naylor (1971) mentioned that, in *T. congolense* infection in his group of cattle, "although there was a considerable drop in blood platelet levels, this lower value was still

within the normal range". Unfortunately, he did not present his platelet data. Thrombocytopenia has not previously been reported in *T. vivax* infection of cattle. Thrombocytopenia was observed in four human patients with *T. rhodesiense* infection (Robins-Browne, Schneider and Metz, 1971). Two mechanisms were believed to be responsible for the thrombocytopenia, namely, splenic pooling of platelets and increased peripheral destruction of platelets. There was evidence of disseminated intravascular coagulation (DIC) in three of these cases, but thrombocytopenia also occurred in one case without evidence of DIC, leaving the role of DIC in causing thrombocytopenia uncertain. Immunologically mediated thrombocytopenia was postulated, but anti-platelet antibodies were not demonstrated.

SUMMARY

Twenty five calves were infected with *T. vivax*, 25 with *T. congolense*, and 25 served as controls. Trypanosomes were first detected in the peripheral blood in both infected groups on day 5, reached peak concentrations on days 7 and 8, and thereafter fluctuated with considerable inter-animal variation. Infection with either *T. vivax* or *T. congolense* was followed in one week by the development of macrocytic, normochromic anemia, leukopenia, and persistent thrombocytopenia. There appear to be significant differences in the response of cattle to the two infections.

ACKNOWLEDGEMENTS

We are grateful to the Director of EAVRO for permission to publish the paper. We thank the technical staff members of the Division of Pathology and Protozoology for assistance and Dr. A. E. Sollod of the University of Nairobi for *T. theileri* culture. We are also grateful to the Director of the East African Trypanosomiasis Research Organization for the stabilates used. This research was supported by the East African Community and the International Development Research Center (Canada) but the opinions presented herein are not necessarily those of the Center.

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THE EFFECT OF TREATMENT ON THE ANAEMIA OF AFRICAN TRYPANOSOMIASIS

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INTRODUCTION

The aetiology of the anaemia associated with trypanosomiasis continues to remain an area of considerable controversy. This in many respects is remarkable because anaemia has long been recognised as an outstanding symptom of the disease in both man and animals and therefore the subject of considerable interest for many years. Much of the controversy has probably been engendered by the use of abnormal experimental hosts, which tend to show disease syndromes somewhat atypical of those encountered in more usual hosts such as cattle. During the past two decades there has been only scattered reference to experimental studies in cattle (Fiennes, 1954; Naylor, 1971), and few attempts to correlate these findings with those obtained in small laboratory animals. Furthermore conventional techniques can only provide static information of the nature of the anaemia associated with trypanosomiasis. It was only the recent application of isotopic tracer techniques to study the kinetic aspects of the anaemia associated with murine trypanosomiasis (Jennings *et al.*, 1974) and bovine trypanosomiasis (Mamo and Holmes, 1975; Holmes and Mamo, 1975) which provided direct evidence of the rate of red cell production and removal and the role of haemodilution in causing the development of the anaemia. Recent studies in cattle (Holmes and Mamo, 1975) experimentally infected with *T. congolense* indicated that the severe anaemia which developed was initially due to haemodilution with an accelerating red cell loss eventually taking over as the major factor causing the progressive fall in the haematocrit. Erythropoiesis in all cases was elevated to about three times the normal level, though this was insufficient to prevent the continuing fall in red cell concentration.

In order to investigate these phenomena further we undertook a series of experiments using radioisotopic techniques to measure the erythrokinetic response of rabbits and cattle following infection with trypanosomes and subsequently treated with a trypanocidal drug.

Most evidence to date suggests that the anaemia is basi-

cally haemolytic in origin and is accompanied by erythrophagocytosis especially in the spleen (MacKenzie and Cruickshank, 1973; Jennings *et al.*, 1974; Murray *et al.*, 1974). The majority of workers have postulated that the underlying mechanism causing increased red cell removal from the circulation, and eventual haemolysis, is immunological (Ingram and Soltys, 1960; Woodruff, 1973) although earlier work by Fiennes (1950) suggested that a haemolysin may be produced by trypanosomes. In the present studies it was hoped that, in addition to providing direct information on haematological parameters, the rate of recovery following treatment might give evidence on the role of living trypanosomes *in vivo* on the aetiology of the anaemia.

In one 7 week study, weekly measurements of red cell volume and plasma volume were calculated by the dilution technique from injections of ^{51}Cr labelled homologous red cells and ^{59}Fe labelled rabbit transferrin respectively, whilst the level of red cell production was assessed from the rate of disappearance of ^{59}Fe from the plasma during three hours post injection. Three groups each of four rabbits were used in this experiment. One group was infected with *T. congolense*, another with *T. brucei*, whilst the remaining group were uninfected and served as controls.

In other experiments red cell survival was measured by the rate at which ^{51}Cr and di-isopropyl (^{32}P) phosphorofluoridate ($DF^{32}P$)-labelled red cells disappeared from the circulation of rabbits infected with *T. congolense* and normal controls.

All samples were counted on a gamma scintillation counter*, with the exception of the $DF^{32}P$ radioactivity which was measured in a M6 liquid counter tube†. The animals were treated once during each experiment between three to five weeks post-infection by intramuscular injection of diminazine aceturate‡ at a dose of 25mg per kilogram of body weight. Both infected and control animals were treated at the same time in each experiment.

A pilot study in eight male yearling Zebu cattle, four of which had been experimentally infected with *T. congolense* from a recently isolated field strain nineteen days previously, was also undertaken using ^{51}Cr-labelled red cells. At 27 days post-infection the cattle were treated with diminazine aceturate at a dose rate of 5 mg/kg body weight.

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* Nuclear Chicago Corporation, Illinois.
† 20th Century Electronics Ltd., Croydon, Surrey.
‡ Berenil, Hoechst Pharmaceuticals Ltd.

RESULTS

Haematological Indices

The haematological indices of rabbits infected with *T. congolense* and *T. brucei*, together with control animals are shown in Figure 1.

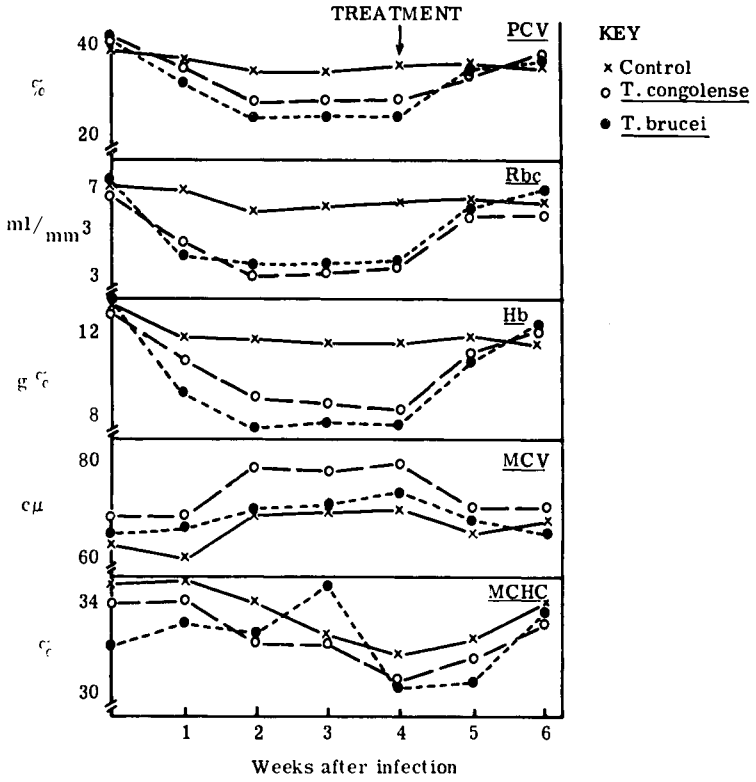

Fig. 1. The haematological changes in rabbits infected with trypanosomes and subsequently treated.

As a result of the infection a progressive fall in PCV, RBC and haemoglobin occurred during the first two weeks, and these values remained depressed until treatment. There was a rise in the MCV in the rabbits infected with *T. congolense* during the first two weeks and it remained elevated until treatment. After therapy there was a rapid return to normal values of all the indices, apart from the MCHC which had remained relatively constant throughout the experiment.

Changes in Circulating Red Cell Volume and Plasma Volume

The effects of trypanosome infection on red cell volume and plasma volume in rabbits are shown in Figure 2.

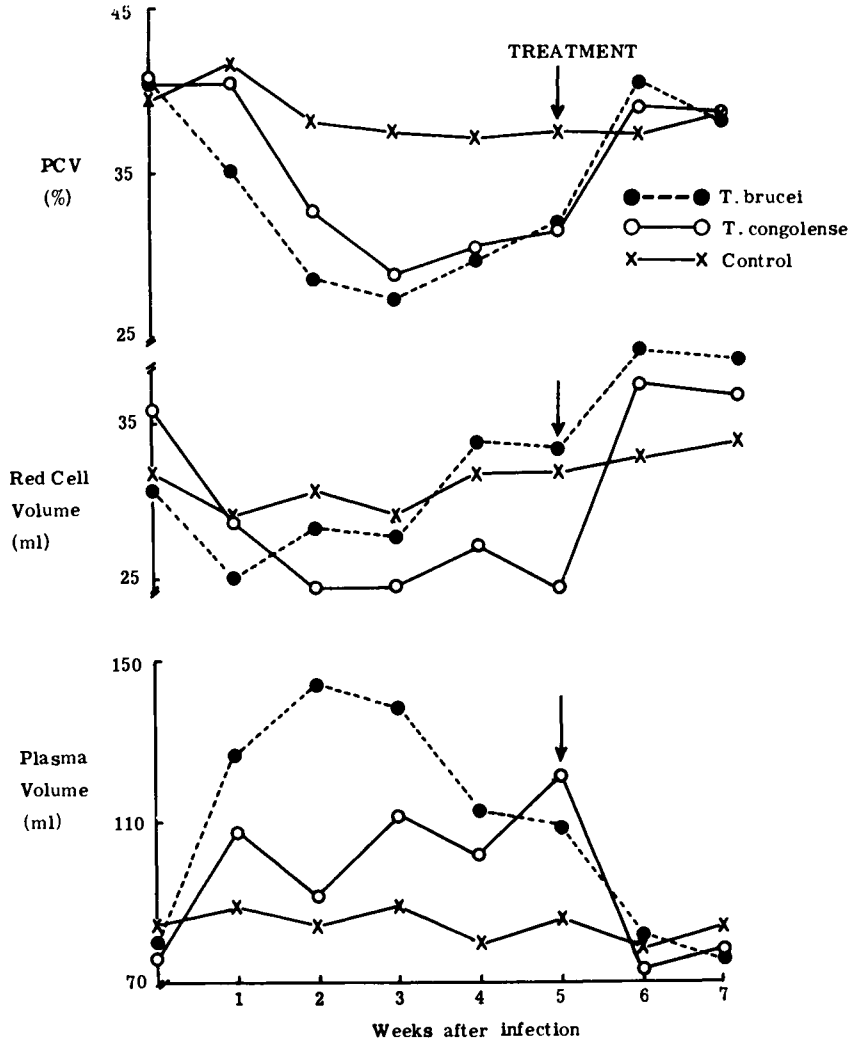

Fig. 2. Changes in PCV, Red Cell Volume and Plasma Volume in rabbits following infection with trypanosomes and subsequently treated.

The results clearly show that a marked haemodilution occurs in both infections, although this was most pronounced in the rabbits infected with *T. brucei*. Furthermore it is apparent that in these animals there was a particularly close

correlation between the fall in PCV and the increase in plasma volume.

The change in circulating red cell volume showed differences between the two infections. In the case of rabbits infected with *T. congolense* a marked decrease was found during the first two weeks of the infection, yet the rabbits infected with *T. brucei* showed no significant alteration. After three weeks post-infection an increase in the circulating red cell volume of both groups of rabbits was detected. The correlation between PCV and red cell volume was especially noticeable in the rabbits infected with *T. congolense*.

The effect of treatment on both parameters was very dramatic in all the infected animals. Within one week of therapy the plasma volume had fallen to control values, whilst the circulating red cell volume had risen temporarily to above normal levels.

Changes in Erythropoiesis

When ^{59}Fe transferrin was injected intravenously into the infected rabbits it disappeared at a progressively faster rate than from uninfected control rabbits. The increased rate of removal from the plasma and incorporation in red cells was reflected in alterations in the plasma iron turnover rate (Fig. 3).

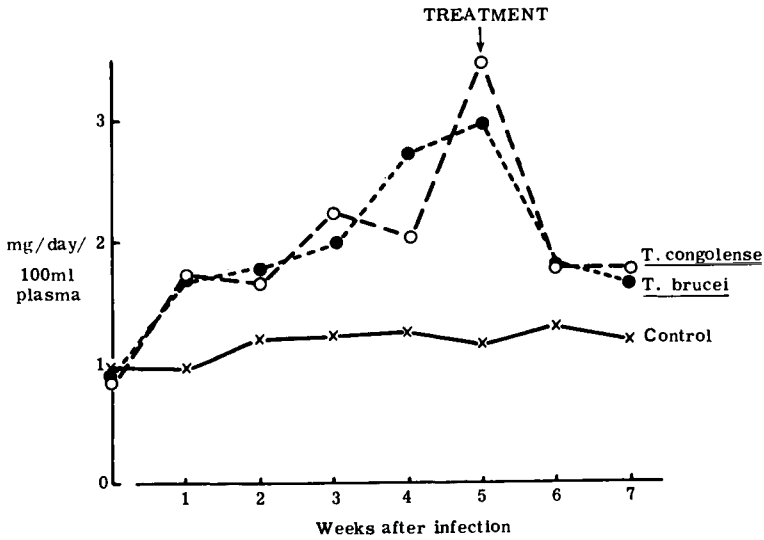

Fig. 3. Changes in the Plasma Iron turnover rate in rabbits following infection with trypanosomes and subsequently treated.

The increasing level of erythropoiesis was especially marked after two weeks post-infection, and was correlated with the increase in PCV found in both infected groups from week three onwards until treatment (Fig. 2). With treatment there was a very pronounced fall in erythropoiesis to near control values, although the fall in red cell production did not prevent the temporary rise in circulating red cell volume and PCV illustrated in Figures 2 and 5.

Red Cell Survival Studies

The simultaneous use of both ^{51}Cr-labelled red cells and DF^{32}P-labelled red cells in rabbits infected with *T. congolense* showed the fall in circulating red cell volume (Fig 2) was closely associated with an increased rate of disappearance of labelled red cells from the circulation of infected animals (Fig. 4).

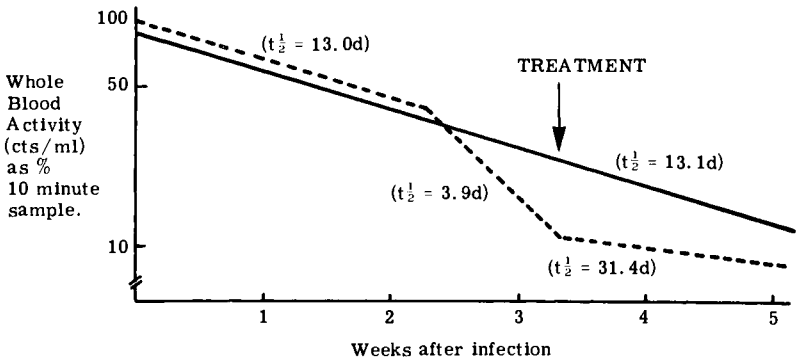

Fig. 4. DF^{32}P-Red Cell disappearance in a rabbit following infection with T. congolense *and subsequently treated, and in a normal rabbit (solid line).*

Furthermore the beginning of the increased loss was closely correlated with the first detection of parasites in peripheral blood samples, and the fall in the haematocrit. Equally significantly, the rate of red cell removal from the circulation was dramatically reduced following treatment (Fig. 4) as shown by an increase in the red cell half-life (Table 1).

TABLE I

^{51}Cr- and $DF^{32}P$-labelled Red Cell Disappearance Rates in Normal Rabbits and Rabbits Infected with T. congolense *and subsequently treated*

Rabbits	Phase	Half-life (days)†	
		^{51}Cr	$DF^{32}P$
Infected	Pre-parasitaemic	11.2 ± 3.5 (10)*	14.7 ± 4.4 (5)
	Parasitaemic	5.4 ± 3.1 (10)	7.1 ± 2.8 (5)
	Post-treatment	7.9 ± 1.3 (10)	22.2 ±14.0 (5)
Control		11.0 ± 1.5 (11)	12.9 ± 1.3 (7)

† mean ± SD
*( ) number of animals

Another method of examining the red cell loss is to monitor the ^{51}Cr radioactivity excreted in the urine since when ^{51}Cr is released from the labelled red cells either by elution or haemolysis it is not re-incorporated into new red cells but is excreted in the urine. When daily total urine collections were used to obtain a daily urine 'blood' clearance (by relating urine activity to blood activity in terms of counts per ml of blood at the beginning of each 24-hour urine collection period) it was found that as the rabbits became anaemic and parasitaemic there was a fall in the red cell activity of the blood, and a corresponding rise in the activity of urine (Fig. 5). Furthermore following the removal of the parasites by treatment a similar but opposite sequence of events was observed (Fig. 5), with a rise in the haematocrit and red cell survival time and a reduction in the daily urine 'blood' clearances.

A preliminary treatment experiment in Zebu yearlings experimentally infected with *T. congolense* using ^{51}Cr-labelled red cells showed similar changes to those described in rabbits (Fig. 6), although in this case total daily urine collections could not be obtained.

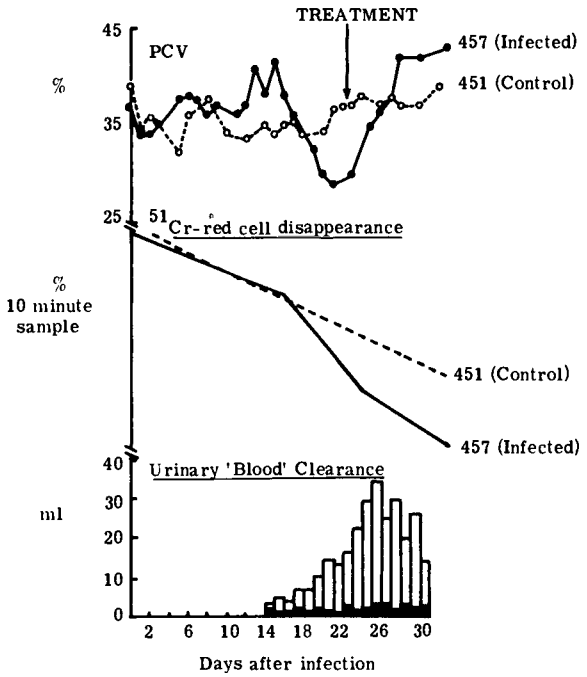

Fig. 5. Changes in haematocrit, ^{51}Cr-red cell activity and urinary excretion of ^{51}Cr in a normal rabbit and in a rabbit infected with T. congolense *and subsequently treated.*

The yearlings had been infected nineteen days prior to the injection of ^{51}Cr-labelled red cells and were showing a well-developed anaemia, which was found to be associated with an increased red cell loss. Again, following therapy a marked increase in the haematocrit occurred and this was associated with an abrupt reduction in red cell loss.

DISCUSSION

The changes in erythrokinetics observed in rabbits infected with *T. congolense* were essentially similar to those previously described in cattle (Holmes and Mamo, 1975), namely that haemodilution played a significant part in the initial development of the anaemia, along with a progressive increase in the rate of red cell removal from the circulation. Furthermore, erythropoiesis was in no way impaired, rather the reverse, it was elevated at least three fold in an apparent attempt to maintain circulating red cell levels in the face of increased removal of erythrocytes. The present results

also confirm the finding of Jennings *et al.* (1974) that the anaemia is essentially haemolytic in origin.

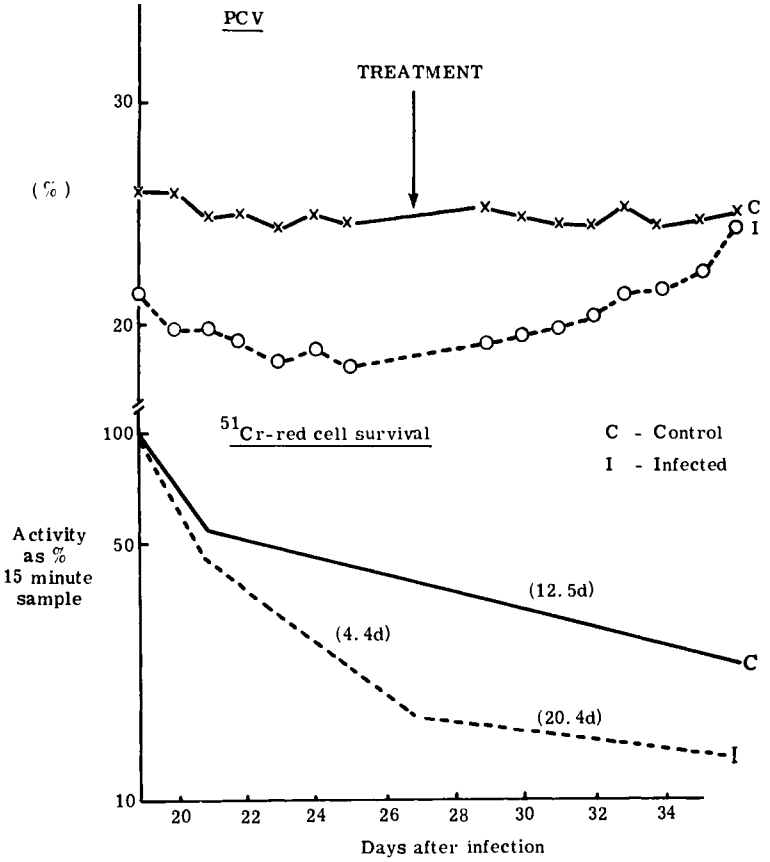

Fig. 6. Changes in haematocrit and ^{51}Cr red cell survival in zebu calves experimentally infected with T. congolense *and subsequently treated.*

The alterations in the haematological indices observed during the course of the infections are consistent with the findings of previous workers using rabbits infected with *T. brucei* (Boreham and Goodwin, 1966; Jenkins *et al*, 1974) in which falls in haematocrit, erythrocyte counts and haemoglobin concentration were described. There was no significant difference in the haematological changes between the two infections in the present studies, with the possible exception of the MCV values which were elevated in the rabbits infected with *T. congolense* but not in those infected with *T. brucei*.

The effects of treatment with a trypanocidal drug on the anaemia were dramatic, and confirmed the findings that in acute infections haematological parameters generally recover rapidly (authors' unpublished observations).

The changes in the plasma volume and circulating red cell volume were particularly striking and in essence support the findings of previous workers that haemodilution plays a significant part in the aetiology of the anaemia of rabbits infected with *T. brucei* (Boreham and Goodwin, 1966) and ruminants infected with *T. congolense* and *T. vivax* (Clarkson, 1968; Naylor, 1971; Holmes and Mamo, 1975). Some differences however were detected between the infections. The plasma volume was markedly elevated in the rabbits infected with *T. brucei* whilst the circulating red cell volume showed no significant reduction. On the other hand, in the rabbits infected with *T. congolense* the rise in plasma volume, though significant, was less marked though the circulating red cell volume showed a pronounced fall.

It was suggested by Boreham and Goodwin (1967) that false high results for plasma volume estimations may be obtained in trypanosome infected animals because of increased vascular permeability associated with increased kinin concentrations in the tissues. In order to investigate this possibility ^{125}I-rabbit albumin was injected into both normal rabbits and animals with four week infections of either *T. brucei* or *T. congolense*, and an estimation of vascular permeability obtained from the rate of disappearance of the labelled protein from the circulation over a three hour period (Wraight, 1974). In no cases was it possible to demonstrate any significant difference between the normal and infected rabbits (authors' unpublished observations). Rather it seems that the increased plasma volume is associated with increased peripheral vasodilatation.

The rapid reduction in plasma volume and the increase in circulating red cell volume following treatment indicates that the living trypanosomes had a direct effect on these parameters.

Similarly the marked elevation in erythropoiesis observed in all the infected rabbits was found to be dramatically reduced following treatment. Several earlier reports have indicated that increased erythropoiesis is associated with trypanosome infections of rabbits (Boreham and Goodwin, 1966) rats and mice (Jennings *et al.*, 1974) and cattle (Holmes and Mamo, 1975). Others have suggested that a depression of erythropoiesis may be an important factor in causing the anaemia associated with trypanosomiasis of cattle (Fiennes, 1951; Naylor, 1971) though the latter workers were basing

their conclusion on the absence of reticulocytes. However these cells have been shown to be present in cattle only in conditions of extreme anaemic stress (Schnappauf *et al.*, 1967).

The demonstration, using ^{51}Cr- and DF^{32}P-labelled red cells, that an increased rate of red removal from the circulation was closely associated with the development of the anaemia, parasitaemia and increased urinary excretion of ^{51}Cr, provides corroborative evidence that the anaemia is essentially due to haemolysis (Naylor, 1971; Jennings *et al.*, 1974). However the exact mechanism initiating red cell removal from the circulation remains obscure. There is considerable evidence of immune mechanisms operating in trypanosome infected animals, which may predispose the erythrocytes to phagocytosis (Zoutendyk and Gear, 1951; Ingram and Soltys, 1960; Woodruff, 1973; Woo and Kobayashi, 1975), although their role in the intact animal has yet to be convincingly demonstrated.

In contrast the close correlation of changes in haematological indices, circulating red cell volume, plasma volume, 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 in the present studies clearly supports the view that the anaemia is not immune in origin, but is the result of damage caused either directly by living trypanosomes or substances released by them. The latter suggestion, which was originally postulated by Fiennes (1950) has recently received considerable support from Nguyen Huan *et al* (1975) who have reported the characterization and purification of haemolytic factor produced *in vitro* by *T. brucei*.

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FEVER: A CRITICAL FACTOR IN THE PATHOGENESIS OF EAST COAST FEVER (*THEILERIA PARVA* INFECTION) IN CATTLE

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INTRODUCTION

Fever is a pathogonomic syndrome of East Coast Fever (ECF) a rapidly fatal protozoan infection of cattle caused by *Theileria parva*. The factors responsible for fever in ECF are unclear, but they may be due to one or more of the reactions associated with septicaemia, toxaemia and hyperthermia, which occur in infected animals.

Previous work on ECF by Cowdry and Danks (1933), de Kock (1957) and Barnett (1960) indicated that the pathogenesis of ECF is mainly due to hyperplasia, depletion and massive cellular degeneration and necrosis of primary lymphoid tissues and aggregation of lymphoid cells in non-lymphoid areas. Also it has been suggested by these and other workers that autoimmunity, toxin production by schizonts, toxic substances derived from degenerating cells, and the physiological loss of large numbers of lymphocytes may contribute to the pathogenesis of ECF. In all, the pathogenesis of the disease is not clearly understood and the significance of fever as a critical factor in the pathogenesis of the disease has yet to be evaluated. The present study evaluated the effects of fever as a pathophysiological factor in the course and pathogenesis of ECF.

MATERIALS AND METHODS

Animals: 54 *Bos taurus*, mostly Friesians and 49 *Bos indicus*, mostly Boran crosses and a few Zebu cattle were used. They were infected with isolates of *T. parva* from Uganda or with *T. parva* (Muguga) a laboratory strain of the organism. The Ugandan *Theileria* infection was induced in 76 cattle by feeding infective ticks of the species *Rhipicephalus appendiculatus* on them and into 20 cattle by inoculating infective blood while 7 cattle were inoculated subcutaneously with *T. parva* (Muguga) stabilates prepared by the East African Veterinary Research Organization (EAVRO), Muguga. Clean *R. appendiculatus* ticks were fed on 34 clean control cattle (10 *Bos indicus*, and 24 *Bos taurus*), during the experiments.

Temperature: Temperatures were taken twice daily, in the morning between 9:00 and 10:00 a.m. and in the afternood between 2:00 and 3:00 p.m. In critically ill animals, the temperatures were taken a third time at 6:00 p.m.

Necropsy: Post-mortem examinations were conducted on 56 cattle, 19 *Bos indicus* and 37 *Bos taurus*.

Weather: Minimum and maximum indoor and outdoor temperatures and rainfall were recorded throughout the study.

Statistical analyses: Analyses were made on the temperature differences between the experimental animals using the Chi-squared test (χ^2) and the Students' t Test. Comparison of temperatures were made between the daily temperatures of a group of infected animals which died or recovered, on one hand, and those of non-infected control group of animals, on the other hand; and also between those infected animals which died and those which recovered.

RESULTS

Figure 1 presents the mean daily temperatures of animals exposed to clean (non-infected) *R. appendiculatus* ticks and animals variously infected with *T. parva* and which either died or recovered from the infection.

NORMAL ANIMALS EXPOSED TO NON-INFECTED TICKS. The feeding of clean (non-infective) *R. appendiculatus*, on 34 control animals caused no significant rise in the temperatures of these animals. Elevated temperatures occurred intermittently in a few animals (on 5 occasions) during the 147 days of observations.

Subnormal temperatures (less than 37.6°C), occurred on 7 occasions in some of these animals. These were associated with periods of cold and wet days (3 to 5 days) with little or no sunshine.

ANIMALS INFECTED WITH EAST COAST FEVER

Species of Cattle. A significant difference at the 1% level ($t = 4.57$) was evident between temperatures of *Bos taurus* and *Bos indicus* which died of ECF, but there was no significant difference in the temperatures of animals of the two species which survived the infection.

Temperature Differences: The temperatures of 103 infected

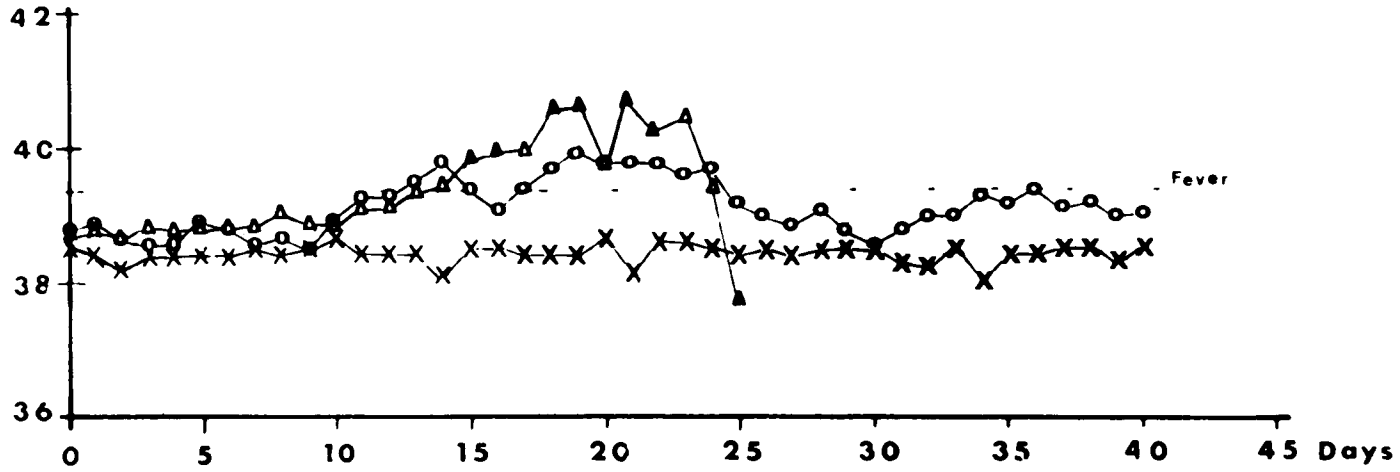

Fig. 1. Average mean daily temperatures of animals exposed to clean (non-infected) ticks (Crosses), animals infected with T. parva *and which died (triangles) (solid symbols represent a death) and animals infected with* T. parva *and which survived (circles).*

cattle showed very significant variations from the control animals either before death or at recovery. Thus the 56 animals which died of ECF between 19 to 40 days after infection showed temperature rises up to 42°C. Continuous, unabated, fever (39.4°C or higher) occurred in all these animals for 3 to 12 days and this was followed by a fall to subnormal temperatures which occurred one to 5 days before death.

Diurnal temperature variations were minimal (about 0.5°C) during periods of fever, regardless of whether animals ultimately survived or died. However, in animals which recovered by 45 days after infection, a diurnal temperature variation of 1.5° to 3.0°C was seen for 182 days. The temperature variation was most marked below 39.3°C and was associated with marked diurnal changes in weather.

The mean temperature difference between cases of ECF which were fatal or recovered was very highly significant ($P<0.001$) especially with a temperature range below 39.3°C. At temperatures between 40.6°C to 41.1°C differences were significant at the 5% level. Variations in the morning and afternoon temperatures of control and infected recovered animals were evident and were very highly significant especially between temperatures 37.6° to 39.3°C. At temperatures between 38.2° and 39.3°C a very highly significant difference was evident between the infected animals which died and those which recovered from ECF.

Clinical Signs: The common clinical signs which occurred in all animals but varied in severity were; fever, lacrimation, nasal discharges, swelling of the superficial lymph nodes, oedema of the eye-lids, keratitis, anorexia, constipation followed by diarrhea, grinding of teeth, rapid loss of condition, lethargy, weakness and prostration.

The majority of the animals which died had acute to peracute reactions, characterised by high, continuous fever, purulent nasal discharge, constipation followed by bloody diarrhea, frothing and laboured breathing and prostration which occurred terminally. Animals which recovered showed fever and clinical reactions of a less severe and prolonged nature. Early onset of diarrhea, watery and purulent nasal discharges and lacrimation were more common in animals which recovered than in the animals which died.

Haemotological Changes: No significant changes were observed in packed cell volumes, red cell counts, haemoglubin levels or mean corpuscular haemoglobin concentrations of infected animals compared with non-infected animals.

The total leucocyte counts decreased from 12,000 to 8,100 cells/mm^3, followed by a very sharp rise to about 20,000 cells/mm^3 before death in some animals. The platelet counts remained normal (between 150,000 and 670,000 cells/mm^3) but there was an increase in the number of giant platelets in the infected animals.

Differential leucocyte counts showed a general fall in the number of lymphocytes, fluctuating between 1,000 and 3,000 cells/mm^3. The neutrophil and eosinophil counts were little affected by the infection. Monocyte counts in the non-infected animals were from 200 to 800 cells/mm^3: in infected animals monocytes counts were 450 to 5,000 cells/mm^3, and the number of "active" monocytes varied between 50 to 400 cells/mm^3, none being present in non-infected animals.

Parasitaemia: An occasional parasitaemia of 20%, for up to a week, was seen in individual animals, but in the majority of cases parasitaemia was 1 to 5%, even in fatal cases.

Necropsy Finding: The majority of animals were in poor to very poor conditions and general emaciation of the carcasses was evident. Vascular congestion, gelatinous appearance of the fatty tissues, sero-fibrinous haemorrhages on the omenta and surfaces of the internal organs, prominent mesenteric lymph nodes and haemolymph nodes and congested mesenteric blood vessels, distended gall-bladder and urinary bladder were the common necropsy findings.

Variable degrees of pneumonia occurred and the lungs were often congested and oedema occurred at the interlobular septa. Froth occurred in the lungs of 50% of the cases examined, and it extended along the larynx, trachea and the bronchial tree of the lungs. Sero-fibrinous haemorrhages occurred on the parietal surfaces of the lungs, the base of the heart, along the coronary grooves and at the apex and the pericardial sac of the heart. Oedema of the gastrointestinal tract and gastroenteritis associated with lymphoid areas of the gut were constant features. Vascular congestion and oedema of the meninges and fatty tissues around the blood vessels were common in the central nervous system.

DISCUSSION

Fever, unlike hyperthermia, is a clinical syndrome indicating a pathological change of conditions in the body. It is associated with various infectious diseases, examples of which in the case of parasitic infections include malaria East Coast Fever, anaplasmosis and babesiasis.

In the present study, it was demonstrated that fever which occurred in the *T. parva* infected animals was not due to a rise in environmental temperature or to the feeding of ticks on to the animals. Hence, the febrile condition was due to the infection with the parasite. It is not yet known how fever is produced in theilerial infections.

In other infectious diseases, it is known that effects on the hypothalamus (Ruch and Fulton, 1960), increased tissue metabolism (Baldwin, 1963) and massive cellular degeneration and toxaemia (du Bois, 1927) individually or collectively, produce a marked rise in body temperatures.

In most cases, fever is usually due to the result of some derangement in the mechanism of heat dissipation leading to excessive storage of heat in the body (du Bois, 1927; Ruch and Fulton, 1960; Blood and Henderson, 1971). The effects of fever in the metabolism of body tissues have been well documented (du Bois, 1927; Best and Taylor, 1950; Sumner and Somers, 1953; Dixion and Webb, 1958; Smith and Jones, 1961; Putnam, 1963,). The animal body responds to changes in temperature according to Van't Hoffs law (du Bois, 1927). Enzyme-catalysed body reactions proceed according to this law up to the optimun temperature of between 30° and 50°C.

The earliest effects of fever in the body is the increase in the basal metabolic rate. In fever due to infections such as malaria, pneumonia and typhoid in man (du Bois, 1927) the sugar content of the body fell markedly and metabolism of proteins was elevated by 20 to 50% above normal. Degeneration of parenchymatous organs and body tissues susceptible to heat occurred in affected patients (du Bois, 1927). Based on these studies, a temperature due to acute ECF of between 41.5° to 42.0°C, which is an increase of 3° to 3.5°C above normal would increase the metabolic rate by 40 to 46%. Necropsy findings in carcasses of cattle which have died of ECF (de Kock, 1957; Neitz, 1957; Oteng, 1969 & 1973), strongly suggest a marked increase in body metabolism. (de Kock, 1957; Neitz, 1957; Oteng, 1969).

Fever is associated also with an increase in the number of phagocytic and lymphoid cells in the circulation (Best and Taylor, 1950; Smith and Jones, 1961; Putnam, 1963). The differential leucocyte counts in the ECF infected animals in this study showed an increase in the "active" monocytes in the peripheral blood, which would indicate increased phagocytosis.

Observations in man and other animals have shown that moderate fever induces rapid cell replacement, phagocytosis, antibody formation and an increased level of cellular degeneration (Best and Taylor, 1950; Smith and Jones, 1961;

Putnam, 1963). Similar changes were recorded by Cowdry and Danks (1933) Barnett (1960) and de Kock (1957) in ECF.

Water, salt, protein and energy reserves in the body are usually affected by fever. Thus, the sugar level falls within a few hours (du Bois, 1927) and thereafter much of the body energy is obtained by oxidation of tissue fat and proteins (Baldwin, 1963). Depletion of fatty tissues leads to rapid wasting and replacement of fatty tissues with gelatenous material (Baldwin, 1963). Consequences of this are evident in infection, in that anasarca, oedema of dependent parts of the body and of the visceral and pericardial fat are common.

The effects of fever on the cardiovascular and respiratory systems have been discussed by Best and Taylor (1950), Ruch and Fulton (1960) Smith and Jones (1961) and Blood and Henderson (1971). In general, there is a loss of the integrity of the systems leading to a generalized failure in the functions of these systems. Circulatory failure is characterized by generalized vascular congestion and haemorrhages in the visceral organs, oedema and an increase in the fluid of the pleural and visceral cavities. In ECF infection widespread internal haemorrhages, generalized vascular congestion of the visceral organs and brain, hydrothorax and ascities have been recorded (Henning, 1956; Neitz, 1957; de Kock, 1957; Oteng, 1969 & 1973). These findings suggest that the cardiovascular manifestations of ECF are related to the fever produced by the infection.

The respiratory system is also affected by fever. The lungs of animals with high fever (up to 42°C) which died of acute and peracute ECF were highly congested and oedematous with froth extending throughout the bronchial tree. The rapid collapse of the respiratory system could be attributed to the rapid collapse of the respiratory system could be attributed to the rapid circulatory failure associated with persistent fever (Ruch and Fulton, 1960; Blood and Henderson, 1971) in ECF.

It is known that high and persistent fever leads to rapid derangement of the central nervous system, and the most readily damaged part of the brain is the hypothalamus which, among other of its functions, is the "physiologic thermostat" of the body (Ruch and Fulton, 1960; Blood and Henderson, 1971). Damage to the thermo-regulatory centre in the hypothalamus usually leads to lack of control of normal body temperatures and affected animals tended to become poikilothermic.

In ECF, there is evidence that damage occurred in the brain of cattle and this affected the thermo-regulatory

mechanism in the hypothalamus. In the animals which died of acute and peracute ECF, death was heralded by a marked fall of body temperatures to subnormal levels within a few days.

The body temperatures of the animals which recovered fluctuated greatly and these were associated with changes of weather, especially at a temperature range between 38.2° to 39.3°C. The diurnal variations of 1.5° to 3°C in such animals were statistically significant and persisted for at least 220 days after recovery from infection. This suggests, that the effects of fever in ECF, led to damage of the hypothalamus.

SUMMARY AND CONCLUSIONS

Fever in ECF appears to play a critical and significant role in the course and pathogenesis of the infection. It may be largely responsible for death in acute and peracute cases of the disease. The effects of high and persistent fever consist of increased metabolism and later degeneration of tissue cells, anascarca due to rapid metabolism of fatty tissues, increased heat retention by the body, probably due to damage of the thermoregulatory mechanism of the body and failure of the circulatory and respiratory systems.

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PATHOPHYSIOLOGY OF THE IMMUNE RESPONSE TO PARASITES*

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INTRODUCTION

Resistance to parasites in general is either innate (natural) or acquired. The fact, that more than 99% of protozoa parasitic for various hosts are not pathogenic for man, underlines the importance of natural resistance (Cohen, 1974), but recent developments have also stressed the importance of acquired immunity in parasitic infections (Ogilvie, 1974). The immune response, a biological (physiological) adaptive mechanism of host defense against a parasite can lead to different levels of immunity in the host. Unfortunately, it can also induce undesirable reactions causing harm to the host; these are regarded as pathophysiological or more appropriately, immunopathological reactions (hypersensitivities, in the terminology of Coombs and Gell, 1975).

The purpose of this paper is to review the mechanisms involved in immunopathology of parasitic diseases, e.g. situations in which immunological reactions play an important role in the pathogenesis of lesions associated with parasitic infections. It will not be a comprehensive account of all of them but rather a discussion of some typical examples, such as immune complexes and complement, autoimmunity, cell mediated granuloma formation and immunosuppression. Those immunological reactions which may not be regarded as physiological, but for which we do not have evidence of their immunopathological effect (increased levels of IgM in trypanosomiasis, big spleen disease believed to be associated with malaria, defects of native resistance and/or specific immune responses, escape mechanisms of the parasites from immune responses, genetic influences etc.) have not been included.

IMMUNE COMPLEXES

The formation of immune complexes by reaction between antigens and antibodies represents one of the pathogenic

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mechanisms which has been quite well established in parasitic infections, such as malaria, trypanosomiasis and schistosomiasis. The antigens may be released from whole organisms (living or dying, especially during treatment), at different stages of their life-cycle as well as from their products, for example from eggs in schistosomiasis. The immune complexes may, therefore, be formed at the site of parasite penetration (if antibodies are already present in circulation), in circulation and/or in extravascular spaces.

The fate of circulating immune complexes depends on their size, composition and on the activity of the macrophages and reticuloendothelial system. Small complexes may persist in circulation for some time, whereas larger complexes are quickly removed from circulation. When the reticuloendothelial system is saturated (or blocked in experimental conditions) and when macrophages (and other phagocytic cells) fail to remove complexes of larger size, these too may circulate for longer time (Mannik *et al.*, 1974). Circulating complexes may localise in filtering membranes of target organs, such as vascular basement membranes, glomerular basement membranes, basement membranes of the chorioid plexus etc. and cause tissue damage. However, one should not consider the circulation of immune complexes and their localization in target organs (such as in kidney) as a prerequisite for their involvement in the pathogenesis of the disease. Several other factors may play an important role in the persistance of circulating complexes, their deposition and perpetuation of the immunopathogenic lesions, such as affinity of antibodies involved, complement activation and others. Soluble antigens and/or immune complexes circulating in the blood have been demonstrated in malaria, trypanosomiasis and schistosomiasis (McGregor *et al.*, 1968; Wilson *et al.*, 1969; Williams and Houba, 1972; Houba and Williams, 1972; Houba and Lambert, 1974; Lambert and Houba, 1974; Nash *et al.*, 1974; de Raad, 1974). Recently, we have been able to demonstrate the *in vitro* binding of IgG containing specific antibodies to *T. parva*, into complexed form when incubated with sera from cattle infected with *T. parva*, indicating the presence of soluble antigens in the serum (Houba and Wagner, unpublished observation).

Malaria represents one of the best studied models for immune complex type of renal lesions. In acute infection, such as *P. cynomolgi* and *P. berghei* in mice and *P. falciparum* in man (Ward and Conran, 1969; Ehrich and Voller, 1972; Boonpucknavig *et al.*, 1972; Bhamarapravati *et al.*, 1973) the deposition of immune complexes (e.g. immunoglobulins, antigen and C3 component of complement) in glomerular capillary

walls has been detected as early as 6 to 9 days after infection. After treatment with antimalarials (when the supply of antigens has been stopped) the clinical symptoms and proteinuria disappear and the renal functions normalize in several weeks. In chronic infections, such as *P. brazilianum* in Aotus monkeys and *P. malariae* in man (Voller *et al.*, 1971; Houba *et al.*, 1971; Lambert and Houba, 1974; Houba *et al.*, 1975) the renal lesions develop later and progress into a chronic stage. These lesions do not respond to antimalarials and their response to other therapy such as corticosteroids is rather poor (Houba *et al.*, 1971; Houba and Lambert, 1974). It seems that the beginning stage is due to the deposition of malarial complexes in glomerular capillary walls (antigens in the deposits can be detected during the initial period of 3 to 6 months only) whereas later other mechanisms (autoimmune ?) are responsible for the perpetuation and the chronic progress of the disease. Although these strains of *Plasmodia* used for detailed studies may not be relevant to veterinary problems, they represent quite good models for immune-complex type of lesions induced by similar organisms such as the *Babesia* spp., *Theileria parva*, etc.

The lesions in African trypanosomiasis are another example of immunopathology due to immune complexes, and perhaps even better one than in malaria as there is a disseminated vasculitis with lesions in different tissues. Glomerular deposits of immunoglobulins and complement were found in mice infected with *T. brucei* of bovine origin but the antigens were detected in about half of them (Lambert and Houba, 1974). Similarly deposits of immunoglobulins and antigens were found along and between the fibres of the myocardium of the heart and, in about 10% of infected mice, also in the choroid plexus and perivascular areas of the brain. Deposits of immunoglobulins and complement in glomeruli were also described in monkeys infected with *T. rhodesiense* (Nagle *et al.*, 1974).

In schistosomiasis (*S. mansoni*), the presence of glomerular lesions due to localized immune complexes was reported in hamsters (Berggren and Weller, 1969; Gold *et al.*, 1969), chimpanzees (von Lichtenberg *et al.*, 1971), mice (Natali and Cioli, 1974), baboons (Houba, Butterworth and Sturrock, to be published) and man (Silva *et al.*, 1970). However, the antigen(s) responsible for these lesions have not yet been identified.

COMPLEMENT

The significance of complement in parasitic infections has been known for a long time. In the past, the only known way

of complement activation was the so called classical pathway, in which C1, a complex of three different proteins, C1q, C1r and C1s, combines reversibly with immune-complexes containing IgM and IgG and induces sequential reaction of 9 components of complement (C1 to C9) leading to lysis of parasites, cells and/or damage of tissues. Recently, an alternative pathway of complement activation has been described which involves the properdin system and can be stimulated by other immunoglobulins (such as IgA) as well as by some products of bacteria, plants and fungi without participation of immunoglobulins (Müller-Eberhard, 1974).

The biological effects of both pathways are similar: the activation of intermediate products such as C3 and C5 generate low-molecular fragments of C3a and C5a which release vasoactive amines, increase vascular permeability and which are chemotactic for polymorphonuclear leucocytes. The further sequence to the final stages may or may not continue. The activation of complement is double edged sword. In some situations it may be part of the effective immune response: e.g. lysis of microorganisms or cells coated with antibodies, or removal of immune-complexes through complement receptors on phogocytes. In other situations it may be part of the pathophysiological reactions such as an anaphylactic reaction, a chemotactic effect with accumulation of leucocytes and release of their lysosomes which cause the damage to basement membranes in immune-complex lesions, etc. The significance of complement activation has been clarified in the pathogenesis of some immune complex type lesions in tissues (Dixon *et al.*, 1965) and in dengue hemorrhagic shock syndrome caused by B-group of arboviruses (Bokisch, 1974). In the parasitic infections the complement activation may be achieved by both pathways (Lambert and Houba, 1974) but its significance in immunopathology (apart from immune complex lesions in tissues) has not been established.

AUTOIMMUNITY

Increased prevalence of different auto-antibodies has been reported in subjects suffering from malaria and trypanosomiasis, such as antiglobulins of the Rheumatoid Factor type, anti-nuclear antibodies, anti-fibrinogen, anti-tissue antibodies and heterophile antibodies (Houba and Allison, 1966; Houba *et al.*, 1969; Seed and Gam, 1967; Greenwood *et al.*, 1970, 1971; Voller *et al.*, 1972; Mackenzie *et al.*, 1972; Houba *et al.*, 1974). The formation of antiglobulins seems to be due to the circulating immune complexes, as demonstrated in malaria (Greenwood *et al.*, 1971) whereas the anti-

nuclear antibodies may represent cross-reacting antibodies induced by deoxyribonucleic acids of parasitic origin (Voller, 1974; Lambert and Houba, 1974). The majority of these auto-antibodies just reflect the autoantigenic stimulation and/or breakdown of tolerance but have no significant pathogenetic effect. However, some of them may be responsible for pathological consequences, especially for anaemia. It has been repeatedly discussed that the degree of erythrocyte destruction in many malarial infections is disproportional to the parasitaemia and, therefore, the possibility that immunological mechanisms might play a part in haemolysis has been considered from several aspects, e.g. transient binding of immune-complexes to red cells (immune adherence), drug-dependent hypersensitivity and autoimmune haemolysis. Although IgM anti-erythrocyte antibodies have been described in *P. falciparum* infections and the presence of complement demonstrated on the surface of uninfected erythrocytes in rodents infected with *P. berghei* (ref. from Voller, 1974) definitive evidence for an autoimmune haemolytic mechanism is still missing.

In trypanosomiasis, the anaemia may be very severe and frequently is considered as the main pathogenic factor of mortality, especially in *T. congolense* and *T. vivax* infections (Goodwin, 1974). However, the immunological mechanisms by which it is initiated have not been clarified. The findings of immunoglobulins, complement, agglutinating and complement-fixing antibodies on red blood cells as well as the absorption of antigen to normal erythrocytes *in vitro* with subsequent lysis in presence of complement and antiserum are quite suggestive for a possible immunological sequence leading to haemolysis (Woodruff *et al.*, 1973; Herbert and Inglis, 1973; Murray, 1974). In addition, Lambert's (unpublished) observations of antiglobulin (Coombs) positive reactions in mice infected with *T. brucei*, confirmed in our laboratory on vervet monkeys infected with *T. brucei* (positive direct Coombs test in animals which developed severe anaemia) indicate the possibility of autoimmune involvement.

CELL MEDIATED REACTIONS

A typical example of cell mediated induced immunopathology reaction in parasitic diseases is the anti-egg response in tissues in schistosomiasis. A wide spectrum of antigens produced by *S. mansoni* has been reported. These include membrane antigens of schistosomula and adult worms, soluble fractions of adult worms, circulating antigens and egg antigens, but only some of them are related to immunopathological

lesions, e.g. egg antigens to granuloma formation and perhaps other antigens to deposition of immune complexes in kidney tissue. The mechanism of the host response to *S. mansoni* eggs in the tissue granuloma has been shown to be a cell mediated type of reaction, transferable passively by cells but not by serum, and markedly suppressed *in vivo* by neonatal thymectomy, anti lymphocyte serum and other measures which are considered to be particularly related to cellular immunity reactions (Warren *et al.*, 1967; Boros *et al.*, 1973; Warren, 1972). The cellular (granuloma) reaction is elicited not only by whole eggs but also by saline extract of eggs (soluble egg antigens) as demonstrated by bentonite particles coated with these soluble antigens and has been correlated with delayed type of hypersensitivity reactions (Boros and Warren, 1970).

During the chronic stages of schistosomal infection the formation of granulomas around eggs recently trapped in the tissue diminishes; this phenomenon, which is more or less related to the improvement in the hepatosplenic disease, seems to be a form of immunological depression (blockade) probably mediated by antibodies and/or immune complexes (Warren, 1974).

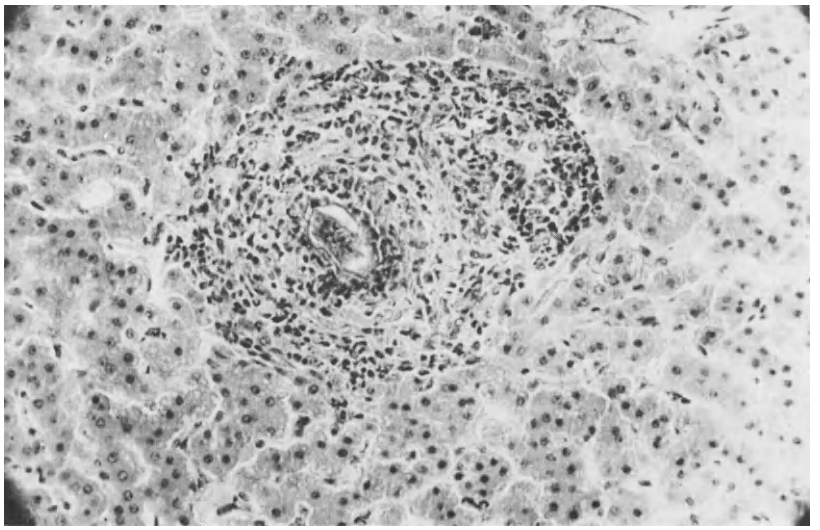

Fig. 1. Granuloma lesions around the egg in the liver of baboon infected with 1000 cercariae of S. mansoni *and killed 30 weeks after infection.*
Cellular infiltration around the egg in light microscopy (hematoxylin-eosin) (X 380.)

In recent investigations of the granuloma lesions formed around the eggs of *S. mansoni* in baboons (Fig. 1) we have found by direct immunofluorescence staining (Houba, Butterworth and Sturrock, prepared for publication) fine precipitates positive for IgM, C3 component of complement and soluble egg antigen(s), suggesting the formation of immune complexes within the granuloma. These immune complexes seem to be phagocytized by cells such as eosinophil and neutrophil leucocytes, macrophages and giant cells and macrophage origin present in granuloma lesions (especially in the peripheral part). We have demonstrated all three components of the complex (e.g. IgM, complement and soluble egg antigen) in these cells of the liver granuloma of baboons as soon as 6 to 7 weeks after infection and up to 8 months later. Interestingly enough, it was always IgM and not IgG which was present in the cells (Fig. 2) although in some of them a proportion of the cells had also IgE and the cells in similar lesions in the large bowel had also IgA. In older lesions, IgG was detected as a granular type of staining between the cells (Fig. 3).

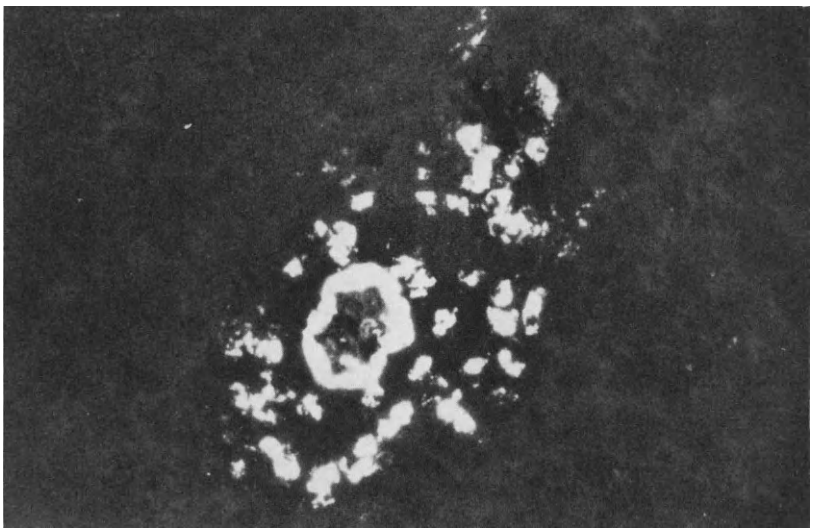

Fig. 2. Granuloma lesions around the egg in the liver of baboon infected with 1000 cercariae of S. mansoni *and killed 30 weeks after infection.*

Positive staining inside cells around the variable egg with anti-IgM conjugate in fluorescence microscopy. The fine granular staining is outside cells. The egg-shell in the centre has its own autofluorescence (X 480.)

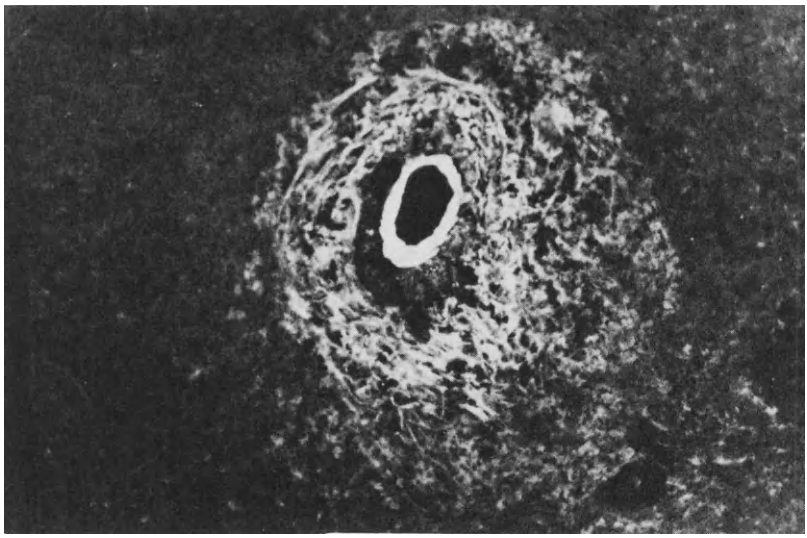

Fig. 3. Granuloma lesion around the egg in the liver of baboon infected with 1000 cercariae of S. mansoni *and killed 30 weeks after infection.*
Positive granular staining between cells with anti-IgG conjugate around the dead egg. Autofluorescence of the egg-shell in the centre (X 480.)

The significance of these findings is difficult to assess at present; obviously the presence of immune complexes within the granuloma may interfer with the cell mediated reactions (blockade ?) but as yet no evidence for this has been demonstrated.

IMMUNODEPRESSION

Immunodepression is an example of a modified immune response which, at least in some parasitic infection, should be considered as a pathophysiological mechanism. It is characterized by impaired reactions of humoral and/or cellular (or both) effectors of the immune response to antigenic stimulation in an otherwise normally developed immune competent system. Immunodepression can be achieved artificially by different means such as irradiation, immunosuppressive drugs and antilymphocyte sera and for obvious reasons has found practical application in transplantation and tumour immunology. However, in the context of this symposium, only those

situations in which immunodepression is due to the effect of parasitic infections on the immune system will be mentioned.

In malaria, the depression of immune response was demonstrated in experimental animals and man. In mice infected with *P. berghei* depressed antibody response to tetanus toxoid, aggregated human gamma-globulin, sheep red blood cells (Salaman *et al.*, 1969; Barker, 1971; Greenwood *et al.*, 1971; Voller *et al.*, 1972) as well as to Moloney lymphomagenic virus (Weddoburn, 1974) was found. Greenwood (1974) described a diminished antibody response to tetanus and *Salmonella typhi* vaccines in Nigerian children with acute *P. falciparum* infections. However, their cell mediated immune responses to purified protein derivative (PPD), *Candida*, streptococcal antigens (delayed type hypersensitivity) as well as response of lymphocytes to phytohaemagglutinin were normal as compared with non-infected controls. From results of other *in vivo* and *in vitro* experiments Greenwood (1974) concluded that immunodepression in malaria was related to the function of macrophages in processing antigens, but perhaps other mechanisms may be involved.

In trypanosomiasis, the immunodepression to different antigens has been reported in rabbits and rodents infected with *T. brucei* (Goodwin *et al.*, 1972; Longstaffe *et al.*, 1973; Murray *et al.*, 1973) as well as in human infections with *T. gambiense* (Greenwood *et al.*, 1973) and cattle with *T. congolense* (ref. from Murray, 1974). In contrast to malaria, trypanosomiasis diminishes responses to *Candida* and PPD antigens and, therefore, induces a more extensive immune defect, involving both humoral and cellular immunity. The state of immunodepression depends on the continuation of trypanosomal infection, since after treatment the immunological competence is rapidly restored (Murray, 1974).

Immunodepression has been described in other infections, such as in *Leishmania donovani* infected hamsters and their response to chicken ovalbumin (Clinton *et al.*, 1969), in *Toxoplasma gondii* infected mice and their response to infection with *P. berghei* (Strickland *et al.*, 1972) and in *Trichinella spiralis* infected mice in which a progressive reduction in thymus weight associated with the intestinal phase of the infection has been demonstrated. The pathophysiological significance of immunodepression is firstly related to the increased succeptibility of subjects with these diseases to secondary infections and, secondly, to depressed responses after vaccination. However, it is difficult to estimate how far the immunodepression in a certain disease contributes to the perpetuation and perhaps progression of the disease itself.

CONCLUSION

There is an increasing evidence that immunological phenomena play an important role in the pathogenesis of clinical manifestations of the parasitic diseases. Some typical examples of immunopathological mechanisms are discussed in this paper.

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ANTICOMPLEMENTARY SUBSTANCES ASSOCIATED WITH TAENIID METACESTODES

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INTRODUCTION

Many helminth parasites are able to survive for prolonged periods in host tissue although infected animals may become resistant to superinfection. In cysticercosis acquired resistance is antibody mediated (Campbell, 1938; Leid & Williams, 1974a) yet the cystic larval stages in tissues contain many host serum components including immunoglobulins. In recent work with *Taenia taeniaeformis* in the rat we have shown that antibodies in the IgG_{2a} subclass are primarily responsible for this acquired resistance (Leid & Williams, 1974a) and that their protective effect is dependent upon complement (Musoke & Williams, 1975). In an effort to explore the relationship between this immunological effector mechanism and survival of the established metacestode cyst we have therefore studied the effects of *T. taeniaeformis* on the complement system. We report here on the detection and partial characterization of certain parasite-derived substances which are able to inhibit complement-dependent haemolysis, deplete C3 levels and generate anaphylatoxin activity in normal serum *in vitro* and cause a profound depression of rat serum complement *in vivo*.

EXPERIMENTAL PROCEDURES AND OBSERVATIONS

In gel diffusion tests using heavy chain specific antisera, prepared as described by Leid & Williams (1974a), IgM, IgG_1 and IgG_2 immunoglobulins were detected in fluid aspirated from the bladders of *T. taeniaeformis* and in washings from within the fibrous host capsule surrounding the organism. Neither fluid provided complement activity for a haemolytic assay employing rat IgM-sensitized sheep red blood cells, performed according to the method of Kabat and Mayer (1971). On the contrary, we observed that the addition of parasite cyst fluid (CF) inhibited the complement activity present in normal fresh rat or human serum. Inhibitory activity was also demonstrable in buffered saline extracts of tissue of *T. taeniaeformis* homogenized in either saline or

cold ethanol. Comparable activity in this assay was also demonstrated in extracts of metacestodes of *T. crassiceps*, *T. saginata*, *T. hydatigena* and *Echinococcus granulosus*, and in bladder fluid from *T. crassiceps*, *T. hydatigena*, *T. pisiformis* and *E. granulosus*.

When fifty 80 day-old larvae of *T. taeniaeformis* were maintained under sterile conditions *in vitro* in a mixture of 10 mls of fresh normal rat serum and 10 mls of Hanks' BSS, at 37°C, the haemolytic complement activity of the rat serum was depleted by 85% after 30 minutes. In further experiments soluble inhibitory factors were shown to be released into the supernatant fluid when larvae were maintained *in vitro* at 37°C in protein-free Earle's basal tissue culture medium over a 24 hour period as described by Leid & Williams (1974b). *In vitro* products (IVP) and CF samples were fractionated and the active components were partially characterized in the following experiments.

Gel filtration chromatography of CF and IVP samples was performed in 90 X 2.5 cm columns of Sephadex G-200 equilibrated with 0.1 m Tris HCl buffer, pH 8, and the elution profiles monitored at 280 nm as shown in Fig. 1. Cyst fluid was collected from 80 day-old larvae in rat livers at necropsy and was used without further concentration. *In vitro* products were collected from 200 larvae maintained for 24 hours at 37°C in Earle's basal tissue culture medium and concentrated 300 fold before equilibration with the column buffer. Host serum proteins detectable by gel diffusion in CF provided internal markers for approximate molecular weight calibration of the column. IgM was present in tubes 48-60, IgG in tubes 60-103 and albumin in tubes 90-113. Five ml of each starting material were applied to the columns and 2.5 ml fractions were collected. Fractions were pooled as indicated in Fig. 1 and concentrated back to one half original starting volume for assay. One tenth ml from each pool was assayed for anticomplementary activity by incubation at 37°C for 30 minutes with 0.5 ml of a 1:20 dilution of fresh normal rat or human serum prior to the addition of 1 X 10^8 sheep red blood cells sensitized with rat (IgM) antibody. The mixture was further incubated at 37°C for 15 min. and haemolysis was qualitated spectrophotometrically at 541 nm.

Inhibitory factors in CF eluted in two distinct areas: the first corresponded to the void volume and contained detectable quantities of rat IgM in double diffusion tests and the second occurred in the terminal portion, after elution of host albumin. The distribution of activity in IVP was more heterogenous but there was a distinct absence of inhibition in the initial portion of the major void volume peak.

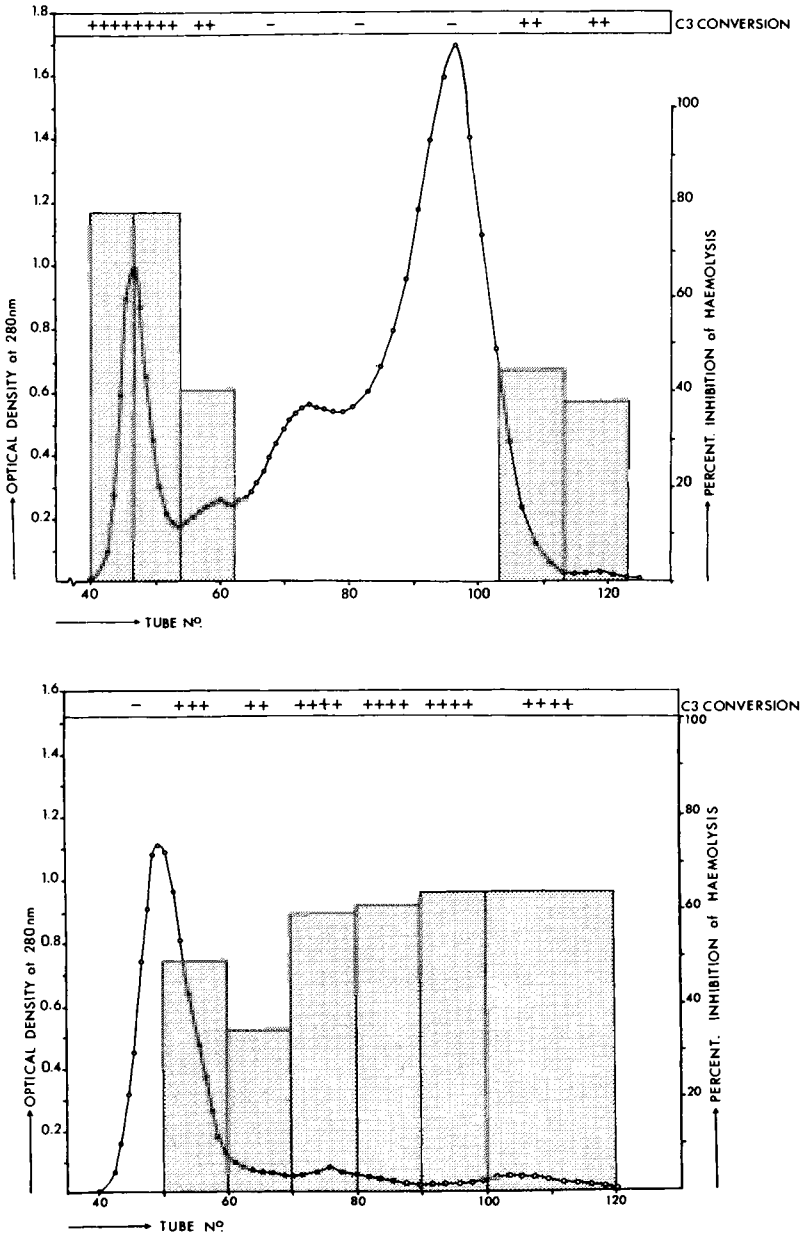

Fig. 1. Gel filtration of cyst fluid (CF) from T. taeniaeformis *(above) and products released from larvae* in vitro *(IVP)(below). Inhibition of complement-dependent haemolysis is indicated by vertical stippled bars and activity in the C3 conversion tests is scored from - ++++ for each fraction.*

Pooled fractions from each chromatogram were also tested *in vitro* for activity in converting C3 in normal human serum to a faster migrating species. The procedure described by Muller-Eberhard and Fjellström (1971) was employed. The extent of conversion was scored visually from - to ++++ and the values are included in Fig. 1. High rates of conversion clearly paralleled anti-complementary titers in the hemolytic assay.

Pool 1 from Sephadex G-200 gel filtration of CF (Pool 1-CF) was also examined for its ability to generate smooth muscle contracting activity in normal serum, detected by the Schultz-Dale assay as described by Dias da Silva, Eisele, and Lepow (1967). Contractile activity was analogous to that produced by C5A since it caused tachyphylaxis to C5A generated in normal rat serum using agar (Dias da Silva and Lepow, 1966). Conversely, C5A produced tachyphylaxis to contractions caused by addition of rat serum incubated with pool 1-CF at 37°C for one hour. There was no detectable contractile activity in CF applied directly to the system.

In order to determine if the active substances in CF could depress serum complement activity *in vivo*, 0.5 ml of pool 1-CF was injected intravenously in rats. Serial samples were taken and tested in the haemolytic assay. Complement activity was reduced by more than 85% within 5 minutes, falling to 93% by 30 minutes, before gradually returning to normal levels by 20 hours post injection (Fig. 2). Unfractionated CF (0.5 ml IV) depressed complement activity to a lesser degree.

We considered the possibility that some portion of the complement inhibitory activity in pool 1-CF could be attributable to antibody antigen complexes or aggregated immunoglobulins. In order to test this, pool 1-CF was passed through a column of glutaraldehyde-polymerised sheep anti-rat Fab. After absorption, the protein concentration of pool 1-CF, determined by the method of Lowry, Rosebrough, Farr, and Randall (1951), was found to have been reduced by 50% and immunoglobulin determinants were no longer detectable in gel diffusion tests. However, there was no reduction in complement inhibitory activity of the fraction. Furthermore, trypsinization of pool 1-CF for 8 hours at 37°C, pH 8.0, caused no loss of inhibitory activity, although once again there was a marked reduction (33%) in total protein concentration following dialysis. In a final effort to rule out a role for host-derived immunoglobulins in the inhibitory functions of the fraction, we inoculated rats intravenously with 40 mgs of homologous aggregated immunoglobulin prepared as described by Christian (1960). The results, shown in Fig. 2, indicate

that these preparations, containing far more immunoglobulin than normally present in pool 1-CF, were infective in reducing complement levels *in vivo*.

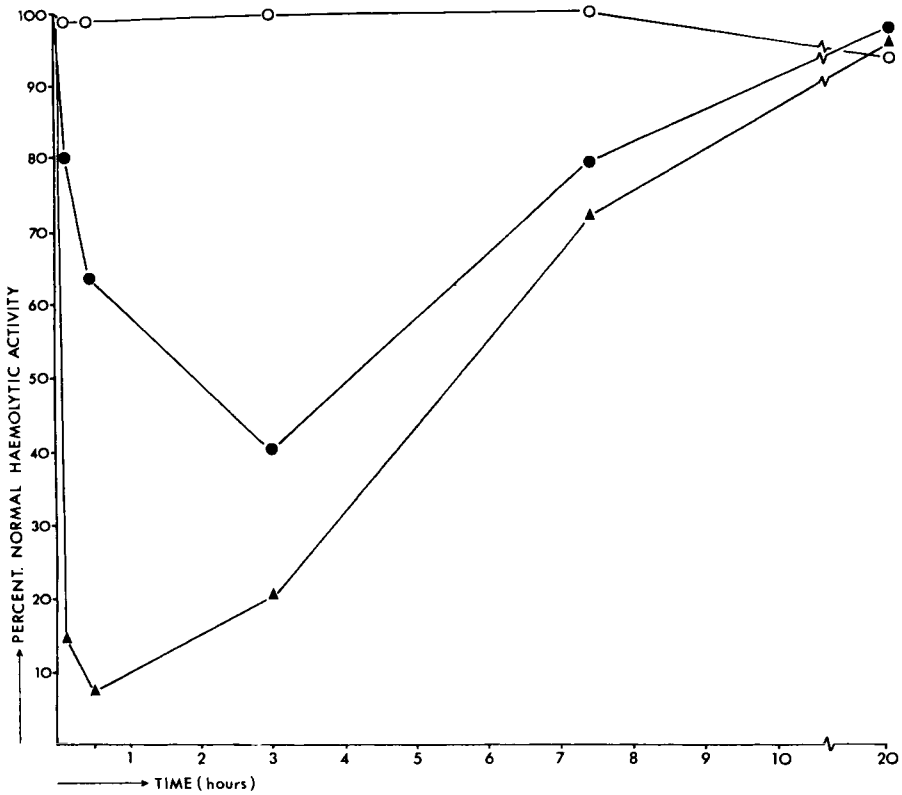

Fig. 2. Depression of serum complement activity in rats following intravenous inoculation of CF from T. taeniaeformis (●——●), *pool 1-CF* (▲——▲) *or aggregated rat globulins* (○——○). *Samples were inoculated in 0.5 ml volumes and pre- and post-inoculation serial bleedings were taken from the orbital plexus. Total serum complement levels were assayed using the method described by Kabat and Mayer (1971). The percent depression below pre-injection levels was calculated for each rat and the means plotted for the 5 rats in each group.*

The ability of parasite-derived factors to convert C3 and generate C5A-like activity *in vitro* suggested the possibility that they might produce changes in vascular permeability

in vivo as a result of anaphylatoxin formation (Lepow, Dias da Silva and Patrick; 1969). All gel filtration fractions of CF and IVP active in C3 conversion were therefore inoculated intradermally (i.d.) into rats previously prepared by i.v. injection of 0.5 ml 1% Coomassie Brilliant blue. Bluing occurred at all sites within 10 minutes reaching a maximum diameter of 8 mm in some cases, whereas control inoculations of NRS or buffer diluent produced only minor bluing at the point of needle trauma. Inoculation of 0.05 ml of each fraction i.d. in 4 normal human subjects resulted in the development of marked wheal and erythema reactions. Maximum reactivity was apparent by 10 minutes after inoculation and in one case an irregular wheal 2.8 cm in maximum width was produced, accompanied by pruritus. Vascular permeability changes were not completed ablated in rats by prior administration of antihistamines (diphenhydramine HCl at 20 mg/Kg, mepryamine meleate at 20 mg/Kg or cyproheptidine at 20 mg/Kg). At these doses each drug abolished skin bluing by i.d. inoculations of 10 µg histamine. Thus it appeared unlikely that the vascular permeability changes could be attributed wholly to anaphylatoxin generation since anaphylatoxins are known to act via mast cell release of histamine (Lepow *et al.*, 1969).

DISCUSSION AND CONCLUSIONS

In terms of their potential role in the host parasite relationship *in vivo*, the significance of the effects that we have demonstrated on the host complement system is difficult to assess at this time. Interactions between metazoan parasite products and host complement have not been reported previously but inhibition of haemolytic complement has been associated with gram negative bacterial parasites (Lepow, 1971) and is attributed to C3 utilization by the properdin pathway (Pillemer, Blum, Lepow, Wurt and Todd; 1956). This is considered to result in cytotoxicity via membrane fixation of complement (Wardlaw and Pillemer, 1956), although it may also enable the host to respond rapidly to the generation of complement mediated chemotactic factors (Austen, 1971). In our system C3 conversion clearly occurs but the tissue phases of *T. taeniaeformis* apparently suffer no detrimental effects from the consumption of C3 and generation of anaphylaxtoxin. Histopathologically there is no evidence of intense inflammatory changes around the 80 day-old cysticercus in the tissue (Bullock and Curtis, 1924) which might be expected to result from the potent biological activities of rat anaphylatoxin (Dias da Silva *et al.*, 1967) although in the early phases of differentiation there is an intense local eosino-

philia which could be mediated by this mechanism (Aboulatta and Williams, 1975).

Haemolytic complement inhibition was produced by soluble substances present in cystic stages of *Echinococcus granulosus, Taenia crassiceps, Taenia saginata, Taenia pisiformis* and *Taenia hydatigena,* although we do not yet know if they act in a similar manner to those in *T. taeniaeformis.* Nevertheless, the occurrence of these factors suggests that interaction of parasite products and complement may be a general characteristic of taeniid infections and should be considered in the interpretation of clinical and experimental studies on these host parasite systems. Non-specific reactivity in immunodiagnostic skin tests, for example, might be attributable in part to the generation of plasma factors which mediate vascular permeability changes and cellular infiltration. Similarly the clinical manifestations associated with rupture of hydatid cysts may result in part from the abrupt release of substances which produce anaphylatoxins in the systemic circulation.

Quite apart from the potential involvement of anticomplementary substances in clinically important reactions, it is tempting to speculate that the acquisition of a means of interference with complement function by cestode larvae could serve to enhance survival in an immunocompetent host. Early post-oncospheral stages of *T. taeniaeformis* are susceptible to complement dependent antibody mediated attack (Musoke and Williams, 1975) but rapidly become resistant to this mechanism. We are currently attempting to determine if resistance is correlated with the onset of production of anticomplementary factors. If so this may provide an alternative to present hypotheses regarding concomitant immunity, and one which may deserve investigation in other host-parasite systems.

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INFLUENCE OF MACROPHAGES AND LYMPHOCYTES OF RATS ON *NIPPOSTRONGYLUS BRASILIENSIS IN VITRO*

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INTRODUCTION

The self-cure phenomenon, an immune reaction against nematode infections in the digestive tract has been known for some time. Detailed studies of the phenomen have been carried out using the rat *Nippostrongylus brasiliensis* model and the reaction has been variously attributed to the different globulin fractions of serum of sensitized animals or to cellular components (Dineen, Ogilvie and Kelly; Keller and Keist, 1972). Clarification of the phenomen is very difficult *in vivo* because the various defense mechanisms of the host animal present a complex interelationship. Consequently, we have approached the problem by exposing *in vitro* maintained parasites to individual components of the immune response. Previous studies with this technique had been fruitful and had demonstrated, for example, that immune sera alone or in combination with mast cells and secretory antibodies of the immunoglobulin A class had no effect on the development of the larval stages and had no killing effect on adult *N. brasiliensis* (Zander, Hörchner and Gerber, 1974). As an extension of these experiments we examined the extent to which cell-dependent immunity was involved in the self-cure phenomenon and its influence on the viability of the adults of *N. brasiliensis* maintained in culture.

MATERIALS AND METHODS

Immune competent Sprague-Dawley rats were infected once or up to three times each with 3,000 infective larvae of *N. brasiliensis*. They served as donors of lymphocytes, macrophages and sera.

Lymphocytes were isolated from the lymph nodes of the small intestine of normal and immune rats. The cells were washed several times in physiological saline containing 100 mg BSA and 50 mg heparin per liter. For *in vitro* studies they were used at a concentration of 8×10^6 in 0.1 ml of diluent. Macrophages were obtained by the stimulation of the peritoneal cavity of rats by the injection of a glycogen so-

lution (0.15 mg glycogen/ml PBS). The macrophages were separated from other cell-types in the peritoneal liquid by Ficoll-gradient-centrifugation. They were used at a concentration of 6 x 10^6 cells in 0.1 ml of diluent. Sterile adult *N. brasiliensis* worms were isolated from the small intestine of rats infected 7 days previously. They were maintained in a culture medium containing yeast extract, casein, Krebs-Ringer solution and rat serum, with added penicillin-streptomycin and thiomersalate, as described by Weinstein and Jones (1956).

Three experiments were conducted and each consisted of 5 parts in which the effect of sensitized or non-sensitized lymphocytes and macrophages was assessed over the period of a week in replicate cultures of *N. brasiliensis*. The design of the experiment is given in Table 1. The separate experiments (I, II, III) assessed the contribution of normal serum, serum from infected rats and serum from three times infected rats, respectively.

The culture tubes each contained an average of 20 adult worms, the medium was changed once and cells were replaced four times over the period of study.

TABLE I

Design of experiment to assess the *in vitro* activity of cells and serum on adult *N. brasiliensis*.

Experimental group	Replicate Tubes	lymphocytes	macrophages
A	10	sensitized	non sensitized
B	10	sensitized	sensitized
C	10	non sensitized	sensitized
D	10	non sensitized	non sensitized
K	10	-	-

RESULTS

The effect of Normal Serum. Fig. 1 illustrates the mean percentage mortality of adult worms incubated with normal rat serum and various combinations of sensitized or non-sensitized macrophages or lymphocytes. Cultures containing both sensitized lymphocytes and macrophages (Group B) or sensitized macrophages and non-sensitized lymphocytes (Group C) showed the greatest mean mortality. The lowest mortality was seen in the control tubes.

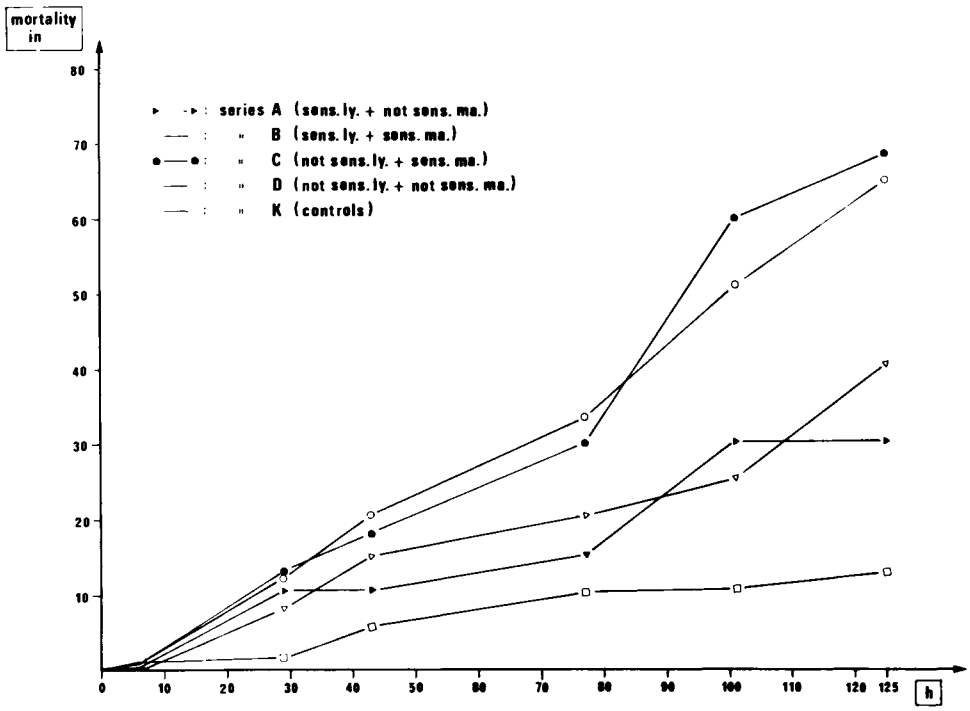

Fig. 1. Mean percentage mortality of adult N. brasiliensis *exposed* in vitro *to normal serum and various combinations of normal or sensitized lymphocytes or macrophages.*

Effect of Serum from previously infected animals. Fig. 2 illustrates the mean percentage mortality of adult worms *in vitro* incubated with various combinations of cells and serum from animals once infected with *N. brasiliensis*. The highest mortality occurred in cultures containing both sensitized lymphocytes and macrophages (Group B).

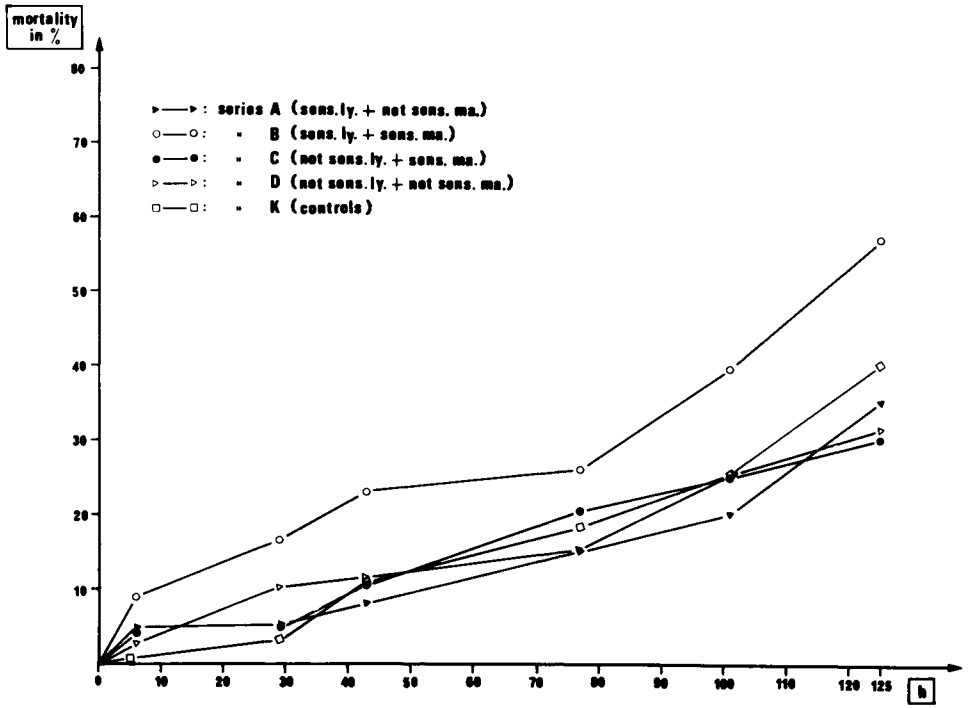

Fig. 2. Mean percentage mortality of adult N. brasiliensis *exposed* in vitro *to serum from animals infected once previouly and various combinations of normal or sensitized lymphocytes or macrophages.*

Fig. 3 illustrates the results obtained with serum from rats infected three times previously. As in the previous experiments, cultures containing both sensitized lymphocytes and macrophages (Group B) or sensitized macrophages and normal lymphocytes (Group C) showed the greatest mortality.

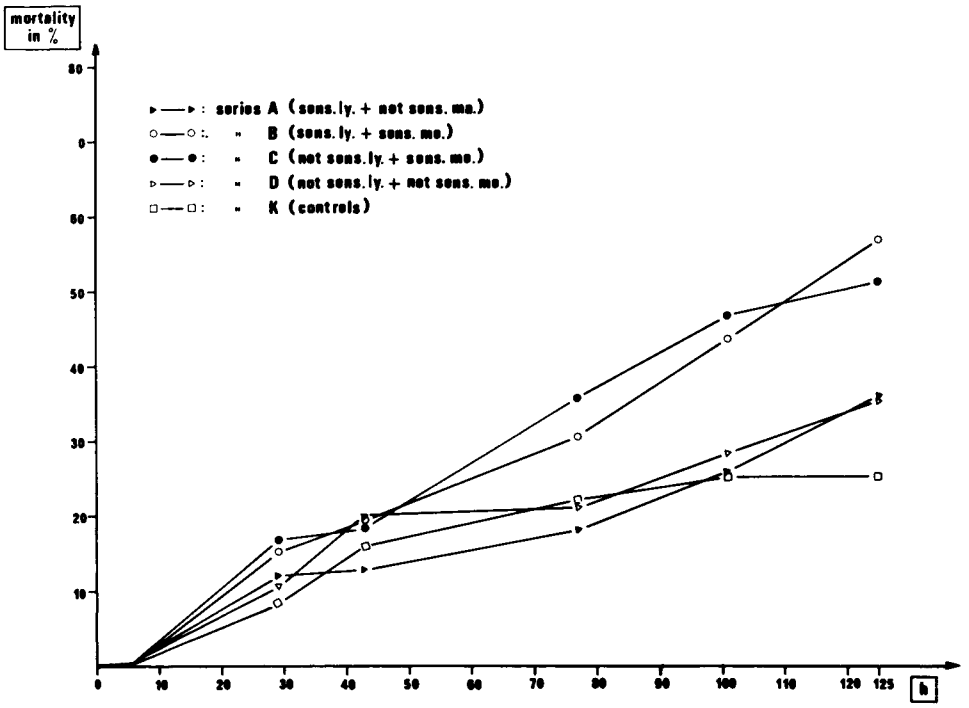

Fig. 3. Mean percentage mortality of adult N. brasiliensis *exposed* in vitro *to serum from animals infected three times previously and various combinations of normal or sensitized lymphocytes or macrophages.*

DISCUSSION

These studies indicate that adult *N. brasiliensis* worms, incubated in the presence of macrophages harvested from previously infected animals, have a reduced survival time compared with control preparations.

The need for specifically sensitized lymphocytes for this effect was not evident from this study since though increased killing of adult worms occurred in cultures containing sensitized macrophages and sensitized lymphocytes, the latter alone, or with non sensitized macrophages, failed to modify

the survival of parasites in the test system. It is also clear that serum from animals variously infected with *N. brasiliensis* had no significant effect on the survival of the parasite under *in vitro* conditions.

Consequently, these studies suggest that cell dependent immune reactions rather than humoral components are involved in the responses to parasites of the digestive tract.

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THE EFFECT OF ACUTE TRICHINOSIS ON ALLOGRAFT REJECTION

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INTRODUCTION

Like many other parasitic agents *Trichinella spiralis* exerts a pronounced and long lasting demand on the immune mechanisms of the host. In the early phase of the infection there is a local accumulation of parasites in the mucosa of the small intestine while during the migration and muscular stages of the infection, parasites are distributed throughout the body. It has been demonstrated that experimental trichinosis in mice is accompanied by a decreased immune response to sheep red blood cells (SRBC) (Faubert and Tanner, 1971; Lubiniecki and Cypess, 1975; Barriga, 1975) and an increased susceptibility to Japanese B encephalitis virus (JBV) (Cypess, Lubiniecki and Hammon, 1973). The latter effect was most pronounced when the virus was given 7 days after infection. In contrast, mice infected with *T. spiralis* were less susceptible to *Listeria monocytogenes* given 7 or 21 days after infection than were control mice (Cypess, Lubiniecki and Swidwa, 1974) Svet-Moldavsky and his colleagues have demonstrated a prolonged survival of skin allografts in mice infected with *T. spiralis* 23 days earlier (Svet-Moldavsky *et al.*, 1970). These observations indicate that infection with *T. spiralis* significantly changes the immune responses of the host.

EXPERIMENTAL OBSERVATIONS

Thymus

Male CBA mice, aged 8 weeks, were infected orally with 500 *T. spiralis* muscle larvae (Ljungström, 1974). The most striking feature of the changes in the structure and function of the immune system was an early reduction in the size of the thymus. Thus there was a progressive loss of weight of the thymus during the first 11 days, ($p<0.001$) after which a comparatively rapid return to normal size occurred. If the mice were challenged with 500 larvae on day 21 after infection a still more drastic reduction occurred (Fig. 1). In the infected mice histopathological examination of the thymus showed cortical depletion but no significant changes in the

medulla. In the thymus-dependent areas of spleen and lymph nodes no marked depletion of small lymphocytes was observed. On the other hand proliferation of large pyronophilic cells was seen round postcapillary venules in this area. These cells did not contain immunoglobulin as assessed by immunofluorescence (Ljungström and Ruitenberg, 1976).

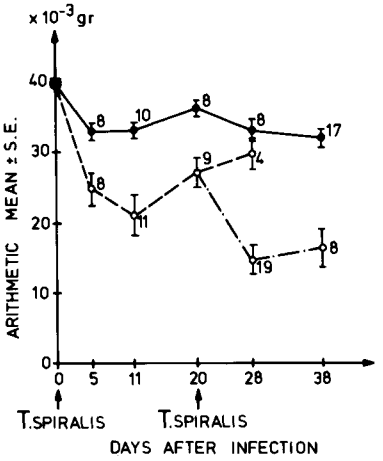

Fig. 1. Weight of the thymus in 8-weeks-old CBA mice infected with 500 T. spiralis *larvae (---), challenged day 21 after initial infection with 500 larvae (-•-•) and normal mice (——) expressed as means and standard error (S.E.). The number at each point indicates the number of animals investigated.*

To investigate the extent to which thymic depletion in male CBA mice was due to adrenal cortical steroids, *T. spiralis* infected mice (500 larvae orally) and normal mice were adrenalectomized and 2 days later were challenged with 500 larvae. A group of intact infected and challenged mice served as controls. The results are presented in Fig. 2. There were no significant differences in the thymus weight between the various groups 3 days after challenge infection. A significant increase ($p<0.001$) in thymus weight in previously uninfected; adrenalectomized mice was evident 7 days after infection. In previously infected, adrenalectomized mice, the mean weight of the thymus was significantly ($p<0.15$) lower than normal at 7 days. As has been noted in other work previously infected, non-adrenalectomized mice showed a signi-

cantly lower ($p<0.001$) thymus weight at 7 days. These results indicate that cortical steroid mediated stress plays only a minor role in the thymic depletion. However, the mechanism of this effect is not clear and it deserves further study.

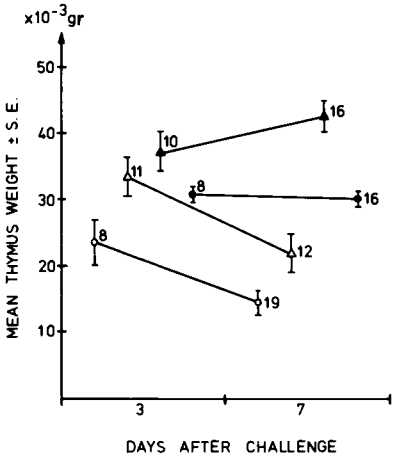

Fig. 2. Weight of the thymus of 8-weeks-old CBA mice, intact and adrenalectomized, given 500 T. spiralis *larvae. Thymus weights are recorded 3 and 7 days after challenge and expressed as means and standard error (S.E.). The number at each point indicates the number of animals investigated.*

○ T. spiralis *infected.* △ *adrenalectomized,* T. spiralis *infected.*

● *normal.* ▲ *adrenalectomized.*

Allograft Rejection

The marked depletion of the thymus might be expected to be accompanied by changes in the T cell mediated immune response. Since the rejection of allografts has been considered as mainly dependent on T cell function, a study of the ability of *T. spiralis* infected mice to reject allografts was initiated. For this purpose heart grafts were made as described by Huff, Liebelt and Liebelt (1968) and Judd and Trentin (1971) following the modifications of Svehag and Schilling (1973).

Hearts from 12-36 hrs old C57B1 ($H\text{-}2^b$) baby mice were sectioned along the ventricular septum and each half inserted into a subcutaneous pouch on the dorsal part of the ear of the recipient CBA ($H\text{-}2^k$) mouse. The survival of the transplants was monitored by measuring the electric activity with a Tektronix 410 cardiocope.

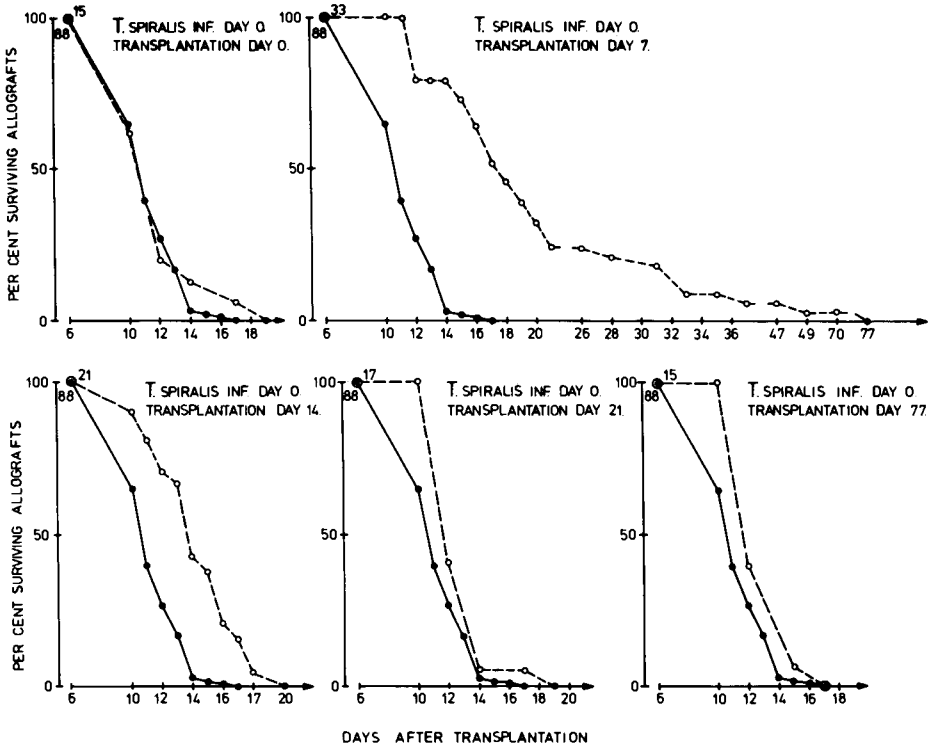

Fig. 3. Percent surviving allografts transplanted on days 0, 7, 14, 21 and 77 respectively after oral infection with 500 larvae of T. spiralis. *The number of transplants investigated is shown in the figure at day 6 after transplantation.*

●—● *normal 8-weeks-old CBA mice.*
○-○ T. spiralis *infected 8-weeks-old CBA mice.*

In Figure 3 the survival of allografts transplanted on days 0, 7 (intestinal phase), 14 (migration phase), 21 (early muscle phase), respectively, after oral infection with 500 larvae is shown. Rejection of the allograft was greatly delayed in *T. spiralis* infected mice. Although the effect was long lasting it was most pronounced in mice transplanted one week after infection at the time when the majority of parasites were developing in the intestinal mucosa. Thus the time for depression of allografts rejection coincides with the maximal thymic depletion.

DISCUSSION

The mechanism underlying this strong immunodepressive effect is not clear, but it seems that immunodepression is mainly associated with the intestinal phase of the infection. The high susceptibility to JBV virus demonstrated 7 days after infection (Cypess *et al.*, 1973) supports this hypothesis whether the immunodepressive effect is associated with a factor produced by the developmental stages of the parasite in the intestine which affects T lymphocytes or whether such a factor is produced by host cells, remains to be determined.

It is conceivable that the large antigenic load presented by the parasite might occupy the host's T cell-mediated defence mechanisms to such an extent that the capacity to reject allografts is significantly altered. There are indications that *T. spiralis* infection can stimulate cell mediated immunity (Kagan, 1960) and that such immunity plays a significant role in protection (Machnicka, 1972; Ruitenberg, Teppema and Steerenberg, 1974) which is associated with the rejection of larvae and adults from the small intestine. Inflammatory cell infiltrations, containing lymphocytes, are seen in the intestinal mucosa of *T. spiralis* infected animals (Walls *et al.*, 1973), but in T cell deprived (Walls *et al.*, and nude mice (Ruitenberg, 1975) such infiltrations are absent. The origin of these cells is the outer cortical zone of thymus and the T cells leave the organ via cortico-medullary junction (Joel, Hess and Cottier, 1971). It has been demonstrated that when certain antigens are given in large doses the subsequent antibody response to unrelated antigens can be significantly depressed. However little is known about the possible competitive effect of unrelated antigens on the mechanisms responsible for allografts rejection.

A number of parasitic infections such as malaria (Salaman, Wedderburn and Bruce-Chevatt, 1969; Greenwood, Playfair and Torrigiani, 1971) trypanosomiasis (Goodwin *et al.*, 1972) and toxoplasmosis (Strickland, Pettitt and Voller, 1972; Huldt,

Gard and Olovson, 1973) have also been shown to be accompanied by profound immunodepression. These infections are generalized, but the present results indicate that even when the parasite is localized in a certain tissue, e.g. in the bowel, in the case of *T. spiralis*, a marked effect on the immune responsiveness of the host can occur.

SUMMARY

Several parasite infections induce immunodepression and it is likely that different mechanisms are involved in this. More detailed investigations of these phenomena are required, especially with regard to their significance in parasitic infections in man.

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INDEX

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

R

S

T

U

V

W