INVESTIGATION INTO THE ROLE OF SIALIDASE-INDUCED GALACTOSE EXPOSURE IN THE ERYTHROPHAGOCYTOSIS OF BOVINE TRYPANOSOMIASIS

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DECLARATION

I hereby declare that this thesis is a record of my own research and has not been submitted for the award of a degree at any other University. Information and excerpts from both published and unpublished work of others have been acknowledged in the text.

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CERTIFICATION

This dissertation entitled "Investigation into the role of sialidase-induced galactose exposure in the erythrophagocytosis of Bovine trypanosomiasis" by Omage, Jebba James meets the regulations governing the award of the degree of Ph.D. in Biochemistry of Ahmadu Bello Univeristy, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to Mrs. Christiana Omage and Mrs. Esther Omage for their love and devotion, my wife Abiodun, my brothers and sisters for their sincere prayers and to JEHOVAH for the uncountable things He has done in my life.

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ABSTRACT

The mechanism that mediate recognition and subsequent sequestration of erythrocytes during bovine trypanosomiasis in *Trypanosoma vivax* infected calves with and without lactose infusion was investigated. Sialidase induced exposure of carbohydrate on erythrocytes following removal of sialic acid with increasing parasitemia, were examined using an *in vitro* cell binding assay that allows spontaneous formation of cell contacts.

The results obtained indicate a preferential recognition of erythrocytes taken from the *T. vivax* infected animals, as compared to those from non-infected animals by rat peritoneal macrophages. This recognition manifested itself by adhesion of the complementary cells in the form of rosettes and hence could explain partly the removal of erythrocytes from circulation during bovine trypanosomiasis. Adhesion was shown by inhibition studies with monosaccharides and oligosaccharides. The inhibition pattern of D-galactosc > Lactose > Maltose > D-glucose > Mannosc was observed.

freshly isolated rat peritoneal macrophages *in vitro* strongly bound desialylated erythrocytes of calves infected with *Trypanosoma vivax*. A consistently higher level of binding was observed in the lactose infused infected calves adduced to a consistent presence of the trypanosome parasite in the peripheral circulation of the lactose - infused calves. A concomittant higher level of free serum sialic acid concentrations was also observed in the lactose-infused infected calves throughout the experimental period. Correlation studies showed a strong positive association ($P<0.001$) between serum sialic acid and rosette formation.

An initial but non-significant ($P>0.05$) increase in free serum sialic acid

concentration was observed in both infected groups of calves and this coincided with increased parasitemia. The initial increase coincided with increased rosette formation and increased parasitaemia. Parasitaemia in both groups of infected calves continued to rise after the establishment of patent infection and peaked 5 days alter infection. While trypanosomes flourished in numbers large enough to be counted throughout the period of the study in the infected calves that were injected with lactose, they were too scanty to be counted by day 8 alter infection in the infected calves that were not injected with lactose.

These observations demonstrate that phagocytosis of erythrocytes early in bovine trypanosomiasis may partly be due to the cleavage of sialic acid, exposure of p-galactosc residues and binding of the erythrocyte by perhaps p-galactose specific lectin on the host macrophages. The persistent parasitaemia, higher concentrations of free serum sialic acids and higher binding and resetting observed in the lactose infused infected calves strongly suggest more desialylation of the erythrocytes, inspite of the lactose infusion.

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CHAPTER 1

INTRODUCTION

Trypanosomiasis is a severe disease in animals and man caused by protozoan *Trypanosoma* parasites mostly transmitted by tsetse flies, *Glossina species*. Different species of the genus *Trypanosoma* infect cattle, sheep, goats, pigs, horses, donkeys, camels and many types of wild animals and man. The disease occurs in scattered foci throughout the subsaharan tsetse belts of Africa, an area of some 10 million square kilometers and it restricts cattle rearing in many areas of the continent (Tropical Diseases, 1990). In highly tsetse infected areas, it is extremely difficult to keep livestock at all and even human habitation may be threatened. The pathogenic animal trypanosomes are transmitted cyclically, mechanically or by coitus (Hoare, 1972; Anosa, 1983; Roeder *et al.*, 1984; Sekoni, 1994). The diseases caused in animals are collectively called Nagana, while in human beings they are known as sleeping sickness (Apted, 1970; Tropical Diseases, 1990; Engstler and Schauer, 1993). Some 50 million people and 25 million cattle in Africa are at risk of infection with these trypanosomes (Turner, 1982; Tropical Diseases, 1990). Seven million cattle die annually of trypanosomiasis throughout a large portion of sub-saharan Africa (Engstler and Schauer, 1993).

The potentials for appreciable increases in livestock production in many parts of Africa and Nigeria in particular is hampered by manpower and animal diseases. Many diseases in Africa such as rinderpest, East Coast fever (ECF) and tsetse-transmitted trypanosomiasis affect animals. Trypanosomiasis has remained a major force militating against livestock production in tropical Africa including Nigeria, where the disease is endemic.

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The most commonly occurring pathogenic trypanosomes in animals are *Trypanosoma congolense*, *T. brucei* and *T. vivax* which affect cattle, buffalo, sheep, goats, pigs, donkey, horses, camels, cats, dogs and laboratory animals. Others are *T. evansi* which affects dogs, buffalo, camels, horses, sheep and goats, *T. equinum* which affects dogs and horses, *T. equiperdum* affects horses, while *T. suis* and *T. simiae* cause disease in pigs. Laboratory animals such as rats, mice, rabbits and guinea pigs are highly susceptible. *T. brucei* rhodesiense and *T.b. gambiense* affects humans. (Hoare, 1972; Tachibana *et al.*, 1988; Brun *et al.,* 1989; Tropical Diseases, 1990). *T. vivax* causes the most important form of trypanosomiasis in farm animals in West Africa (Soulsby, 1982), particularly in Northern Nigeria (Leeflang, 1978).

Pathogenic animal trypanosomes are causative agents of the most common livestock diseases which have an important economic impact on many African countries. These diseases usually cause debilitating symptoms manifested by anaemia and cachexia which may result in death (Sekoni, 1994). The economic losses resulting from death of the affected animals, or stunting, debilitation and poor reproductive performance have not been well quantified, but must be staggering. Ilemobade and Balogun (1981) have shown that pigs infected with *T. simiae* exhibited impaired feed conversion efficiency, growth failure, poor carcass traits and extremely high and uneconomic feed cost per unit of weight gain apart from the anaemia of the infection.

Affected animals generally show poor growth rates, low milk yields, reduced capacity for work, poor reproductive performance (ILRAD, 1986) and at slaughter, carcasses are of low weight and poor quality, hence leading to large economic losses. The animals also show a wide range of reproductive disorders which include

degeneration of the hypothalamus, pituitary glands and gonads with consequent disruptions in the secretions and plasma concentrations of the hormones necessary for normal reproductive processes in both sexes (Sekoni, 1994). These reproductive disorders in animals are also of significant economic importance, especially in sub-saharan Africa, where the disease is endemic.

In animals, trypanosomiasis may be peracute, in which case the animal dies within a few hours or it may be acute, sub-acute or chronic. In chronic form, the animal is usually emaciated, anaemic with low total plasma proteins and intermittent pyrexia (Apted, 1970; Akol and Murray, 1981; Esievo *et al.*, 1982a; Boch *et al.*, 1984;; Sekoni, *et al.*, 1990).

Anaemia, a clinical feature in animal trypanosomiasis (Fiennes, 1954; Mackenzie and Cruickshank, 1973; Mamo and Holmes, 1975; Kobayashi *et al.*, 1976) has long been established as a major pathological feature of animal trypanosomiasis (Fiennes, 1954; Losos and Ikede, 1972) caused by *T. brucei*, *T. congolense* or *T. vivax* infection. The anaemia is hemolytic in origin but the mechanism of hemolysis is the subject of numerous investigations as reviewed by Esievo and Saror (1991). Of the numerous factors advanced as being responsible, emphasis has been given to erythrophagocytosis and the reduction of erythrocyte life-span due to the effects of trypanosome sialidases (neuraminidase) on surface sialic acid on the red blood cells as reviewed by Esievo and Saror (1991).

Sialic acid occurs as the terminal sugar on the glycoconjugates on the surfaces of erythrocytes of mammals where they mask ligands or recognition sites on the surfaces of erythrocytes and also give a general shield on the cell surfaces by their negative charge (Muller *et al.*, 1981; Esievo *et al.*, 1986; Kelm, *et al.*, 1986ab; Engstler and Schauer,

1993). Removal of this terminal sialic acids results in the exposure of subterminal β-glycosidically bound galactose residues which then binds with the β-D-galactose specific lectin on macrophages (Kolb and Kolb-Bachofen, 1978; Kuster and Schauer, 1981; Kelm and Schauer, 1986; Mohr *et al.*, 1987). This attachment leads to erythrophagocytosis (Kelm *et al.*, 1986a). This attachment of erythrocytes to lectin on the macrophages which leads to erythrophagocytosis can be inhibited or prevented by other saccharides similar to galactose (Kolb and Kolb-Bachofen, 1978; Ashwell and Morell, 1978; Muller *et al.*, 1981, 1983a; Mohr *et al.*, 1987) thus keeping the erythrocytes longer in circulation. The above investigations were accomplished using commercially-produced bacterial sialidase (neuraminidase). With the production of sialidase by *T. vivax* (Esievo, 1979, 1983) the development of severe anaemia in Zebu cattle has been associated with a significant reduction in erythrocyte surface sailic acid concentrations during the acute phase of experimental *T. vivax* infection (Esievo *et al.*, 1982a). Thus during bovine trypanosomiasis, sialidase is produced by *T. vivax* which cleaves erythrocyte surface sialic acids, rendering the erythrocytes more prone to phagocytosis by the expanded mononuclear phagocytic system (reticuloendothelial system) perhaps due to some physico-chemical damages on such erythrocytes (Esievo *et al.*, 1982a). Therefore, erythrocytes, surface sialic acids and trypanosome sialidase have significant roles in the pathophysiology and pathogenesis of animal trypanosomiasis (Esievo and Saror, 1991). It is important therefore to investigate if the anaemia observed during bovine trypanosomiasis is due to the removal of erythrocyte surface sialic acids, exposure of β-galactose and subsequent removal of such desialylated erythrocyte from circulation by β-D-galactose specific lectins on macrophages. It is also important to investigate if this process of erythrophagocytosis

can be inhibited *in vivo* thereby increasing the survival time of the desialylated erythrocytes in circulation. There is no information on this aspects in bovine trypanosomiasis. This study was therefore designed to:

1. establish the exposure of galactose on erythrocyte surface during trypanosome infection;
2. examine the relationship between erythrophagocytosis in bovine trypanosomiasis, desialylation of erythrocytes by sialidase produced by the invading trypanosome and the exposure of β-D-galactose residues as the subterminal sugar and it's subsequent trapping by rat peritoneal macrophage; and,
3. examine and compare the inhibitory effect of lactose when used in an *in vitro* cell binding assay and when repeatedly infused intravenously to *T. vivax* infected calves.

CHAPTER 2

REVIEW OF LITERATURE

Across much of tropical Africa, cattle, goats, sheep and camels provide smallholder farmers with two of their most important sources of food - milk and meat (ILRAD, 1989a). Livestock also provide fertilizer and tractive power for cultivation. At whatever level of production, livestock provide millions of families with better nutrition, family income and employment opportunities, draft power and improved agriculture.

There is an imbalance in the growth of human population when compared with food production, particularly livestock production where in Africa for instance, ILRAD (1989a) reported that the human population has an average annual increase of 3 percent while the cattle production has risen little with an annual increase of only a little over 2 percent.

The problems of beef production in developing countries are extremely diverse. This is more pronounced in a country like Nigeria with various climatic and ecological differences ranging from hot savannah zone where livestock production is based on pastoral system, to humid zones with rainfall in the south where utilization of abundant pasture resources and water is limited by tse-tse-flies and social land pressures (Nuru, 1982). One of the most important constraints on livestock productivity in tropical environment is disease (ILRAD, 1989b).

The adverse effects of East Coast fever (ECF), rinderpest, trypanosomiasis and other livestock diseases on food and agricultural production must be reduced as quickly and as effectively as possible. In Nigeria, for instance, with the eradication or control of major epidemic diseases such as Rinderpest and Foot and Mouth disease, livestock

production is now faced with insidious economic diseases such as mastitis, trypanosomiasis, streptothricosis, endo- and ectoparasites and reproductive diseases like brucellosis leading to breeding inefficiency and infertility (Nuru, 1982). In Nigeria, trypanosomiasis and streptothricosis are of great economic importance in the loss of value of hides, premature culling of potential beef bulls and even death or slaughter of emaciated beasts.

Trypanosomiasis is a livestock disease that is of major economic importance in Africa (ILRAD, 1989b) limiting the growth of beef cattle industry since only the trypanotolerant breeds can thrive in those areas heavily infested by tsetse flies (Nuru, 1982). Efforts to increase livestock production in many areas south of the Sahara depend on improving methods used to control trypanosomiasis. Effective animal production is dependent on prevention and/or treatment control of detrimental diseases including trypanosomiasis. Therefore, in planning for increased cattle productivity in tropical Africa a principal requirement is for information to predict accurately the usefulness of production systems, management levels, disease situations, nutritional resources and major breed types for different ecological zones (Trail, 1984).

The protozoan parasites (trypanosomes) that causes trypanosomiasis are transmitted by the bite of tsetse flies to domestic animals in which severe and often fatal infections may develop. Trypanosomiasis is one of the few serious cattle diseases that cannot be effectively controlled by veterinary practices (Oldfield, 1983) and it is indeed a more important problem than human sleeping sickness (Willet, 1970).

Tsetse flies inhabit about 10 million square kilometers or roughly one third of the African continent, an area containing some of Africa's best watered and most fertile land. In these vast areas that are otherwise suitable for livestock production, the

presence of tsetse flies limits the production of most breeds of cattle and small ruminants except those kept under close veterinary supervision and given frequent and costly drug treatment or the use of trypanotolerant breeds of cattle. Therefore, a large proportion of livestock on the continent is kept in tsetse-free areas, most of which are fragile, arid and increasingly over-grazed (ILRAD, 1989a). As a result of this, the human population suffers from malnutrition due mainly to lack of animal protein, while agriculture which is heavily dependent on fertilizers in many places remains in the primitive state of shifting cultivation due to lack of cattle manure (Hornby, 1952; Willet, 1970; Hoare, 1972;). About 75 percent of Nigerian land is known to be infested by tsetse flies and only trypanotolerant breeds can thrive in these areas.

Tsetse fly (*Glossina*) belongs to the insect order of Diptera, family Muscidae, sub family Stomoxydinae. Other Diptera especially species of Stomoxys and the Tabanidae, that live by blood sucking, can participate in the transmission of trypanosomes from one host to another (Ford, 1971). There are 34 species, sub-species and races of *Glossina* now recognized. There are 3 subgeneric groups of tsetse, *Glossina fusca*, *Glossina morsitans* and *Glosinna palpalis* (Ford, 1971). The fusca group as a whole live in humid environment, the palpalis group is generally confined to water side habitats and the morsitans group is associated with more and 'Savannah' type vegetation (Ford, 1971).

The trypanosomes (*Trypanosoma*) are flagellated protozoa belonging to the class zoomastigophorea, family Trypanosomidae and order kinetoplastida (or Protomonadina). The kinetoplastida represent a prominent order of flagellated protozoa containing several species threatening man and his domestic animals, especially among the genera Trypanosoma and Leishmania (Engstler *et al.*, 1995). They have single

flagellum and the genus includes a number of species which are dangerous parasites to man and domestic animals, though many of the same and other species are non-pathogenic in wild animals (Hoare, 1972). *Trypanosoma vivax* used in this study is a specie belonging to the B section salivaria and subgenus Duttonella which are represented by monomorphic forms with a free flagellum.

The role of tsetse-flies as vectors of Nagana is closely related to the sources of their food, represented primarily by wild ungulates, a large proportion of which are symptomless carriers of trypanosomes that are pathogenic to domestic animals. Most of the available information about Nagana involves bovines and there are over 100 million head of cattle in tropical Africa (WHO, 1969; Hoare, 1972) hence they are economically the most important hosts affected by trypanosomiasis.

The disease varies according to the degree of challenge by tsetse and the resistance of different breeds of cattle to infection. In northern Nigeria for instance, the Muturu and N'dama breeds are tolerant of the disease while the Zebu cattle are highly susceptible. However, due to improved conditions of husbandry, cattle exposed to tsetse can be protected by prophylactic and therapeutic drug treatment.

Thus, in this review, African trypanosomiasis is examined. The contribution of past research work on the anaemia observed in trypanosomiasis particularly the role of erythrocyte surface sialic acids, sialidases and galactose exposure on the sequestration of erythrocytes by a process of erythrophagocytosis need to be examined.

2.1 METABOLISM OF TRYPANOSOMES

The metabolic activity of a parasite may affects its host in two ways; by depleting essential nutrients and/or producing toxic metabolites (Newton, 1978). The mode of

nutrition of trypanosomes is of the saprozoic type aided by pinocytosis. Carbohydrates, especially glucose fulfil most of the essential requirement for trypanosome metabolism. The amount of glucose consumed per hour by blood stream forms of African pathogenic trypanosome for instance is equivalent to 50 - 100 percent of their body weight and they die within a few minutes when deprived of this sugar *in vitro* (Brand, 1951; 1966) because of their low endogenous energy reserve (Opperdoes *et al.*, 1976). The trypanosome utilize the sugar that is available to them in the host's blood under natural conditions while in cultures it is incorporated in the media.

In blood where there is abundance of carbohydrate, the haematozoic trypamastigotes do not metabolize glucose completely but break it down to pyruvate, which is excreted into the blood of the host. Here the parasites can do without the requisite enzymes and their mitochondrial system is weakly developed. In the gut of the insect vector with lower glucose content which diminishes progressively as the blood meal is digested, the trypanosomes adopt a more economical method for obtaining energy by enlarging the mitochondrion and synthesizing it's enzyme which enables it to fully utilize pyruvate to liberate energy. Trypanosomes contain glycosomes, microbodies containing most of the enzymes of the glycolytic system (Opperdoes and Borst, 1977; Borst *et al.*, 1983;) and several additional enzymes (Opperdoes *et al.*, 1981; Hammond *et al.*, 1981; Opperdoes and Cottem, 1982). They also contain a fatty acid β-oxidation system and enzymes of plasmalogen biosynthesis (Borst *et al,* 1983). According to Opperdoes and Borst (1977), these enzymes in blood stream *T. brucei* breakdown glucose to glycerol-3-phosphate (G-3-P) and 3 phosphoglycerate (3-PG) which passes into the cytoplasm. G-3-P is oxidized by molecular oxygen in the outer membrane of the mitochondrion and the dihydroxyacetone phosphate produced is

shipped back to the glycosome for recycling. The 3-PG is converted to pyruvate in the cytoplasmic matrix, with linked phosphorylation of adenosine diphosphate (ADP) and the pyruvate is excreted by the trypanosomes. Hill (1976) observed that there is no functional tricarboxylic acid cycle and no cytochromes in trypanosomes. Only two molecules of adenosine triphosphate (ATP) are produced per molecule of glucose. Dixon (1966) similarly observed that lactate dehydrogenase is absent and reduced nicotine amide adenine dinucleotide (NADH) is reoxidized by a unique glycerophosphate oxidase system consisting of glycerol-3-phosphate oxidase with a shuttle between the glycosome and the undeveloped mitochondrion. Investigations (Gutteridge and Coombs, 1977; Gutteridge and Rogerson, 1979) showed that no ATP is produced by the oxidase system and that the system is not sensitive to cyanide but it is inhibited by hydroxamic acids like salicyl hydroxamic acid (SAHM). In aging trypanosomes, when parasitaemia begins to fall, Bowman and Flynn (1977) reported that 2-oxoglutarate can replace glucose for energy supply to the trypanosomes to some extent.

Pyruvate produced by the trypanosomes is readily used by host tissues, it accumulates in the blood in amounts directly proportional to the numbers of parasites present in laboratory infections (Grant and Fulton, 1957). High concentrations of pyruvate could lead to depletion of alkali reserves, acidosis, and a lowered affinity of haemoglobin for oxygen (Newton, 1978). Haematic trypanosomes like *T. congolense* and *T. vivax* do not produce as much pyruvate per molecule of glucose metabolized as compared to *T. brucei* which is humoral and infections with these parasites are not characterized by extensive inflammatory, degenerative and necrotic changes (Newton, 1978).

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Blood forms of *T. brucei* are also capable of ingesting and digesting proteins (Langreth and Balber, 1975). The availability of ingestible proteins, amino acids and other nutrient has been suggested (de Raadt and Seed, 1977) to be an important factor in determining when, during an infection, trypanosomes begin to develop in the cerebrospinal fluid (CSF). The parasites may have access to the CSF throughout an infection (Peruzzi, 1928) but be unable to grow until it has been enriched by proteins and other nutrients from degenerating tissues. In the present study, lactose, a disaccharide of glucose and galactose was injected intravenously into calves infected with *Trypanosoma vivax* as a possible inhibitor of erythrophagocytosis.

2.2 CYCLE IN MAMMAL.

The course of development of trypanosomes in mammalian hosts is initiated by the metatrypanosomes introduced by the insect vector either passively by contamination of the mammal's mucous membrane or skin (in the case of stercorarian species like *T. lewisi* and *T. cruzi*) or actively by their inoculation through the bite (in the case of tsetse-borne salivarian species) (Hoare, 1972). The metatrypanosomes then find their way into the bloodstream or tissues, where they are transformed into trypomastigotes possessing the characteristic structure of the species to which they belong.

The pattern of multiplication in the mammalian host, as well as the stages in which it takes place vary considerably in different groups and/or species of trypanosomes. Multiplication in the stercorarian is discontinuous being either limited to a reproductive period (for example in *T. cruzi*) giving rise to adult trypomastigotes in both cases, which do not divide any more in the bloodstream and constitute the established phase of the infection. In the salivarian, there is no special reproductive

period but the trypanosomes multiply more or less continuously throughout the infection (Hoare, 1972).

The site of multiplication and the method of division as well as the morphological stages involved are also characteristic of various groups and species of mammalian trypanosomes. In the stercorarian subgenera Herpetosoma and schizotrypanum (Davis, 1952; Hoare, 1972) reproduction takes place either in (a) the mastigote stage and is confined to the tissues where the flagellates divide by equal binary fission (for example in *T. cruzi*) or equal multiple fission or (b) the epimastigote stage in the bloodstream where the flagellates divide by unequal binary fission (for example in *T. theileri*) or by multiple fission which may be equal (as in *T. rabinowitschae*) or unequal (as in *T. lewisi*). This reproduction in the bloodstream appears to be characteristic of those species which divide in the epimastigote stage, whereas in species dividing in the mastigote stage reproduction is confined to the tissues and/or capillaries (Molyneux, 1970).

Reproduction of the salivarian normally takes place in the bloodstream by binary division in the trypomastigote stage. The cycle of these trypanosomes includes a tissue phase comparable to that in the stercoraria (Hoare, 1972). In the stercorarian, the tissue phase is an integral part of the life cycle concerned exclusively with reproduction whereas in the salivarian, the tissue phase proceeds in the bloodstream irrespective of whether the tissues are involved or not (Hoare, 1972). In the salivarian, invasion of the tissues by trypanosomes is responsible for lesions leading to profound pathological changes whereas development of the trypanosomes in the stercoraria causes no damage to the tissues (Goodwin, 1970).

2.3 AFRICAN TRYPANOSOMIASIS.

Infection by one or other of the African trypanosomes is known as African trypanosomiasis. It is a disease complex caused by infection with certain salivarian trypanosomes and it is a serious threat to man and domestic animals in vast areas of Africa. The spectrum of diseases includes:

(a) African human trypanosomiasis known as sleeping sickness in tropical Africa and chagas' disease (or South American trypanosomiasis) in the Americas; and,

(b) Animal trypanosomiasis known as Nagana in tropical Africa, Surra in Africa north of the Sahara, in tropical and sub-tropical areas in Asia, as well as Central and South America and Dourine which has practically a cosmopolitan distribution (Hoare, 1972). In general, trypanosomiasis may be grouped together as zoonoses, that is diseases caused by parasites of animals which accidentally become established in man or his domestic symbionts, cattle, sheep, goats, horses, pigs and dogs. There can be as many trypanosomiases for a single host species as there are separate strains of trypanosomes (Hornby, 1952). However, the severity of infection depend on a number of factors which includes the virulence of the strain of parasite, the innate resistance of the host, nutritional factors and the presence or absence of secondary infections (Anosa, 1981). Other factors include, the level or size of challenge, duration of exposure to the tsetse-flies, species and breed of the animal host and the age of the animal. In all the cases, the pattern of pathological process includes erythrocyte sequestration characterized by anaemia, damage to tissues including those of the reticulo-endothelial system and the nervous system and fever.

In both man and animals, the disease has been reported in Tropical Africa, Central and South America, the Middle East and Asia (Hoare, 1972).

2.4 AFRICAN ANIMAL TRYPANOSOMIASIS.

There are four groups of trypanosomes that affect domestic animals.

1. The brucei group which includes *T. brucei,* which are salivarian and are cyclically transmitted by tsetse flies, *T. evansi* and *T. equinum* which are transmitted mechanically by stomoxys and tabanids and *T. equiperdum* which requires veneral transmission.
2. The congolense group which includes *T. congolense* and *T. simiae* which are salivarian.
3. The vivax group which includes *T.* vivax and *T. uniforme* which are salivarian; and,
4. *T. theileria* which is a large non-pathogenic species that often develops in tabanids.

However, the main species that produce disease in livestock are *T. vivax, T. congolense* and *T. brucei. T. vivax* is of central interest in this investigation hence it is described below.

T. vivax is the causative agent of one of the most important and widespread forms of trypanosomiasis in livestock in tropical Africa. *T. vivax* is prevalent throughout the wide area of distribution of its tsetse-fly vectors, though in some localities it is found outside the tsetse-belt. It occurs in most parts of West, Central, East and South Africa. It is pathogenic to various ungulates including cattle, sheep, goats, horses and camels, causing the disease known as Surra. It has also established itself in bovines in West Indies, Central and South America and Mauritius (Hoare, 1972).

During its development in the mammalian host after a bite from tsetse, the parasites are restricted to the subcutaneous tissues during the first 48 hours when the

metatrypanosomes are transformed into blood forms, which then invade the lymph glands and the blood. Apart from cyclical transmission by tsetse flies which are the true intermediate hosts of *T. vivax*, transmission by mechanical inoculators represented by other blood sucking Diptera like Tabanid flies occurs (Hoare, 1972). *T. vivax* is made up of a number of strains differing in their mode of transmission as follows: (a) strains in the tsetse belts which are maintained by cyclical transmission through the natural intermediate host Glossina spp. (b) strains in tsetse-free areas of Africa which are transmitted mechanically by other insects with or without loss of transmissibility by Glossina, according to the degree of contact with tsetse present in adjacent territories, and (c) strains outside Africa that depends exclusively upon mechanical transmission by local vectors, while their ability to develop in Glossina has been lost irretrievably (Hoare, 1972).

The course of infection with *T. vivax* varies in different hosts and according to the strain of parasite. The incubation period in cattle may vary between 9 to 14 days in the case of virulent strains and 9 to 59 days in the case of mild ones (Hoare, 1972). In established infections, parasitaemia fluctuates considerably, the trypanosomes appearing and disappearing from the blood at irregular intervals. Sometimes they may be numerous in the morning and scanty in the afternoon of the same day (Van Hoof *et al.*, 1948) but as a rule there is an initial period of parasitaemia lasting up to several months, after which the trypanosomes are undetectable in the blood, or alternating periods of crises and relapses follow. In the early stages of the infection, *T. vivax* may invade the lymph glands.

The infection in different areas varies according to the strain of parasite and the resistance of the host. In some localities the disease may be severe and of short duration

while in others it runs a chronic course (Hoare, 1972). Animals usually succumb to the infection if left untreated. Thus in Nigeria, the infection in Zebu cattle may result in 100 per cent mortality 3 to 4 months after exposure to tsetse (Unsworth, 1953).

The clinical symptoms of the disease depends largely upon the immune response of the host. In Nigeria, the course of the disease varies with the breed of cattle affected (Chandler, 1952; Desowitz, 1959). In tsetse areas, Zebu are highly susceptible to infection which may run an acute course with high mortality or a chronic course with eventual recovery, but the N'dama and Muturu breeds posses a high degree of tolerance, the disease in these breeds being transient and may terminate in self cure. But animals born and bred in tsetse-free localities are as susceptible as Zebu, the disease being chronic in the case of N'dama and acute and fatal in Muturu. In general, N'dama cattle are able to survive under conditions which are rapidly fatal for Zebu. Chandler (1958) believed that this tolerance to *T. vivax* infection is an inherent property of N'dama cattle while Desowitz (1959) believed that it is due to acquired hyper-immunity. Though the exact type of immunity to *T. vivax* which develop in Nigerian cattle is not clear, it appears to be the premunition type (Hoare, 1972) since in N'dama cattle previous exposure to infection enhances their resistance to a new challenge (Chandler, 1958).

The reaction of cattle to infection with *T. vivax* is also influenced by their state of nutrition, the disease being severe in under-nourished animals and benign in those that are well fed (Hoare, 1972).

2.5 GENERAL PATTERN OF TRYPANOSOMIASIS.

The disease process of animal trypanosomiases is complex and variable depending upon the nature and stage of the infection. The severity of trypanosome

infection depends on a number of factors, including the virulence of the strain of parasite, the innate resistance of the host, nutritional factors and the presence or absence of secondary infections. Different combinations of these factors produce varying severities of disease ranging from subclinical infections with no apparent ill-health to chronic, sub-acute, acute and hyperacute diseases (Anosa, 1981). The pathological process in all forms of animal trypanosomiasis is probably similar (Fiennes, 1970), however, with a given species of trypanosome the severity of anaemia is related to the level of parasitaemia.

Generally, trypanosome infection can be divided into two main phases, a 'crisis' phase and a 'post-crisis' or recovery phase (Anosa, 1981).

CRISIS PHASE: This phase follows the first appearance of parasites in blood. During crisis, the intensity and frequency of parasitaemia are high, red cell volumes begin to drop progressively, macrocytosis develops in most cases and leucopenia is also present (Naylor, 1971a; Anosa *et al.*, 1977; Valli *et al.*, 1978; Anosa and Isoun, 1980). In this phase, the disease may be peracute (hyperacute), acute or subacute (chronic) and animals usually die during this phase.

(a) Peracute (hyperacute) course: This form of the disease is characterized by severe haemolysis accompanied by hydraemia and it is rapidly fatal. This has been reported in West African cattle (Stephen, 1970).

(b) Acute Course: In this form, the haemolysis is less severe in the early stages. There is initial hydraemia which is followed by dehydration. Death occurs at the time of haemolytic crisis.

(c) Subacute course: The early stages of the subacute course are similar to those of the acute course but hydraemia persists without dehydration. The haemolytic crisis

if present, does not prove fatal.

(d) **Chronic course:** The animal enters a chronic wasting disease which persist for months and may eventually prove fatal.

POST-CRISIS OR RECOVERY PHASE: Animals that survive the crisis phase lapse into a post-crisis phase during which the frequency and intensity of parasitaemia wane, red cell and leucocyte values begin to recover, sometimes attaining normal values, and normocytosis and microcytosis supervenes (Naylor, 1971b; Anosa et al., 1977; Valli *et al.*, 1978; Anosa and Isoun, 1980). Fiennes (1954) reported that this phase may become a wasting period characterized by 'physiological starvation' in which inanition exists and animals eventually die despite abundant supplies of food.

2.6 CLINICAL MANIFESTATION.

The first evidence of infection is a rise in body temperature 5 to 7 days after infection. Symptoms then develop over weeks, however lactating animals develop symptoms more quickly with reduced milk yield. The animals occasionally go off feed, become dull and lack energy. There is also a significant loss of body weight. The major clinical symptom of the disease in cattle is anaemia and intermittent fever which are directly related to levels of parasitaemia. In less severe cases in cattle, the hair coat becomes staring, the superficial lymph nodes, particularly the prescapulars and prefemorals are enlarged. In the terminal stage of the disease, the animals becomes very emaciated and swellings may appear along the belly and dew lap. The animal now extremely weak and lethargic often is unable to rise and dies in a state of exhaustion between 3 to 12 months after infection. However, in peracute cases, especially with *T.*

vivax in cattle, the disease resembles a septicaemia and the animal may die within a week.

Generally, the outcome of African trypanosomiasis in the bovine may be death, complete or partial recovery, or more commonly a syndrome characterized by stunting, wasting and persistent low grade anaemia, which have been termed "chronic trypanosomiasis syndrome" (Murray, 1978).

The manifestations of cellular injury in trypanosomiasis are many and varied and arise in a number of different ways. Variations occur in different hosts and with different trypanosome species. Though there may be common underlying mechanisms of pathogenesis, different abnormalities may result because of the predominance of one or more of the mechanisms. The main lesions are anaemia with *T. congolense* and *T. vivax* and generalized inflammation with the *T. brucei* sub-group organisms. Many different interacting and sequential events occur in trypanosomiasis that the actual outcome of any particular infection will depend on which of the pathological pathways breaks first. Furthermore, under field conditions, animals may be infected with more than one species of trypanosome, and the resulting pathologic consequences may take a variety of forms (Ford, 1971; Hoare, 1972).

2.7 ANAEMIA IN TRYPANOSOMIASIS.

The cardinal sign and major disease-promoting factor of cattle African trypanosomiasis is anaemia. It is the consensus that the anaemia is haemolytic in origin, occurring intravascularly in the acute phase and also extravascularly in the subacute and chronic course of the disease, but the mechanism of the haemolysis is the subject of numerous investigations. Despite it's importance it has many unexplained aspects about

which reports frequently appear to conflict. Several excellent reviews (Losos and Ikede, 1972; Clarkson, 1976; Jennings, 1976; Murray, 1978; Tabel, 1978; Ikede *et al.*, 1988; Esievo and Saror, 1991) are available on the pathogenesis and pathology of animal trypanosomiasis. Numerous factors have been advanced as being responsible, such as haemodilution, bone marrow depression, erythrophagocytosis, haemolysis of erythrocytes either by haemolytic factors or by immunological means and reduced erythrocyte life-span (Murray, 1978; Esievo and Saror, 1991).

Based on the presence or absence of trypanosomes and on clinical and pathological findings, Murray (1978) in a review divided the diseases into three distinct but overlapping phases. In the first phase which may last from 3 to 12 weeks resulting in the death of the animal, the onset and severity of the anaemia is directly related to the level of parasitaemia. The anaemia is haemolytic (Mamo and Holmes, 1975; Dargie and Alesandro, 1978) and red cell destruction is mainly by phagocytosis by the expanded and active mononuclear phagocytic system, MPS (Murray, 1974; Murray *et al.*, 1978a). There is massive red cell sequestration and erythrophagocytosis as well as striking lymphoid hyperplasia resulting in marked splenomegaly. Erythrophagocytosis occurs in the spleen, the liver, lungs, hemal nodes, bone marrow and circulation (Murray, 1978). The anaemia in this phase depends on the presence of trypanosomes and has a multifactorial etiology that may include haemolytic factors produced by the trypanosome, immunologic mechanisms, fever, disseminated intravascular coagulation (DIC) and an active MPS.

Each of these factors may function independently, or they may interact together thus causing erythrophagocytosis. Cattle that survive phase one pass into phase two which may last for several months. This phase is characterized by transient and scanty

parasitaemia (often parasites cannot be detected) and by a persistent low grade anaemia. Erythrocyte destruction by the expanded and active MPS continues, although splenomegaly is no longer a consistent feature (Dargie and Alesandro, 1978; Murray *et al.*, 1978b). There is also increased hemosiderin deposition throughout the body during this phase.

Cattle that survive phase two pass into phase three which may be ongoing or may end in the animals death or recovery. This phase is characterized by the absence of parasite in blood and tissues which means that the disease continues in the absence of parasites (Murray, 1978). In this phase, there is continued red cell destruction, iron trapping and dyshemopoiesis. Even in the absence of splenomegaly, erythrophagocytosis is still found throughout the body. Hemosiderosis is widespread in the spleen, lungs, liver and bone marrow, and the femoral marrow is often yellow, gelatinous, and inactive. Animals may die, but many remain alive in poor health characterized by wasting or stunting.

The continuing erythrocyte destruction by the MPS in the absence of trypanosomes in phases two and three may be due to the initial stimulation of the MPS by the massive trypanosome parasitaemia long before the disappearance of the parasite, thereby causing hypersequestration and increased red cell haemolysis. A similar observation has been reported in rats (Jandl *et al.*, 1965).

Esievo and Saror (1991) in a review on the possible causes of anaemia laid emphasis on erythrophagocytosis and reduced erythrocyte life-span from loss of erythrocytes sialic acids.

Phagocytosis of erythrocytes is a phenomenon that contributes to the anaemia observed in animal trypanosomiasis (Evievo and Saror, 1991). This phenomenon has

been observed in *T. brucei* infected rabbits (Boycott and Price-Jones, 1913). *T.* brucei infected rats and mice (Jennings et al., 1974; Anosa and Kaneko, 1983 a,b) *T. congolense* infected sheep (Mackenzie and Cruickshank, 1973) *T. vivax* infected goats (Saror, 1980) and *T. congolense* infected cattle (Fiennes, 1954; Naylor, 1971ab; Losos *et al.*, 1973; Valli and Forsberg, 1979). Among the causes of erythrophagocytosis are the attachment of trypanosome antigen to the surface of red cells, their subsequent combination with anti-trypanosome antibody in the plasma and eventual sequestration by erythrophagocytosis (Jennings, 1976). Mackenzie *et al.* (1978) demonstrated trypanosomal material on erythrocytes of sheep infected with *T. congolense* and concluded that the phagocytosis of the erythrocytes was due to the coating of the surface of red cells with trypanosomal antigen.

However, with the production of sialidase (neuraminidase) by *T. vivax* (Esievo, 1979) and the cleavage of erythrocyte surface sialic (neuraminic) acid during bovine trypanosomiasis (Esievo *et al.*, 1982a) reveal new antigenic sites on affected erythrocytes (Pirofsky, 1969). Esievo *et al.* (1982a) therefore postulated that probably with time during the course of the disease, antibody would be produced against these new antigenic sites on the erythrocytes to render them suitable for erythrophagocytosis.

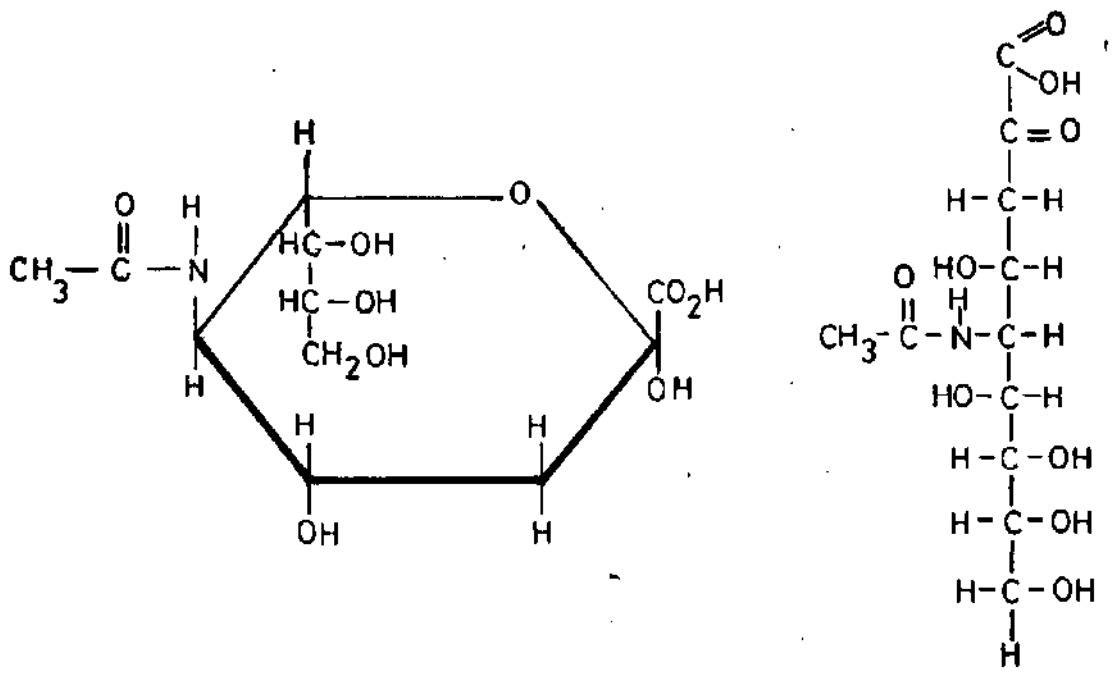
The contribution of reduced erythrocyte life-span due to the removal of surface sialic acid to anaemia was also reviewed by Esievo and Saror (1991) based on some erythrokinetic studies in animals infected with pathogenic African trypanosomes. The existence of an increased rate of red cell destruction characterized by the presence of haemosiderosis and erythrophagocytosis in the cells of the reticuloendothelial system (Boycott and Price-Jones, 1913; Fiennes, 1970; Naylor, 1971b) and bilirubinaemia (Fiennes, 1970) is recognized in the anaemia observed in trypanosomiasis. Studies using

^{51}Cr-labeled red cells also confirmed the existence of accelerated red cell destruction in *T. brucei* infection of rodents (Jennings *et al.*, 1974; Anosa *et al, 1977) T. congolense* infection of cattle (Mamo and Holmes, 1975; Valli *et al.*, 1978; Dargie *et al.*, 1979ab), *T. vivax* infections of sheep (Anosa and Isoun, 1980) and *T. rhodesiense* infections of man (Woodruff *et al.*, 1973). The use of ^{51}Cr-labeled red cells has shown that red cell survival and total red cell mass are reduced, and that the spleen and in some cases the liver is the major site of red cell destruction in trypanosomiasis (Anosa, 1981). While there was a consensus that red cell life-span is reduced, the mechanism involved is not firmly established, but it was speculated that sialic acid removal might be partly responsible since sialic acid concentrations is high in young red cells and low in old red cells and loss of sialic acid is recognized as an ageing process (Yaari, 1969; Durocher *et al.*, 1975).

2.8 SIALIC ACIDS.

Sialic acids (sia, derived from the Greek word 'siales' meaning saliva) form a class of important ketoses that contain nine carbon atoms (ketononoses) and are acylated derivatives of 2 keto-5-amino, 3,5-dideoxy D nonulosonic acid called neuraminic acid. It is made up of a family of 36 naturally occurring derivatives of neuraminic acid that are all N-acylated to form N-acetyl-neuraminic acid (Neu5Ac) or N-glycolylneuraminic acid (Neu5Gc) as fundamental molecules and 12 of them are found as components of glycoproteins from the submandibular glands of cow, pig and horses (; Corfield and Schauer, 1982; Reuter *et al.*, 1983; Reuter and Schauer, 1988; Varki, 1992; Engstler *et al.*, 1993; Engstler and Schauer, 1993; Schauer *et al.*, 1995). As a result of the variation of substituents at carbon 4, 5, 7, 8 and 9, additional modifications may result from 0-

acetylation at one or several of the hydroxyl functions at these carbon atoms or to the introduction of a double bond between C-2 and C-3 (Smith *et al.*, 1985a; Schauer *et al.*, 1995).



N-Acetyl-D-neuraminic acid.

These acidic sugars are found throughout in the deuterostomate (echinoderms to mammals) branch of metazoa, viruses and in some microorganisms such as bacteria and protozoa (Schauer *et al.*, 1984; Roggentin *et al.*, 1993; Engstler and Schauer, 1993; Schauer *et al*, 1995). Sialic acids are also found in a large number of higher animals, and on cell membranes and in body fluids of all mammals (Schauer *et al.*, 1995).

The saturated sialic acids usually occupy terminal, non-reducing positions of oligosaccharide chains of complex carbohydrates on outer and inner (for example lysosomal) membrane surfaces in various linkages mainly to galactose, N-acetylgalacytosamine, and to sialic acid itself (Schauer *et al.*, 1995). In the higher

metazoans, they are primarily ∝(2-3)- or ∝(2-6)-glycosidically linked to subterminal sugars in oligosaccharides, glycoproteins and glycolipids; ∝(2-8) linkages are found in gangliosides and glycoproteins (Corfield and Schauer, 1982). A few bacterial species produce sialic acids as ∝(2-8), ∝(2-9) or alternatively ∝(2-8)- ∝(2-9) homopolymers (Silver and Vimr, 1990) as an integral component of capsular heteropolysaccharides or as part of the core region in the lipopolysaccharide of Rhodobacter species (Krauss *et al.*, 1988). Most of these bacteria are infectious for animals and use capsules for escaping the host immune system.

N-acetylneuraminic acid (NeuNac, Neu5Ac or NANA) is the most common neuraminic acid derivative which has been found exclusively or together with other neuraminic acid derivatives in many bacteria and animal tissues and in most mammals it may either be N-acetyl or N-glycolyl derivatives (Warren, 1959; Jourdian *et al.*, 1971; Schauer, 1978; Smith *et al.*, 1985a). The ratios of these two derivatives differ in various tissues with different species. For instance, bovine, ovine and porcine mucins of saliva contain mostly the N-acetyl derivative whereas the N-glycolyl group predominates in erythrocytes stroma of these species. Similarly, the 0-acetylated sialic acids is the major type on the surface of erythrocytes in N'dama cattle while the glycolyl groups predominate in the Zebus erythrocyte (Shugaba *et al.*, 1994). In the bacteria, in addition to Neu5Ac (Moran et al., 1991) in few cases Neu5, $9AC_2$ (Varki and Higa, 1993) were identified.

Sialic acids are widely distributed in nature and they are formed in the cells by the condensation of D-mannosamine derivatives and pyruvic acid (Pigman, 1977). Members of the sialic acid family of compounds occurs as integral parts of the Oligosaccharide units in mucins, glycoproteins and gangliosides (Warren, 1959;

Jourdian *et al.*, 1971; Reuter *et al.*, 1983;). They occur as terminal sugars in many of these Oligosaccharides and glycoconjugates (Nohle and Schauer, 1981; Nohle *et al.*, 1982; Schauer *et al*, 1995). Sialic acids are found in milk oligosaccharide (Jourdian *et al.*, 1971) whey proteins (Bezhorovainy *et al.*, 1970) and certain microbial polymers (Warren, 1959; Jourdian *et al.*, 1971; Smith *et al.*, 1985a). These acids have been found to occur on the surface coats of certain trypanosomes (Schauer *et al*, 1983; Esievo and Saror, 1991) particularly *T. congolense* (Rautenberg *et al.*, 1981) and *T. cruzi* (Schauer *et al.*, 1983).

Sialic acid is an indigenous component of the erythrocytes of many different animal species (Eylar *et al.*, 1962;Aminoff *et al.*, 1977; Esievo *et al.*, 1982a;Schauer *et al.*, 1984; Kelm *et al.*, 1986b;). Glycophorin, the major integral protein of the erythrocyte is a glycoprotein containing both 0- and N-linked oligosaccharide which form a region on the external surface of the cell that provides about 75 percent of the cell surface sialic acid (Brown, 1980; Smith *et al.*, 1985a;). The sialic acid content of circulating erythrocytes varies with the age of the erythrocyte. In older erythrocytes, sialic acid content is 10-15 percent less than in younger erythrocytes (Danon, 1966; Yaari, 1969; Gregoriadis *et al.*, 1974).

In addition to the long established species - specific pattern of sialic acids (Corfield and Schauer, 1982; Varki, 1992) there is also strain, tissue or cell specificity within a given species and a developmental regulation of the expression of certain sialic acids (Schauer *et al.*, 1995). For instance, in a comparative analysis study, Schauer *et al.* (1991) reported a concentration of 0.1-3.1mg total sialic acids/g wet weight in adult bovine tissues while in the corresponding fetal tissues the sialic acid concentration was higher and ranged from 1.1-12.5 mg/g wet tissue. The percentage of Neu5Gc in the total

sialic acid pool was always greater in the adult than in the fetus, ranging from 9 to 66 percent and 0 to 49 percent respectively. The sialic acid composition of mouse erythrocytes also show great variation among the strains analyzed (Klotz *et al.*, 1992). Developmentally regulated expression of sialic acids have also been reported in chicken erythrocytes (Herrler *et al.*, 1987), insects (Roth *et al.*, 1992) hibernating animals (Rahmann *et al.*, 1987) and others.

2.8.1 BIOLOGICAL ROLES OF SIALIC ACIDS.

Sialic acids are among the first molecules encountered by other cells or by compounds coming into contact with the cell, a feature that is important in the expression of their biological role (Schauer, 1982a; Schauer *et al.*, 1991, 1995). The functions of sialic acids may be grouped into three sections:

1) a less specific, more general role, which is related to the fact that sialic acids are relatively large, hydrophilic and acidic molecules that exert physicochemical effects on the glycoconjugates to which they are bound, and on the environmental molecules *in situ* for example in cell membranes. The sialyl groups therefore influence and stabilize the conformation of both the glycan and the protein parts of glycoconjugates, resulting in modified properties such as higher thermal and proteolytic stability of glycoproteins (Schauer, 1982ab). Mucin glycoproteins of vertebrates which have a protective and lubricative effects are highly sialylated. Indeed, many physical and biological functions of glycoproteins and gangliosides can be attributed to their sialic acid component. Sialic acids play important roles in a variety of biological processes including the maintenance of serum glycoproteins in circulation (Ashwell and Morell, 1974, 1978; Aminoff *et al.*, 1977; Schauer *et al.*, 1995) and they also play a major role in the chemical and biological

diversity of glycoconjugates. Cell-type specific expression of glycosyltransferases particularly of sialytransferases (Paulson and Colley, 1989; Van den Eijnden and Joziasse, 1993) leads to specific sialylation patterns of oligosaccharides which can be considered as key determinants in the make-up of cells.

2) The most important role of sialic acids is anti-recognition (Schauer, 1982ab). Sialic acids acts as masks on serum glycoproteins, erythrocytes and a host of other receptors to prevent biological recognition for example of subterminal galactose residues (Schauer, 1982a, 1985; Schauer *et al.*, 1995). The best studied example of the masking function is the binding and uptake of desialylated serum glycoproteins by hepatocytes (Ashwell and Harford; 1982). Sialic acid is an important factor for the regulation of the life-time of erythrocytes and serum glycoproteins in circulation (Ashwell and Morell, 1974, 1978; Schauer, 1982ab). Loss of this sugar leads to rapid and irreversible removal of red blood cells from the circulation, hence it gives a general shield on cell surfaces by their negative charge (Kuster and Schauer, 1981; Schauer, 1982ab; Schauer *et al.*, 1984 Kelm *et al.*, 1986ab). This is also true with lymphocytes and thrombocytes (Kaufmann *et al.*, 1981).

Anti-recognition is also exerted by sialic acids in other system for example, the reactivity of IgG with the Fc receptor of human lymphocytes (Schuloff *et al.*, 1980), the number of receptors for luteinizing hormone (LH) and human chorionic gonadotropin (HCG), in rat ovary (Ueer and Engel, 1981), the amount of carrier sites for dopamine in nerve cell membranes (Dreyfus *et al.*, 1983), the accessibility of β-adrenergic receptors in rat heart, the sensitivity of cells from human cell lines to diphtheria toxin and *Pseudomonas aeruginosa* exotoxin (Mekada *et al.*, 1979) or the complement-dependent cytotoxicity against autologous, invasive human bladder tumor cells (Jacobsen, 1982).

Sialic acids can mask antigenic sites of molecules and of normal or malignant cells (Schauer, 1982ab).

3) Sialic acids may also serve as recognition sites (Schauer, 1982a, b; 1985; Schauer *et al.*, 1984). They are components of receptors for hormones, bacterial toxins and infectious agents (for example viruses and mycoplasma). Thus they play a role in the adsorption of toxins (Hansson *et al.*, 1977), virus (Howe and Lee, 1972; Carroll *et al.*, 1981) and antibodies (Sadler *et al.*, 1979) to cell surfaces and the regulation of certain aspects of cellular communication (Liu *et al.*, 1982) and differentiation (Town and Stanford, 1979). Sialic acids also influence the shape of cells because of their negative charge and the conformation of macromolecules. This has various metabolic (for instance hindrance of the activity of proteases) and biological consequences (for instance influence on the viscosity of some mucins and masking of serum glycoproteins). They also bind positively charged compounds with nutritive and other physiological effects and thus help to regulate the numerous functions of cells (Schauer *et al.*, 1984).

2.8.2 MODE OF MASKING BY SIALIC ACIDS.

Sialic acid is relatively a strong acid (pka = 2.6) and it is essentially deprotonated at physiological pH, thereby providing a negative charge at the surface of cells hence protecting the life-time of molecules and cells. Treatment of cells with neuraminidases or sialidase alters this charge (Pereira, 1983). This masking effect may be caused by the bulkiness of sialic acid molecule, a N-acylated polyhydroxyamino acid (Schauer *et al.*, 1984, Schauer, 1985). The carboxyl group is assumed to give the sialic acid residue a conformation suitable to shield the penultimate sugar effectively. Reduction of the carboxyl groups of sialic acid, or in fetuin or glycophorin led to a decrease of the

masking effects of these molecules (Schauer *et al.*, 1984). However, the masking by sialic acid is not due to the anomeric configuration of the glycosidic bond of this sugar nor is it confined to the side chains of the sugar molecules (Schauer *et al.*, 1984).

2.9 SIALIDASES.

Sialidases (neuraminidase, acylneuraminyl hydrolases; EC 3:2:1:18) belong to a class of glycosyl hydrolases that release terminal N-acylneuraminic acid (sialic acid) residues from glycoproteins, glycolipids and polysaccharides. The efficiency of cleavage by sialidases depends on the source of the enzyme, the nature of the glycosidic linkage and the type of substrate (Roggentin *et al.*, 1993, 1995; Engstler and Schauer, 1993; Schauer *et al.*, 1995). These enzymes as well as their substrates are common in animals of the deuterostomate lineage (Echinodermata through mammalia) and also in diverse micro-organisms that mostly exist as animal commensals or pathogens. Thus parasites, bacteria, viruses, trypanosomes and mammals are known to produce sialidases (Veh *et al.*, 1979; Esievo, 1979, 1983; Muller and Schauer, 1983; Corfield *et al.*, 1983; Schauer *et al.*, 1983; Shehu *et al.*, 1991; Roggentin *et al.*, 1993). Most of the micro-organisms that produce these enzymes are symbionts or pathogens of higher organisms. Sialidases and their sialyl substrates appear to be absent from plants and most metazoans. Among bacteria, sialidase is found irregularly so that related species like *Clostridum sordellii* and *C. bifermentans* (Roggentin *et al.*, 1985) or even strains of one species like *C. butyricum* (Popoff and Dodin, 1985) differ with respect to sialidase production (Schauer *et al.*, 1995). This unusual phylogenetic distribution of the enzyme indicates that sialidases evolved by other mechanisms than their producers in the bacteria kingdom.

Although sialidases have been suggested as pathogenic factors in microbial infections, their specific role still remains unclear (Roggentin and Schauer, 1992). In recent developments, however, trypanosomal sialidases have provided the opportunity to assign a direct pathophysiological role to these enzymes (Esievo *et al*, 1982a; Engstler and Schauer, 1993).

2.9.1 AFRICAN TRYPANOSOMES SIALIDASES.

For more than two decades, African trypanosomes have attracted great scientific interest. Before the extensive investigation by Engstler *et al.* (1995), little information was available about sialidases from African trypanosomes. First reports on the occurrence of sialidase in *T. vivax* was published more than a decade ago (Esievo 1979, 1980, 1983). Trypanosomal sialidases have also been reported from *T. brucei* group and *T. congolense* (Engstler *et al.*, 1992a,b; 1995).

The bloodstream forms of *T. vivax* exhibit sialidase activity *in vitro* and the degree of enzyme activity could be correlated with the number of trypanosomes (Esievo, 1979, 1980, 1983). Equal number of lysed and unlysed *T. vivax* showed approximately the same enzyme activity suggesting that it was membrane bound and was also inhibited by influenza virus antiserum directed against the viral sialidase (Esievo, 1983). The *T. vivax* sialidase is membrane-bound and developmentally restricted to the mammalian life cycle stage (Engstler *et al.*, 1995). Following purification of plasma membrane by density gradient centrifugation (Engstler *et al.*, 1992b) *T. vivax* sialidase activity was exclusively associated with the isolated membranes. The enzyme activity of *T. vivax* sialidase on intact trypanosomes was decreased by trypsin, Neu5Ac2en (a naturally occurring sialidase inhibitor) and chelating agents such as EDTA while the activity

increased in the presence of calcium ions (Ca^{2+}). It has a catalytic optima at pH 6.0 and 37°C in 100mM Bis-Tris buffer while enzyme activity is sensitive towards alkaline pH conditions (Engstler *et al.*, 1995). *T. vivax* bears no trans-sialidase activity.

Engstler *et al.* (1992a) described a novel developmentally regulated sialidase in procyclic trypomastigotes of *T. brucei* residing within the tsetse-fly's midgut however, the mammalian stage blood stream forms display no enzyme activity. The enzyme is a cell surface protein, linked to the membrane via a modified glycosylphosphatidylinositol (GPI) anchor which is probably identical to the anchor of the procyclic acidic repetitive protein (PARP) which coats the surface of procyclic trypanosomas. The sialidase has a molecular mass of 67KDa and is active over a broad pH and temperature range, with optima at pH 6.9 and 35°C respectively. The enzyme has a strong preference for ∝(2-6)- and ∝(2-8)-linked terminal sialic acids. Oligosaccharides are also better substrates than glycoproteins and gangliosides (Murray and Dexter, 1988). A developmentally regulated trans-sialidase activity was described on the surface of procyclic *Trypanosoma brucei* while the blood stream stages display no trans-sialidase activity (Engstler *et al.*, 1993). This enzyme is capable of transferring sialic acids from a variety of sialoglycoconjugates to asialo-acceptor molecules without requirement for CMP-Neu5Ac. The enzyme is linked to the plasma membrane via a GPI - PLC resistant GPI-anchor. Sialic acids are transferred to ∝(2-3)- positions of terminal β-galactose residues of oligosaccharides and glycoconjugates, at various rates. The enzyme utilizes serum glycoconjugates, human and bovine erythrocytes as sialic acid donors, and resialylates sialidase-treated erythrocytes. It transfers sialic acids from the GPI-anchor of procyclic acidic repetitive protein (PARP) onto lactose and vice-versa (Engstler *et al.*, 1993). Also structures within a variant surface glycoprotein (VSG MIT at 1.7) can be trans-sialylated.

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The trans-sialidase from *T. brucei* shares many features with the sialidase activity of *T. brucei*, suggesting that both activities reside within the same protein. This is of particular significance since sialic acids have been detected in the GPI anchor of the main surface component PARP and yet *T. brucei* lacks the key enzymes of sialic acid anabolism (Engstler *et al.*, 1992a). The *T. brucei* transialidase is capable of sialylating not only PARP, but also sialidase treated erythrocytes and structures within variant surface glycoproteins (VSG) (Engstler and Schauer, 1993). Thus, it is possible that the trans-sialidase activity enables procyclic *T. brucei* to sialylate parasite glycoconjugates in order to protect surface molecules from glycosidases in the digestive tract of the tsetse-fly. Acquisition and distribution of sialic acids could be the true function of *T. brucei* (trans)-sialidase (Engstler and Schauer, 1993).

Both *T. brucei* and *T. cruzi* lack the sialic acid biosynthesis apparatus known to exist in other organisms (Schauer *et al.*, 1983; Engstler *et al.*, 1992a). The involvement of *T. cruzi* sialidase in the acquisition of sialic acids on its surface was therefore proposed earlier (Schauer *et al.*, 1983). The biological significance of this unusual sialylation mechanism is to protect these pathogens from the host's immune or proteolytic defence systems (Engstler and Schauer, 1993). African *T. brucei* and American *T. cruzi* sialidases share some striking similarities. It is however not clear yet why and from which origins such geographically distant species have acquired such specialized enzymes.

Similarly, the trans-sialidases from African and American trypanosomas share a number of striking features, some of which are rather unusual for common sialidases such as the relatively neutral pH-Optimum (Schenkman *et al.*, 1992), and lacking requirement for divalent cations (Pereira, 1983). The two enzymes are developmentally

regulated (Pereira, 1983; Engstler *et al.*, 1992a). They are surface glycoproteins and anchored via a GPI-structure (Engstler *et al.*, 1992a). Both enzymes are capable of sialylating surface epitomes (Schenkman *et al.*, 1991). The substrate specificity is very similar for instance, ∝(2-3)-linked sialic acids are much better hydrolyzed and transferred, than the ∝(2-6)-bound sugars and β-galactose is the preferred acceptor saccharide (Vanderkerchhove *et al.*, 1992). Both enzymes utilize neither Neu5Ac nor CMP-Neu5Ac as donor and fail to transfer sialic acids onto methyl-umbelliferon (Schenkman *et al.*, 1992). Also both enzymes are able to sialylate sialidase-treated erythrocytes. All these structural and enzymological similarities lead to the assumption that trypanosomal trans-sialidases might be closely related enzymes (Engstler *et al.*, 1993).

A trans-sialidase is also expressed in the procyclic culture forms of *T. congolense* and it is shed into the culture medium (Engstler *et al.*, 1995). The enzyme has a pH-optimum at pH 7:0, displays sensitivity towards chlorides and is resistant against commonly used sialidase inhibitors (Engstler *et al.*, 1995). It transfers preferentially ∝(2-3)-linked sialic acids onto terminal β-galactose residues. Also hydroxylated sialic acids (Neu5Gc) are transferred. The major glycoprotein GARP from procyclic *T. congolense* was identified as one potential natural sialic acid acceptor on the parasite's surface (Engstler *et al.*, 1995).

A sialidase is expressed in *T. rangeli* without a trans-sialylating activity (Reuter *et al.*, 1987; Engstler *et al.*, 1995). The enzyme is shed into the culture medium. Neither *T. evansi* nor *T. equiperdum* which are taxonomically closely related to the *T. brucei* group express sialidases or trans-sialidases (Engstler *et al.*, 1995). Both species are transmitted mechanically without requirement for the tsetse-fly stage. Similarly, the

absence of both sialidase and trans-sialidase activities was observed in Leishmania, Crithidia, Herpetomonas, Leptomonas and Phytonomas respectively (Engstler *et al.*, 1995). All strains of *T. b. brucei, T. b. rhodesiense* and *T. b. gambiense* species displayed trans-sialidase activity within the procyclic stage while no enzyme activity was detectable in the corresponding mammalian blood stream forms (Engstler *et al.*, 1995).

The occurrence of sialidases and trans-sialidases is not a common feature among the kinetoplastida, but it is restricted to distinct developmental life cycle stages of only a few species (Engstler *et al.*, 1995). The advantage of having a sialidase within the insect vector could possibly be that the sialidase might cleave sialic acids from the ingested blood cells (Engstler and Schauer, 1993). Proteases and other glycosidases act more effectively on glycoproteins after the removal of sialic acids. Accommodation of procyclic *T. brucei* may therefore be an evolutionary advantage for the transmitting flies (Engstler *et al.*, 1992ab). Similarly, the expression of trans-sialidase may be bound up with major biological advantage for the parasite (Engstler *et al.*, 1995). Research so far suggest that the expression of trans-sialidases is restricted to trypanosomes and the expression of active trans-sialidase within the subgenus Trypanozoan may correlate with tsetse-transmission (Engstler *et al.*, 1995). Trans-sialylation is only found in those trypanosomes which have to reside as dividing forms within the tsetse fly's midgut. Species which are transmitted mechanically or by other vectors seem to have no need for a protecting procyclic surface coat or reveal other protective mechanisms (Engstler *et al.*, 1995). Trans-sialylation may therefore be the only way in which Trypanosomes obtain sialic acids (Engstler *et al.*, 1995).

2.9.2 AMERICAN TRYPANOSOMES SIALIDASES

Trypanosomal sialidases have been reported from *T. cruzi* (Pereira, 1983) and *T. rangeli* (Reuter *et al*, 1987). Non-dividing metacyclic and bloodstream trypomastigotes of American *T. cruzi* exhibit surface sialidase activity (Pereira, 1983; Rosenberg *et al.*, 1991). The enzyme is a member of a large gene family (Roggentin *et al.*, 1993) and share conserved sequences with bacterial and viral sialidases (Roggentin *et al.*, 1989, 1993) and has a negative effect on cellular invasion.

A trans-sialidase activity was reported in *T. cruzi* (Cross and Takle, 1993). This enzyme is involved in the assembly of a stage-specific sialylate surface epitome, which is proposed to have a role in the attachment to and penetration of host cells (Engstler and Schauer, 1993). In *T. cruzi* trans-sialylation is involved in interactions between mammalian host cells and the parasites, leading to invasion by the trypanosomes and disruption of the phagosomal membrane (Hall *et al.*, 1992; Schenkman and Eichinger, 1993). Both the *T. cruzi* sialidase and trans-sialidase can be mediated by the same gene product (Engstler and Schauer, 1993).

2.9.3 SIALIDASE DISTRIBUTION AND FUNCTION.

All animals exhibiting sialic acids produce a variety of sialidases with different sub-cellular locations and substrate specificities. Although little is known about these enzymes at the molecular level, the rate of sialic acid cleavage from a respective substrate is a property specific to each sialidase and depends on three factors, viz:

i. The kind of neuraminic acid derivatization;

ii. The type of ketosidic linkage; and,

iii the nature of the penultimate sugar residue (Roggentin *et al.*, 1993). This

specialization may allow animals to control the turnover of their sialoglycoconjugates.

Diverse micro-organisms (some fungi, protozoa, bacteria and viruses) also produce sialidases (Muller, 1974). However, this property is not correlated with the phylogenetic relationship of these species (Roggentin *et al.*, 1993). Similarly, most of the micro-organisms that produce sialidase have close contact with animals as commensals or facultative pathogens. This close interactions between animals and micro-organisms may have led to the exchange of sialidase genes between these groups and also between the micro-organisms involved (Schauer *et al.*, 1995). In some cases, the involvement of these enzyme in pathogenesis has been shown (Esievo, 1979, 1983; Esievo *et al*, 1982b; Shehu *et al.*, 1991; Corfield, 1992; Engstler and Schauer, 1993; Schauer *et al.*, 1995). For instance, these enzymes have been thought to play an important role as a virulence factor by allowing bacterial adhesion to subterminal galactose residues after removal of sialic acids (Gabriel *et al.*, 1984) or as a 'spreading factor' in microbial infection (Ezepchuk *et al.*, 1973). They may also be important for nutrition of the bacteria (Muller, 1992; Corfield, 1992; Godoy *et al.*, 1993) as they initiate the degradation of sialoglycoconjugates (Corfield and Schauer, 1982). Most microbial sialidases preferentially cleave ∝(2-3)-linked sialic acids, which are only common in animals.

2.9.4 SIALIDASE SUBSTRATES.

Natural sialidase substrates are generally terminally bound sialic acids. It is assumed that the diversity of sialic acid structures and their glycosidic linkages have been major factors in sialidase evolution (Roggentin *et al.*, 1993). The substrate specificity of sialidases from viral, bacterial and mammalian sources has been the

subject of considerable interest, with increasing emphasis on the pathological role of the enzymes from micro-organisms and the occurrence of different sialidases in mammalian tissues (Dizeniek, 1973; Rosenberg and Schengrund, 1976). On anomeric specificity studies of six sialidases, Corfield *et al.* (1983), observed that ∝(2-3)-glycosidic linkages are hydrolyzed faster than ∝(2-6) linkage. Sialidase activity was higher with N-acetylneuraminic acid when compared with N-glycolylneuraminic acid. Also core oligosaccharide and protein structure in glycoproteins may influence the rate of release for different glycosidic linkages. Most microbial sialidases including trypanosome sialidases preferentially cleave ∝(2-3)-linked sialic acids which are only common in animals (Roggentin *et al.*, 1993).

2.10 GALACTOSE.

Galactose is a hexose that is present in normal blood and urine in very low concentrations. Increased levels occur occasionally, especially in nursing infants with disorders of digestive function, in rare cases of the familial disease called galactose intolerance, and in the congenital disease galactosemia.

Two general methods were formerly used for quantification of galactose; (a) the orcinol reaction and (b) measurement of reducing substances after the destruction of glucose either by yeast fermentation or treatment with glucose oxidase (Waldstein *et al.*, 1964). Removal of glucose is not complete, however, and other substances in plasma interfere to a greater or lesser extent depending upon the specificity of the particular method chosen for measuring reducing substances. A more specific method involves the use of galactose oxidase which oxidizes galactose at the sixth carbon atom to yield D-galactohexodialdose. In addition to its reaction with galactose, galactose oxidase

catalyzes the oxidation of galactosamine and N-acetylgalactosamine (Sempere *et al.*, 1965).

Early hint on the importance of galactose for cellular interaction were derived from experiments with sialidase-treated lymphocytes or erythrocytes (Woodruff and Gesner, 1969; Jancik and Schauer, 1974) which are rapidly sequestrated from the blood stream. Molecules, particles and cells exposing terminal D-galactosyl and N-acetyl-D-galactosaminyl residues do not stay in circulation but rapidly accumulate in the liver (Muller *et al.*, 1981; Ashwell and Harford, 1982). Treatment of native glycoprotein and erythrocytes with neuraminidase exposes galactose as the terminal non-reducing sugar of the protein-linked carbohydrate chains and serves as a specific determinant for the hepatic recognition of the sialic acid deficient molecules (Ashwell and Morell, 1974). Autologous transfusion of sialidase-treated erythrocytes results in their rapid disappearance from the circulation. This has been observed in several different species such as rats (Gregoriadis *et al.*, 1974; Durocher *et al.*, 1974), rabbits (Durocher *et al.*, 1974; Jancik and Schauer, 1974; Aminoff *et al.*, 1977), dogs (Aminoff *et al.*, 1976), goats (Aminoff *et al.*, 1976) and human beings (Jancik *et al.*, 1975).

2.11 MACROPHAGES.

Macrophages are mononuclear phagocytic cells that originate from bone marrow precursor cells. After differentiating and maturing in the bone marrow they are released into the blood where they circulate as monocytes for about 12 hours before entering the tissues and becoming macrophages.

Macrophage constitute one of the special system of the body for combating the different infectious and toxic agents by: (I) actually destroying invading agents by the process of phagocytosis and; (ii) by forming antibodies and sensitized lymphocytes, one

or both of which may destroy the invader (Guyton, 1981). The wandering or non-fixed macrophage (histocyte) are normally present and function in the spleen, bone marrow, liver, lungs, stomach and intestine and are also found at sites of inflammation and in peritoneal, pleural and synovial fluids. Tissue or fixed macrophages known as kupffer cells, in the liver, reticulum cells in lymph nodes, spleen and bone marrow, tissue histocytes, clasmatocytes or fixed macrophages in subcutaneous tissues and microglia in the brain are also known (Brown, 1980; Guyton, 1981). Both the wandering macrophages and fixed tissue macrophages are collectively called the reticuloendothelial system (RES) or Mononuclear phagocytic system (MPS).

Macrophages constitute a significant proportion of invading cells in the spleen, liver, testes, heart and kidney (Anosa and Isoun, 1980) and they have been implicated as contributing actively to immunosuppression in African trypanosomiasis (Corsini *et al.*, 1977; Wellhausen and Mansfield, 1979). Monocytosis apparently reflecting increased bone marrow production of mononuclear phagocytic cells, has been observed in *T. brucei* infected mice (Anosa, 1980) as well as *T. vivax* infection of sheep and goats (Anosa and Isoun, 1980). Monocytes are the recognized precursors of macrophages in the tissues including kupffer cells of the liver (Furth *et al.*, 1972; Carr and Deams, 1980).

The liver is a heterogenous tissue, about 70% of the cells present sare hepatocytes (parenchymal cells) with the largest population of non-parenchymal cells being the reticuloendothelial cells which line the liver sinusoids. Two types of RES have been functionally and morphologically distinguished in liver; phagocytically active kupffer cells (Scott and Deane, 1966) and other phagocytically inactive endothelial cells (Montgomery-Bissell *et al.*, 1972). The hepatic

reticuloendothelial system is the main filter organ of the body (Benacerraf *et al.*, 1975) and most colloidal and macromolecular foreign materials and bacteria are cleared from the blood by the macrophages by phagocytosis (Brown, 1980; Mohr *et al.*, 1987). Therefore, one important physiological function of these macrophages is to distinguish between "self" and "non self" or altered. Recognition can occur by lectin mediated cell contacts (Kolb *et al.*, 1981; Muller *et al.*, 1981; Mohr *et al.*, 1987) or by the production of antibodies which binds and removes the foreign structure (Tsagumis *et al.*, 1985) with their subsequent binding to the receptors on the liver macrophages. The macrophage is also able to phagocytose old and damaged cells like erythrocytes (Brown, 1980).

These macrophages contain lectin-like receptors on their surfaces (Kolb and Kolb-Bachofen, 1978) and more specifically, they contain β-galactose specific lectin (Muller and Schauer, 1983; Muller *et al.*, 1983a; Kelm and Schauer, 1986; Mohr *et al.*, 1987; Kempka *et al.*, 1990; Esievo and Saror, 1991;). The occurrence of galactose-specific receptors on rat liver hepatocytes (Ashwell and Harford, 1982; Kolb-Bachofen, 1988; Mohr *et al.*, 1987), liver macrophages (kupffer cells) (Kolb and Kolb-Bachofen, 1978; Kolb-Bachofen *et al.*, 1982; Kolb-Bachofen *et al.*, 1984; Kolb-Bachofen, 1988) endothelial cells (Kolb-Bachofen *et al.*, 1982; Kolb-Bachofen *et al.*, 1984), peritoneum (Kuster and Schauer, 1981) is well documented.

Although the receptor activities expressed on the different liver cell types are of similar or identical sugar specificity (Kolb *et al.*, 1980) the galactose specific receptors of hepatocytes and liver macrophages differ in molecular weight, membrane anchorage (Roos *et al.*, 1985) and plasma membrane arrangement (Schlepper-Schafer *et al.*, 1986a). The two receptor activities also differ with respect to their ability in mediating

uptake of particulate ligands. Hepatocytes from adult rat liver endocytose molecular ligands and small particles exposing terminal galactosyl moieties up to 8nm in diameter only (Schlepper-Schafer *et al.*, 1986b) whereas liver macrophages take up large particulate ligands exposing the same terminal moieties as well as desialylated erythrocytes (Kolb-Bachofen *et al.*, 1983) and asialolymphocytes (Woodruff and Woodruff, 1976). Endothelial cells take up small and intermediate sized particles (Kolb-Bachofen, 1988). Macrophages accumulate particulate ligands of all sizes but are ineffective in uptake of molecular ligands. This observed differences in binding and internalization of molecular and particulate ligands between hepatocytes and liver macrophages was hypothetically explained to be due to differences in binding site arrangement on plasma membrane which is statistically distributed on hepatocytes and in preaggregated clusters on liver macrophages (Kolb-Bachofen *et al.*, 1982; Kolb-Bachofen, 1986; Dini and Kolb-Bachofen, 1989). The preclustered receptor arrangement on macrophages and endothelial cells enables them to avidly bind large particles over such a receptor cluster by multiple binding (Kolb-Bachofen *et al.*, 1984).

Galactose-specific receptors on macrophages have been demonstrated via binding of sialidase-treated cells (Kolb and Kolb-Bachofen, 1978; Nagamura and Kolb, 1979; Kuster and Schauer, 1981) or gold particles coated with glycoprotein (Kolb-Bachofen *et al.*, 1982; Kempka and Kolb-Bachofen, 1985; Kolb-Bachofen, 1988;) and by inhibition of these interactions by free galactose or compounds with terminal galactosyl groups (Kolb and Kolb-Bachofen, 1978;Muller *et al.*, 1981; Mohr *et al.*, 1987). Sialidase treatment causes mammalian erythrocytes to become "non self" particles. They are rapidly eliminated from the circulation *in vivo* (Jancik *et al.*, 1978). Phagocytes in liver and spleen participate in erythrocyte sequestration (Ashwell and

Morell, 1974; Jancik *et al.*, 1978).

2.12 LECTINS.

Proteins that possess the remarkable ability to agglutinate erythrocytes and other types of cells are widely distributed in nature. Such agglutinins are found predominantly in the seeds of plants, in particular those of legumes, but they are also present in other parts of plants such as roots, leaves and bark (Sharon and Lis, 1972). They also occur in invertebrate like snails and the horse-shoe crab, and lower vertebrates such as fish (Pardoe and Uhlenbruck, 1970). Plant agglutinins are commonly referred to as phytohaemagglutinins or phytoagglutinins. However, as cell-agglutinating proteins also occur in organisms other than plants, the term lectins was proposed. Lectins are conveniently classified in terms of their ability to agglutinate certain cell types such as erythrocytes or lymphocytes. Another classification employs the ability of certain lectins to stimulate lymphocyte to divide (the mitogenic effect). Occasionally, separate lectins that have haemagglutinin activity, lymphocyte agglutinating activity or mitogenic activity are present in one source (Allan and Crumpton, 1971).

Many plant lectins have been purified and their structures and binding properties have been well characterized. Most of them contain covalently bound sugars and can thus be classified as glycoproteins. The carbohydrates found in lectins include mannose, glucosamine and galactose which are typical constituents of animal glycoproteins. Other sugars such as xylose and arabinose are sometimes found but in smaller amounts. Prominent examples of these lectins are Concanavalin A from jack bean which binds oligosaccharide ligands containing non-reducing terminal mannose, peanut lectin binds to galactose, wheat-germ agglutinin binds to N-acetyl-D-glucosamine, and soybean agglutinin (Lis *et al.*, 1969). For agglutination of cells or for the formation of

precipitates with complementary macromolecules, a lectin molecule must possess at least two binding sites as is found in immunoglobulins. In all cases, the combining sites are homogenous and identical with respect to their distribution in different molecules of the same lectin. In this property lectins differ form immune antibodies in that the combining sites of the latter are neither identical with one another nor are they homogenous.

A host of animal lectins have been characterized (Smith *et al.*, 1985a; Schauer *et al.*, 1995). The rabbit liver galactose lectin is an integral trans-membrane protein which form receptors on the surfaces of hepatocytes. These receptors bind and internalize plasma glycoproteins with terminal galactose after the removal of surface sialic acids. The internalized glycoproteins are subsequently degraded by lysosomes (Smith *et al.*, 1985a). The rat liver hepatocytes and liver macrophages as well as endothelial cells also express galactose-specific receptors which are lectins (Mohr *et al.* 1987; Kolb-Bachofen, 1988; Dini and Kolb-Bachofen, 1989; Kempka *et al.*, 1990). Rat peritoneal macrophages also express galactose-specific lectins (; Kuster and Schauer, 1981; Muller *et al.*, 1983a; Muller and Schauer, 1983; Kelm *et al.*, 1986a; Kelm and Schauer, 1986). The avian and amphibian hepatocytes lectins bind glycoproteins with terminal N-acetylglucosamine (Kawasaki and Ashwell, 1977; Smith *et al.*, 1985a). Mammalian hepatocytes also contain a Ca^{2+} dependent fucose binding lectin that is an integral membrane glycoprotein on the cell surface. Reticuloendothelial cells of liver, but not hepatocytes contain another lectin which binds N-acetylglucosamine and mannose-terminated glycoproteins equally well (Smith *et al.*, 1985a).

2.13 INTERACTION OF ERYTHROCYTES WITH MACROPHAGES (ADHESION).

Since the first recognition of the significance of sialic acid to the lifespan of serum glycoproteins by Morell *et al.* (1968), similar influence of these sugars on the survival of blood cells in circulation have been reported (Yaari, 1969; Ashwell and Morell, 1974).

Sialidase-treated erythrocytes from different species are rapidly sequestrated from the blood stream by liver and spleen (Durocher *et al.*, 1975; Aminoff *et al.*, 1977; Jancik *et al.*, 1978; Muller *et al.*, 1981). Studies *in vivo* and experiments *in vitro* with kupffer cells and peritoneal macrophages demonstrated that the interaction of sialidase-treated erythrocytes with macrophages is mediated by a lectin-like substance with β-galactose specificity present on the macrophage surface (Muller *et al.*, 1981; Kolb and Kolb-Bachofen, 1978; Kuster and Schauer, 1981).

2.13.1 STUDIES *IN VIVO*.

Jancik and Schauer (1974) and Jancik *et al.* (1975) observed a marked reduction of the lifespan of sialidase treated rabbit and human erythrocytes respectively. Removal of about 60% of the sialic acids from human erythrocytes resulted in a reduction of the half life-time of these cells from normally 26 days to 2 hours. Sialidase-treated ^{51}Cr labelled erythrocytes were not lyzed in the blood stream but accumulated in spleen and liver of rabbits and they are bound and phagocytozed by macrophages (Jancik *et al.*, 1978). Sequestration of sialidase-treated erythrocytes from the blood stream seems to be a general phenomenon for mammalian erythrocytes which was also observed with cow (Jancik *et al.*, 1978) and with a variety of other mammals (Schauer, 1978, 1982a).

Ligands as large as blood cells are too large to gain access to the galactose-specific receptors on hepatocytes, they cannot pass the endothelial fenestrae easily (Kolb-Bachofen, 1988). Electron microscopy revealed that *in vivo* both asialolymphocytes (Kolb-Bachofen and Kolb, 1979) and asialo-erythrocytes (Kolb-Bachofen *et al.*, 1984) are found adhering almost exclusively to the liver macrophages shortly after their injection. The *in vivo* homing of desialylated blood cells to the liver is also mediated by galactose-specific receptors (Kolb *et al.*, 1981; Muller *et al.*, 1981). Investigations on the macrophage receptor-activity in both *in vitro* and *in vivo* experiments showed that there is a distinct difference in the handling of small or molecular ligands and large particulate or cellular ligands by the liver *in vitro* although the initial recognition step of the ligands investigated always involves terminal galactosyl groups (Kolb-Bachofen, 1988). With the use of asialofetuin (ASF) or lactosylated bovine serum albumin (LacBSA) adsorbed onto colloidal gold particles of sizes 5nm, 17nm and 50nm, Kolb-Bachofen *et al.* (1983) showed localization differences in the two lectin activities expressed on hepatocytes and liver macrophages. Kupffer cells (liver macrophages) endocytosed these particulate ligands while hepatocytes take up small sized particulate ligands up to a diameter of 8nm (Kolb-Bachofen *et al.*, 1983; Schlepper-Schafer *et al.*, 1986b; Kolb-Bachofen, 1988). All ligand sizes are bound by the same receptor, the route of uptake depends on the ligand size. Small particles up to a diameter of 35nm are taken up via the coated pit/vesicle route (Kolb-Bachofen *et al.*, 1982). Ligands that exceed these size are not packed into coated organelles but transported via non-coated vesicles (Kolb-Bachofen *et al.*, 1983). However, irrespective of the transport route, particles of all sizes are finally delivered to the same endocytotic vesicles (Kolb-Bachofen, 1988).

2.13.2 STUDIES *IN VITRO.*

The mechanism involved in the sequestration of sialidase-treated erythrocytes by macrophages observed *in vivo* can be studied in details using isolated macrophages (rat kupffer cells or peritoneal macrophages) in culture in *in vitro* experiments. The rapid disappearance of sialidase-treated erythrocytes from the blood stream is due to binding and phagocytosis of these cells by macrophages (Kolb and Kolb-Bachofen, 1978; Muller *et al.*, 1981; Kuster and Schauer, 1981). Rat peritoneal macrophages bind and phagocytize homologous sialidase-treated erythrocytes at a rate which is dependent on the amount of sialic acid that has been removed from the cells. Increased binding of erythrocytes is observed after the removal of 10-20% of membrane sialic acids, while for phagocytosis at least 30-40% of the sialic acid must be removed (Muller and Schauer, 1983; Muller *et al.*, 1983a).

Removal of 10-20% of sialic acid have been reported to be sufficient to eliminate erythrocytes from the circulation (Aminoff *et al.*, 1976) while hydrolysis of larger amounts led to an increase of the number of bound and engulfed erythrocytes (Muller *et al.*, 1983a). The amount of bound and endocytozed erythrocytes was proportional to the quantity of erythrocytes present in the incubation mixture (Muller *et al.*, 1983a) and reached maximum 30 and 45 minutes with sialidases from *Vibrio cholerae* and *Arthrobacter ureafaciens* respectively (Schauer *et al.*, 1984). Usually, one macrophage is able to engulf between one and ten erythrocytes (Schauer *et al.*, 1984).

Kolb and Kolb-Bachofen (1978) reported an indirect evidence for an asialoglycoprotein receptor in cell adhesion studies. A lectin-like receptor on kupffer cell surface mediate *in vitro* the adhesion of neuraminidase-treated erythrocytes (Kolb

and Kolb-Bachofen, 1978; Kolb *et al.*, 1979; Schlepper-Schafer *et al.*, 1980) and lymphocytes (Kolb-Bachofen and Kolb, 1979). The specificity of the kupffer cell lectin is very similar to the asialoglycoprotein receptor on hepatocyte plasma membrane described by Ashwell and Morell (1974). Both receptors bind glycoproteins with terminal D-galactosyl residues. Kolb-Bachofen *et al.* (1982) provided a direct evidence for the presence of an N-acetyl-D-galactosamine specific lectin on kupffer cell surface by visualization of ligand binding in electron microscopy. Incubation of freshly isolated kupffer cells with asialofetuin adsorbed onto colloidal gold particles (ASF-gold) resulted in binding and endocytosis of the ligand. The ASF particles are endocytosed via the coated pit/vesicle pathway and appear to be transported to the secondary lysosomes by coated vesicles, as shown by the occurrence of coated areas in the secondary lysosome membrane. Furthermore, a galactose-specific lectin have been isolated and purified from rat serum (Kempka *et al.*, 1990). Comparative analysis of this serum galactose-binding protein with the galactose-particle receptor protein purified from rat liver macrophages and with C-reactive protein (CRP) reveals close relation or identity of these proteins. Kempka *et al.* (1990) therefore concluded that CRP occurs as a membrane-associated protein constitutively expressed on liver macrophages functioning as a membrane receptor mediating galactose-specific binding of particulate ligands. Binding and uptake of *in vitro* aged or senescent rat erythrocytes by isolated liver macrophages also suggest recognition by galactose- specific receptors on the cell surface of the macrophages (Schlepper-Schafer and Kolb-Bachofen, 1988). Recognition of these old erythrocytes is mediated by two systems; old erythrocytes expose different terminal sugar residues or a different arrangement of glycan when compared to young erythrocytes, rendering them recognizable by liver lectins and by autoantibodies

(Schlepper-Schafer and Kolb-Bachofen, 1988). The macrophage lectin not only recognizes β-galactosyl compounds *in vitro* but also *in vivo* as shown by the inhibition of the clearance of sialidase-treated erythrocytes from the blood stream by repeated injection of lactose or asialo-fetuin into rabbits (Muller *et al.*, 1981). The galactose-particle receptor appears therefore to function in recognizing opsonized particles and thus may mediate what formerly has been called "unspecific phagocytosis" of macrophages. Kolb *et al.* (1981) further showed that in *in vitro* uptake studies the galactose particle receptor is recycled back to the plasma membrane as is the asialoglycoprotein receptor (ASGP-R). The recycling of the macrophage receptor however, is blocked when the macrophages were blocked with colloidal carbon (India ink) prior to their isolation.

In this investigation, the role of sialic acid and galactose residues of erythrocyte membrane for the regulation of the life-time of erythrocytes and for the interaction of desialylated erythrocytes and rat peritoneal macrophage lectins in the initial phase of bovine trypanosomiasis was evaluated.

2.13.3 MECHANISMS OF ADHESION.

Sialidase-treated erythrocytes are usually bound to the β-galactose-specific lectin on the macrophage surface and are phagocytozed as a function of their number and binding strength to the macrophages (Muller *et al.*, 1983a). Phagocytosis is therefore induced after the interaction of macrophages with a critical number of β-galactose-residues on erythrocytes, the strength of this interaction and consequently binding and phagocytosis seem to be increased by the addition of serum and perhaps of the lectins (Muller and Schauer, 1983).

Binding and phagocytosis of sialidase-treated erythrocytes occur in the presence of mere buffer, both steps are however, enhanced by native homologous serum in a dose-dependent way (Muller and Schauer, 1983). In their initial reports, Muller and Schauer (1983) noticed significant phagocytosis only in the presence of native homologous serum. Further heat inactivation of the serum leads to a marked decrease of phagocytosis hence indicating the participation of complement in the phagocytosis process of partially desialylated erythrocytes by rat peritoneal macrophages. It was therefore concluded that trapping of these erythrocytes seems to involve two steps, first recognition and binding of the altered red cells by a β-galactosyl specific lectin on the macrophage surface and second uptake of the attached cells mediated by components of the native serum, presumably belonging to the complement system. Recognition by the lectin and binding is a pre-requisite for uptake of sialidase-treated erythrocytes by the peritoneal macrophages (Kuster and Schauer, 1981).

Muller *et al.* (1981) also reported the involvement of β-galactosyl-specific lectin in the mechanism of the sequestration of desialylated erythrocytes both *in vivo* and *in vitro* by a two step model. Binding via demasked erythrocyte surface β-galactosyl residues to the lectin on the macrophage cell membrane is necessary for the subsequent phagocytosis step. Careful experiments, however, using various immunoglobulins, anti-IgG, complement factors or inhibitors of Fc receptors have excluded immunoglobulins and/or complement factors as well as the Fc receptor of macrophages in the sequestration process (Muller *et al.*, 1983b). Similarly, substances known to stimulate phagocytosis such as Fibronectin, tuftsin or "substance P" were also excluded to play a role in the erythrocyte-macrophage system studied (Muller *et al.*, 1983b). Heat inactivation of serum to destroy complement factors of the classical (56°C, 30min) or

alternative (50°C, 20min) pathways reduced phagocytosis with less pronounced effect on binding. Incubation of serum with zymosan A, addition of guinea pig whole complement alone or together with IgM blockage of the complement receptor with 2-deoxy-D-glucose did not influence both binding and phagocytosis, thus excluding the role of complement factors in the sequestration process. Also the addition of IgG and IgM to the incubation mixture and blockage of the Fc-receptor had no influence on binding hence excluding the participation of immunoglobulins (Muller *et al.*, 1983b; Muller and Schauer, 1983). Binding and phagocytosis of sialidase-treated erythrocytes thus occurs by a mechanism independent of opsonins (Muller *et al.*, 1983b). Therefore after binding of sialidase-treated erythrocytes to the β-galactose-specific lectin on the macrophage surface, they are phagocytozed as a function of their number and binding strength to the macrophages (Muller *et al.*, 1983b).

Erythrophagocytosis *in vivo* is mediated by the cells of the reticuloendothelial system and takes place primarily in liver and/or spleen (Vomel and Platt, 1982; Khansari and Fudenberg, 1983; Smedsrod and Aminoff, 1985). Using rat liver macrophages, Mohr *et al.* (1987) reported that binding of human erythrocytes as well as binding of neuraminidase-treated human erythrocytes is mediated by the GalNAc/Gal particle receptors while binding of sheep erythrocyte is mediated by several different mechanisms including the GalNAc/Gal particle receptor. Therefore, the GalNAc/Gal particle receptor mediates or participates in recognition of non-self structures (Mohr *et al.*, 1987). Similarly, recognition of senescent erythrocytes by liver macrophages *in vitro* is mediated by a receptor specific for galactose residues (Schlepper-Schafer *et al.*, 1983) while *in vitro* aged and senescent rat erythrocytes accessibility to the autologous galactose-specific receptor isolated from rat liver hepatocytes as well as to antibodies

eluted from senescent rat erythrocytes have been demonstrated (Schlepper-Schafer and Kolb-Bachofen, 1988). Therefore, *in vitro* recognition of senescent rat erythrocytes by macrophages includes two mechanisms, direct recognition by a galactose specific lectin and indirect recognition by Fc-receptors mediated by antibodies (Schlepper-Schafer and Kolb-Bachofen, 1988). However, Kolb *et al.* (1981) on the basis of experiments with germ free rats suggested that serum antibodies are not involved in trapping of sialidase-treated erythrocytes.

Loss of sialic acid alone is responsible for the recognition of erythrocytes by peritoneal macrophages and resialylation of these cells by purified sialyltransferases causes remasking of the galactose residues and prevents binding of the cells (Kelm *et al.*, 1986a). Though only the amount of released sialic acids is the decisive factor for the binding and phagocytosis of sialidase-treated erythrocytes (Muller *et al.*, 1983a, 1983b), clustering of the ligands leads to a strong increase in binding and this may be due to simultaneous binding of more than one galactose residue (Kelm *et al.*, 1986a) for peritoneal macrophages. Similarly, the interaction of kupffer cell lectins with single asialofetuin molecule is not sufficient to trigger off endocytosis, whereas the reaction with a multivalent particle induces a physiological response hence the clustering of receptors may be of importance (Kolb-Bachofen *et al.*, 1982). The preclustered arrangement of galactose-specific binding sites on liver sinusoidal cells is indeed an absolute requirement for effective recognition and uptake of larger particulate ligands (Kolb-Bachofen, 1988; Dini and Kolb-Bachofen, 1989). The signal for uptake of a ligand is only triggered when more than a few receptors per cluster are occupied, a condition that is fulfilled best by particles with multiple binding sites, but not by soluble glycoproteins (Kolb-Bachofen *et al.*, 1982). Kelm and Schauer (1986) demonstrated the

requirement of Ca^{2+} to distinguish between reversible surface bound and irreversible internalized ligands. Internalization is followed by degradation of the ligands and binding strength depends on the arrangement of galactose residues presented by the ligand. Resialylation of sialidase-treated erythrocytes led to the incorporation of 60-80% of the enzymically released sialic acids (Kelm *et al.*, 1986b). Binding of the resialylated erythrocytes to peritoneal macrophages was reduced compared with corresponding partially desialylated erythrocytes, thus the amount of transferred sialic acids was sufficient to demonstrate reconstitution of the masking effect of sialic acids (Kelm *et al.*, 1986b).

A possible role of sialidase molecules themselves which have been observed to remain attached to cell membrane even after washing the cells in binding and phagocytosis of erythrocytes was excluded by the use of immobilized sialidase or the sailidase inhibitor 2-deoxy-2,3-didehydro-N-acetylneuraminic acid as well as by the addition of very low amounts of soluble sailidase (Muller *et al.*, 1983b; Schauer *et al.*, 1984).

Sialidase treatment therefore exposes penultimate β-galactose residues of the erythrocyte membrane and interaction of these cells with β-galactose specific lectin on the liver and spleen macrophages (Jancik *et al.*, 1978) results in the rapid sequestration of the cells from the blood stream (Muller *et al.*, 1981; Schauer *et al.*, 1984). Although the exposure of β-galactosyl residues on the surface of particles and cells seems to be the only factor responsible for the initiation of uptake of these cells by macrophages (Muller *et al.*, 1983a, 1983b; Schauer *et al.*, 1984) the presence of homologous serum or plant lectins in subagglutinating concentrations was found to enhance both binding and phagocytosis of rat erythrocytes (Kuster and Schauer, 1981;

Muller *et al.*, 1983b; Muller and Schauer, 1983). It is not known however, if the effect of serum is due to a specific compound present in serum or to an unspecific effect (Schauer *et al.*, 1984). The stimulative effect of peanut lectin (galactose-specific) or *Erythrina crystagelil* agglutinin (recognizing galactose and N-acetylgalactosamine) (Muller *et al.*, 1983b) is not yet clearly explained. Lectins could however, cause alterations of the conformation of desialylated glycoconjugate molecule in erythrocyte membrane which leads to exposure of cryptic compounds triggering phagocytosis (Muller *et al.*, 1983b; Schauer *et al.*, 1984). It could also be speculated that lectins are model substances for an explanation of the effect of the unknown serum factor(s) on binding and phagocytosis of sialidase-treated erythrocytes (Muller and Schauer, 1983).

In bovine trypanosomiasis, there is the production of sialidase (neuraminidase) by *T. vivax* (Esievo *et al.*, 1982a) which leads to the cleavage of erythrocyte surface sialic acids (Esievo *et al.*, 1982a). Pirofsky (1969) reported that new antigenic sites would be revealed on affected erythrocytes while Esievo and Saror (1991) in a review hypothesized that antibody may be produced against these new antigenic sites on the erythrocytes hence rendering them suitable for erythrophagocytosis. It was further hypothesized that sialidase-treatment removes the masking effect of the negative charges of the sialic acid residues on the erythrocytes and opsonization by immunoglobulins and complement leads to recognition and degradation by phagocytic cells of liver and spleen (Jancik *et al.*, 1978). In studies using scanning electron microscopy, no membrane abnormalities was observed in cells from cattle infected with *T. vivax* (Esievo, More and Muller; 1982b), nor was the shape of rabbit erythrocytes affected by sialidase treatment (Jancik *et al.*, 1978) however, several alterations were observed in erythrocytes of deer mice infected with *T. brucei* (Anosa and Kaneko,

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1983ab).

Erythrocyte surface sialic acids and trypanosome sialidase therefore may have significant roles in the pathophysiology and pathogenesis of trypanosomiasis (Esievo and Saror, 1991). It will be of interest and particularly of significant contribution to the knowledge of the mechanism of anaemia in bovine trypanosomiasis to know if the phagocytosis of erythrocytes due to the removal of sialic acids during *T. vivax* infection in cattle (Esievo, 1979; Esievo *et al.*, 1982a) occurred through exposure of β-galactose residues and binding by the β-galactose specific lectin on macrophages. The purpose of the present study is to examine if the interaction between erytrocytes desialylated *in vivo* by trypanosome sialidase and rat peritoneal macrophages is due to exposure of β-galactose on the erythrocytes and its subsequent removal by macrophages.

2.14 INHIBITION EXPERIMENTS.

In order to confirm if the exposure of β-D-galactosyl residues on erythrocytes is due to sialidase treatment, hapten inhibition experiments are usually carried out (Kolb and Kolb-Bachofen, 1978). Many inhibitors have been used to prevent desialylated erythrocyte-macrophage interaction and thus keeping the erythrocytes longer in circulation. Such inhibitors include a wide range of monosaccharide oligosaccharides, Neoglycoproteins, gangliosides, IgG (Kolb and Kolb-Bachofen, 1978; Muller *et al.*, 1981, 1983ab; Mohr *et al.*, 1987). Most of the complex carbohydrates possessing one or several terminal β-galactosyl groups are capable of inhibiting both binding and phagocytosis of sialidase-treated rat erythrocytes by homologous peritoneal macrophages. The inhibitory capacity of these compound is rather variable and branched oligosaccharides generally inhibit stronger than linear ones (Schauer *et al.*,

1984).

Among the various oligosaccharides used, lactose was found to give a high percent of inhibition in *in vitro* studies while it kept desialylated erythrocytes longer in circulation in rabbits (Muller *et al.*, 1981) and prevents erythrophagocytosis in cattle infected with *T. vivax* (Umar *et al.*, 1998). In the work reported here, the inhibitory effect of lactose was examined and compared when used in an *in vitro* and *in vivo* cell binding assay. Other saccharides were also used to confirm the exposure of subterminal β-galactose moieties on erythrocytes obtained from *T. vivax* infected calves and prevents erythrophagocytosis in cattle infected with *T. vivax*.

CHAPTER 3

MATERIALS AND METHODS

3.1 ANIMALS AND PARASITES.

Twelve (12) male Zebu (Sokoto Gudali) calves, ranging from 6 to 12 months of age and weighing between 80 and 90kg were purchased from Talata Mafara (Zamfara State) an area known to be free from tsetse-fly (Glossina spp). On arrival in Zaria, the animals were dewormed with Thiabendazole (Merck, Sharp and Dohme) and vaccinated against anthrax, black-quarter, contagious bovine pleuropneumonia and rinderpest. The animals were sprayed against ticks at weekly intervals with chlorfenvinphos (Pfizona, Pfizer, Nigeria Ltd.), housed and fed on a diet supplemented with a mixture of cotton seeds, wheat offal and hay. Water and salt licks were supplied *ad libitum*.

All animals were located at the Faculty of Veterinary Medicine of Ahmadu Bello University, Zaria. The animals were well conditioned to their environment (which is known to be free of tsetse fly) for about three months before infection.

Mature albino rats free from infection were purchased from the Nigerian Institute for Trypanosomiasis Research (N.I.T.R.) Vom, via Jos. The rats were housed in 33 x 55cm stainless steel top-tough polypropylene base rat cages. Water and chick mash (Sanders feed) were provided *ad. libitum* throughout the experimental period.

An isolate of *Trypanosoma vivax* (Samaru stock) was obtained from a cow with pure natural infection and identified by morphological examination. Two (2) ml of infected blood from the cow was used to infect a goat (Red Sokoto goat) to serve as a donor. The goat was housed and fed on a diet supplemented with a mixture of cotton seeds, wheat offal and hay while water was provided *ad libitum*. Parasitaemia was monitored daily by HCT, wet mount and haemocytometer techniques. When

parasitaemia was very high (approximately 11 x 10^6 trypanosomes) the infected donor goat's blood was used to infect the experimental calves.

3.2 CHEMICALS AND EQUIPMENT.

Gelatine, D-Glucose, D-Galactose and D-Mannose were purchased from BDH. Barbitone Diethylbarbituric acid and 5-5-Diethylbarbituric acid sodium salt were purchased from MERCK. Lactose was purchased from Hopkin and Williams, England. Medium 199E and all other reagents were of analytical grade and purchased from Sigma Chemical Company, St. Louis, USA.

Hemle ZK510 refrigerated centrifuge and Gallenhamp cooled $C0_2$ incubator were used for both centrifugation and incubation throughout the study. Counting and examination of cells were carried out using a Zeiss Photomicroscope while photographs of slides were taken using a Leitz Ortholux 2 Laboratory and Research Microscope.

3.3 EXPERIMENTAL DESIGN.

The calves were divided into three groups for the controls, *in vitro* and *in vivo* experiments. Six (6) calves served as the non-infected controls.

Two (2) calves were each infected with 11.0 x 10^6 *T. vivax* by jugular venipuncture. This group of animals were used for the *in vitro* experiment.

Four (4) calves were each infected with 11.0 x 10^6 *T. vivax* by jugular venipuncture and monitored very closely for the development of parasitaemia. This group of animals were used for the *in vivo* experiment.

3.4. SAMPLE COLLECTION.

Blood samples were collected as described by Esievo *et al.* (1982). For about three weeks, ten (10) ml of blood was collected daily from each calf by jugular venipuncture. Of the blood samples collected, 1 ml was used for routine haematology, 3ml was placed in vacutainers containing barbital buffered saline (147mM NaCl, 36mM EDTA, 9mM sodium diethylbarbiturate, 27mM diethylbarbituric acid and 0.1%gelatine pH 7.2) as anticoagulant and the remaining 6ml was placed into vacutainers without anticoagulant for serum preparation. Serum was prepared by allowing the blood to clot undisturbed for about 1 hour and then centrifuged at 2,000g for 10 min. at 4°C. The serum was analyzed for sialic acid.

3.5 ERYTHROCYTE PREPARATION.

Erythrocytes were isolated as described by Muller *et al.* (1983ab). Briefly, blood (3ml) was mixed with 3ml of barbital-buffered saline. The erythrocytes were washed three times by suspension in twenty fold (20) volume of phosphate-buffered saline (Na_2Cl-P buffer: 137mM NaCl, 2.7mM KCL, 8.1mM Na_2H-PO_4 and 1.5 mM KH_2PO_4, pH 7.2) and centrifuged at 850g for 10min. at 4°C using an MSE Mistral 3000 refrigerated centrifuge. The cells were adjusted to 2.5 x 10^5 cells/ml by diluting 0.1ml of erythrocytes to 4ml with phosphate buffered saline and counted in a haemocytometer. Erythrocytes are usually prepared before the isolation of macrophages.

Briefly: A 1:40 dilution of the isolated erythrocytes was made by diluting 0.1ml of erythrocytes to 4.0ml with phosphate buffered saline in a glass (75 x 12mm) tube. After sealing the tube with a tightly fitting bung, the diluted blood was mixed by repeated shaking.

3.3.1 *IN VITRO* EXPERIMENT

Five male Zebu calves were used for this experiment. Two (2) of the calves were each infected by jugular venipuncture with 5ml of the infected donor goat's blood, containing approximately 11.0 x 10^6 *Trypanosoma vivax* while the remaining three calves served as the uninfected control

3.3.2 *IN VIVO* EXPERIMENT

Four (4) calves were each infected by jugular venipuncture with 5ml of the infected goat's blood containing approximately 11.0 x 10^6 *T. vivax*. Parasitaemia was monitored daily by HCT, wet mount and haemocytometer techniques. At the first peak of parasitaemia which occurred five days post-infection (p.i) with associated declining packed cell volume, each calf was given lactose solution at a dose rate of 0.5g lactose per kg body weight thrice daily at intervals of four hours, by jugular venipuncture at a rate of approximately 18ml per minute for four days. The lactose was administered as a solution in physiological saline. Briefly a 50 per cent solution of lactose (or 1.40M) in physiological saline (0.9 per cent NaCl) was prepared and placed in an injection bottle, plugged, autoclaved at 15 lbs pressure for five minutes and allowed to return to room temperature. Normal saline was preferred to aqueous solution to reduce systemic haemolysis. Approximately 80-90ml of the lactose solution was administered to the calves depending on their body weights. The quantity of lactose solution were computed (to give 30mM plasma concentration) and administered as a function of calf blood volume, estimated at 6-7 per cent of body weight.

The counting chamber of the Neubauer haemocytometer with a coverslip already in position was filled without delay with the aid of a pasture pipette which has been allowed to take up the suspension by capillary. The counting chamber was left undisturbed for about 2 minutes for cells to settle. The cells were then counted using the 40 x objective. Cells in 4 or 8 horizontal rectangles of 1.0mm x 0.05mm (80 or 160 small squares) or in 5 or 10 groups of 16 small squares were counted including the cells which touched the top and right hand margins.

Calculations:

$$\text{Red cell count} = N \times \frac{1}{A \times D} \times DF \quad \text{per ml.}$$

where N is the number of cells counted, DF is the dilution factor (i.e. 40), A is the area of chamber counted (i.e. $0.2mm^2$) and D is the depth of the chamber (i.e. 0.1mm).

Therefore,

$$\text{Red cell count} = N \times \frac{1}{0.2 \times 0.1} \times 40 \quad \text{per ml.}$$

$$= N \times 2 \times 10^3 \text{ per ml.}$$

3.6 MACROPHAGE PREPARATION.

Peritoneal macrophages were isolated from adult rats by washing the peritoneal cavity with 20ml of medium 199E (1%) as described by Muller *et al.* (1983ab). Briefly, unanesthesized rat was held by the skin of the neck and turned to reveal the ventral view. The skin of the abdomen was carefully picked using a pair of forceps. A 20ml syringe fitted with a 19 guage needle containing 20ml of 1% medium 199E was carefully pierced into the peritoneal cavity and it's content emptied into the peritonium and left for about 5 minutes, aspirated and re-emptied twice. The extracted medium was

then centrifuged at 320g for 10 min. and counted in a haemocytometer. 2.0-2.5 x 10^5 cells in 2ml medium 199E were used per assay. Macrophages are prepared fresh daily from different rats and used for assay.

Briefly: A 1 in 20 dilution of the isolated macrophages was made by adding 0.1ml of macrophages to 2ml of medium 199E in a 75 x 10mm glass tube. After tightly corking the tube the suspension was mixed by gentle shaking. The counting chamber of the Neubauer haemocytometer with a coverslip already in position was filled by means of a pasture pipette and mounted on a light microscope. Cells found on any of the four corner 1mm^2 areas and those in the central 1mm^2 area were counted.

Calculation:

If N is the number of cells counted, DF is the dilution factor, A is the area counted (i.e. 5 x 1mm^2) and D is the depth of the counting chamber (i.e. 0.1mm).

$$\text{Macrophage count} = N \times \frac{1}{A \times D} \times DF \times 10^6 \text{ cells per litre}$$

$$= N \times \frac{1}{5 \times 0.1} \times 20 \times 10^6 \text{ cell per litre}$$

$$= N \times 4 \times 10^7 \text{ cells per litre}$$

$$= N \times 4 \times 10^4 \text{ cells per ml.}$$

3.7 ASSAY OF ERYTHROCYTE-MACROPHAGE INTERACTION.

Binding assay was done based on the formation of rosettes as described by Mohr *et al.* (1987). Briefly, 100µL erythrocyte suspension (about 2.5 x 10^5 cells) in phosphate buffered saline was mixed with an equal volume of peritoneal macrophages (2.0-2.5 x 10^5 cells) in a plastic tube (75 x 7mm) and centrifuged at 80g for 5 min. and incubated for 60 min. at 7 $\pm$ 1°C in a Gallenkamp cooled CO_2 incubator. The resulting rosettes were resuspended by gentle shaking and a drop of crystal violet added to each tube. A

wet slide was then prepared for microscopic examination using a Zeiss Photomicroscope using the 40 x objective. Percent macrophages binding 3 or more erythrocytes was determined. Each test was performed in triplicate. A minimum of 100 macrophages were examined per individual test. Photographs of slides were taken using a Leitz Ortholux 2 Laboratory and Research Microscope. Macrophages stain dark blue while erythrocytes stain light blue.

3.8 INHIBITION STUDIES.

For in vitro inhibition studies, 100µL peritoneal macrophages were incubated with 100µL of the inhibitors for 5 min. at 4°C before the addition of erythrocytes as described by Mohr *et al.* (1987). Only calves in the *in vitro* experiment were used. The inhibitors used are: Monosaccharides such as D-Galactose (25mM) D-Glucose (25mM), D-Mannose (25mM) and Oligosaccharides such as lactose (25mM) and Maltose (25mM).

Cal culation of Percent Inhibition

The percent inhibition was calculated using the following equation:

Number of cells inhibited = BP - BI

$$\text{Percent Inhibition} = \frac{BP - BI}{BP} \times 100$$

where BI = Binding of erythrocytes in the presence of inhibitor and BP = binding of erythrocytes obtained from infected calves when percent binding was high in the absence of inhibitor.

3.9 ASSAY OF SERUM SIALIC ACID.

The serum samples were assayed for free serum sialic acids as described by Aminoff (1961).

Each sample was assayed as follows:

REAGENTS: Periodate: 25mM periodic acid in 0.125N H_2SO_4 (pH 1.2).

Sodium arsenite: 2% in 0.5N. HCL.

2Thiobarbituric acid: 0.1M adjusted to pH9 with NaOH.

Butanol: n Butanol containing 5% (v/v) of 12N HCl.

Standard sialic acid solution: 10mg of sialic acid in 100ml of distilled water.

PROCEDURE: The reactions were carried out in glass centrifuge tubes with ground-glass stoppers. Serum (0.5ml), water and standard solution were dispensed into tubes labeled test, blank and standard respectively and were treated with 0.25ml of the periodate reagent for 30 min. at 37°C. Arsenite solution (0.2ml) was added to each tube to destroy excess of periodate. As soon as the yellow colour of the liberated iodine has disappeared (1-2 min.), 2.0ml of the thiobarbituric acid reagent was added to each tube which were then covered and heated in a boiling water bath for 7.5 min. and cooled in an ice bath. The chromophor was extracted by vigorously shaking each of the above mixtures with 5.0ml of the acid/butanol mixture and then centrifuged at 3000g for 3 min. The clear organic phase was then transferred to a 10 mm cuvette and the optical density was read at 549nm against the butanol phase of the blank.

Sialic acid concentration was calculated as:

$$\text{Sialic acid (mg/dl)} = \frac{A_T}{A_{std}} \times \text{Conc. std.} \times 100$$

where A_T = Absorbance of samples

Astd = Absorbance of standard

Conc. std. = Concentration of standard.

The samples were assayed in triplicates.

3.10 ESTIMATION OF PARASITAEMIA.

Trypanosomes were detected in the blood by the micro-haematocrit centrifuge technique. The number of trypanosomes was evaluated using the improved Neubauer haemocytometer while Woo (1969) method was used for the preliminary estimation of trypanosomes.

3.10.1 MICROHAEMATOCRIT CENTRIFUGATION TECHNIQUES

Capillary tubes were filled to about 10mm from the top with fresh anticoagulated blood (EDTA 1mg/ml). The top end of the capillary tubes were then sealed with a sealant (cristaseal) and then centrifuged in a haematocrit centrifuge for 5 minutes. The areas of capillary tubes to be viewed were then wiped clean i.e. where the red cell column joins the cellular layer (buffy coat) and plasma with tissue paper. The capillaries were mounted on slides, supported on two strips of plasticine and gently pressed to embed the capillaries in the plasticine. The space between the capillaries were filled with clean water and covered with a cover glass. The plasma just above the buffy coat layer was examined for motile trypanosomes using the 10 x objective while the 40 x objective was used to confirm that motility was due to trypanosomes. The parasitaemia seen in the buffy coat/plasma junction were scored as 1+ (occasional trypanosomes), 2+ (1-2 trypanosomes), 3+ (3-20 trypanosomes) and 4+ (> 20 trypanosomes).

The preparations were examined within a few minutes of the blood being centrifuged to prevent the migration of the trypanosomes into the supernatant plasma.

3.10.2 QUANTITATION OF TRYPANOSOMES USING NEUBAUER HAEMOCYTOMETER

A 1 in 10 dilution of blood (in EDTA) from the infected animals was made with the counting stain (see appendix) and allowed to stain for 5 minutes. A coverslip was fixed onto the counting chamber of the Neubauer haemocytometer. One side of the counting chamber was filled with the diluted blood using a capillary tube and allowed to settle for 2 minutes in the moist chamber and then mounted on the light microscope. The trypanosomes found on any of the large side squares of the haemocytometer were counted.

Calculation:

If N number of trypanosomes were counted in a large square of the haemocytometer in 1 in 10 dilution,

Total number of trypanosomes = N x 10 x 10 x 1000 per ml

= N x 10^3 per ml

3.11 HAEMATOLOGICAL EVALUATION.

Packed cell volume (PCV) was determined by the microhaematocrit method as described by Dacie and Lewis (1975), well-mixed anticoagulated whole blood was taken up into capillary haematocrit tubes until they are approximately 2/3 filled with blood (air bubbles were avoided). One end of the haematocrit tube was sealed in a hot flame and the tubes placed in the radial grooves of the centrifuge head exactly opposite each other with the sealed end away from the centre of the centrifuge and centrifuged for 5 min. The haematocrit was read using the micro-haematocrit tube reading device.

3.12 DATA ANALYSIS.

Data were presented as means $\pm$ standard deviations. Statistical difference between means was assessed by paired students t-test and by correlation studies (Chatfield, 1983).

CHAPTER 4

RESULTS

Results obtained from this study are presented under *in vitro* and *in vivo* experiments respectively

4.1 *IN VITRO* EXPERIMENT:

HAEMATOLOGICAL CHANGES.

Results of the haematological changes obtained in the *in vitro* experiments are presented in fig. I and in Table I in the appendix.

All the infected calves developed trypanosomiasis. The prepatent period following the needle infection with *T. vivax* varied between 2 and 3 days. The course of the disease was mild, the affected animals becoming lethargic soon after the onset of the anaemia with mean PCV falling to about 14.5% on day 8 post infection (p.i).

PARASITAEMIA

Results of the parasitaemia during the *in vitro* experiment are presented in Fig. 1 and Table 2. Soon after establishment of patent infection, the parasitaemia continued to rise and reached a peak 5 days after infection. This was followed by a gradual decline over the next 2 days and by day 8 after infection, trypanosomes were too scanty to be counted. The number of circulating trypanosomes increased again between days 9 and 13 after infection (Fig. 1).

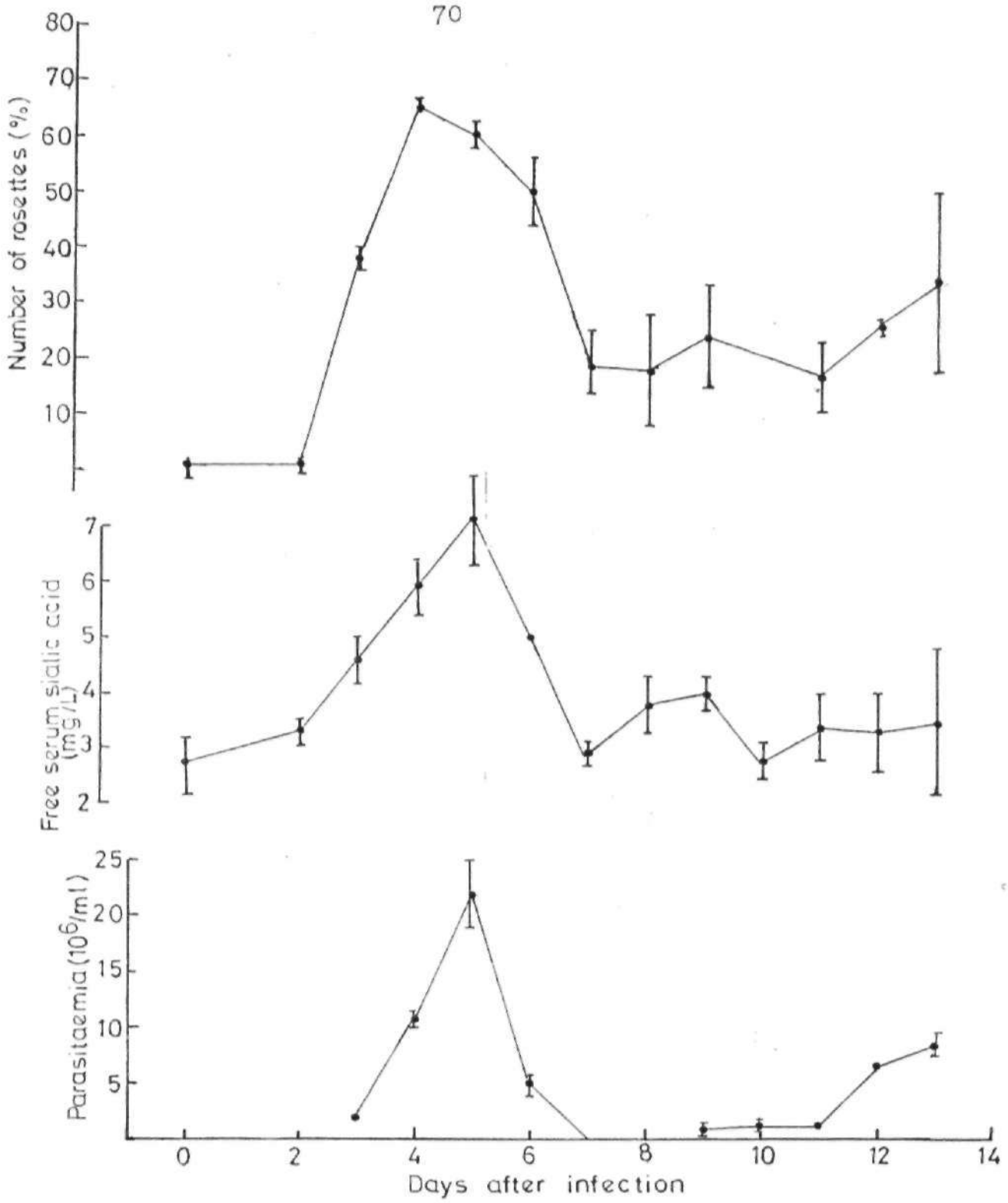


Fig.1: Variation in free serum sialic acid in relation to percentage rosette formation and parasitaemia during experimental *T. Vivax* infection in zebu calves

PRODUCTION OF SERUM SIALIC ACID.

The changes in free serum sialic acid during the infection and post infection periods in the infected calves are presented in Fig. 1. There was gradual increase in free serum sialic acid concentrations that reached a peak 5 days after infection. The values then declined though not below the pre-infection period. There was another appreciation in the free serum sialic acid concentrations between days 8 and 9 after infection and a decline on day 10 after which there was a gradual increase till the termination of the study (Fig. 1, Table 3 in appendix).

BINDING OF BOVINE ERYTHROCYTES WITH RAT PERITONEAL MACROPHAGES

Binding of erythrocytes from non-infected calves with rat peritoneal macrophages are shown in Plate 1 while binding of erythrocytes obtained from the infected calves with rat peritoneal macrophages are presented in Plate 2. Binding of erythrocytes obtained from the infected calves with freshly isolated rat peritoneal macrophages in the presence of 25mM D-galactose are presented in Plate 3.

Erythrocytes from infected calves adhere spontaneously to freshly isolated rat peritoneal macrophages when incubated in the cell binding assay (Plate 2). Fifty nine (59%) percent of the rat peritoneal macrophages bind 3 or more of the erythrocytes at the first peak of the parasitaemia which occurred on day 5 p.i (Fig. 2) while 34 percent of rat peritoneal macrophages bind 3 or more of the erythrocytes at the second peak of parasitaemia which occurred on day 13 p.i (Fig. 3). There was no significant ($P>0.05$) difference in binding at the two peaks of parasitaemia. A gradual increase in the binding was observed from day 2 post infection and peaked on day 4 post infection. The

values then declined though not below the pre-infection period. A gradual increase in binding was again evident between days 11 and 13 post infection (Fig. 1, Table 4 in appendix).

INHIBITION EXPERIMENT

In order to confirm the exposure of β-D-galactosyl residues on the erythrocytes due to *in vivo* desialylation by circulating sialidase and its involvement in binding, hapten inhibition experiments were performed by pre-incubating the rat peritoneal macrophages with monosaccharides and oligosaccharides before introduction of desialylated erthrocytes obtained from infected calves. Adhesion of erythrocytes was inhibited in the presence of D-galactose, lactose, D-glucose maltose, mannose (Table 5 and Table 6 in the appendix). The inhibition pattern of D-galactose > lactose > maltose > D-glucose > mannose was observed. Galactose gave a significantly ($P<0.01$) higher inhibition compared to the other saccharides.

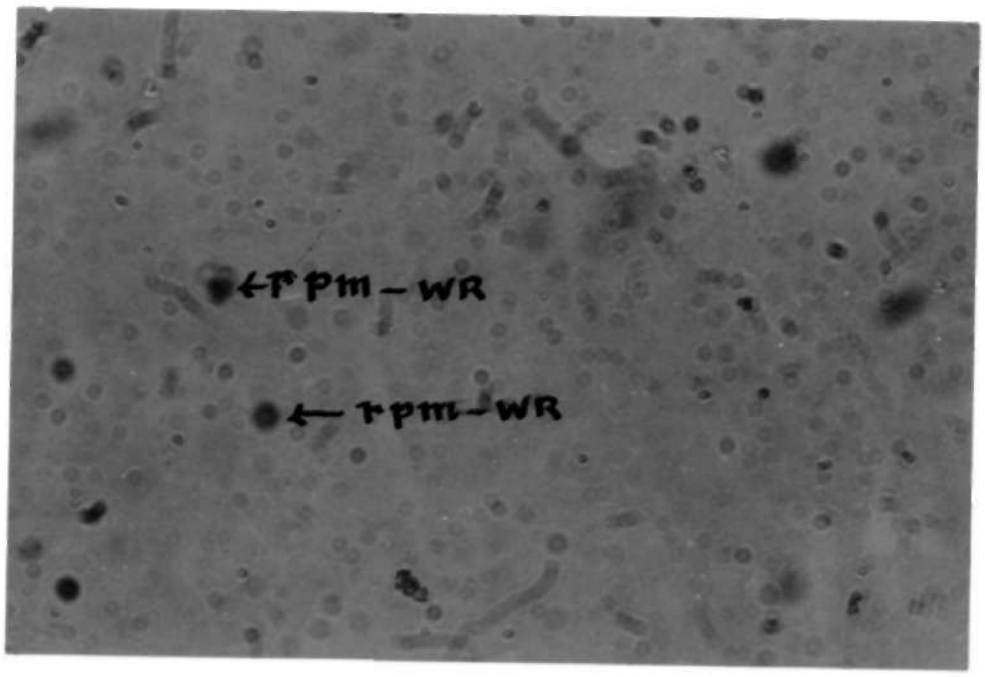


Plate I: Rosette formation by rat peritoneal macrophages with erythrocytes from non-infected calves. x 400
rpm - WR = Rat peritonial macrophages without red blood cells.

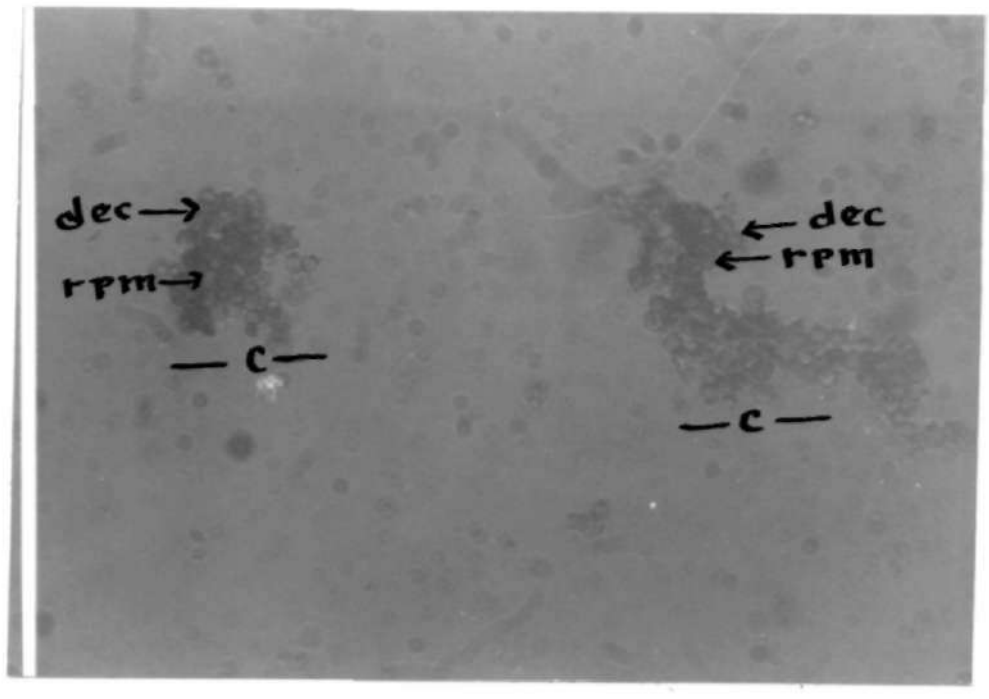


Plate 2: Rosette formation by rat peritoneal macropages with desialylated erythrocytes from *T. vivax* infected calves. x 400
-C- = Clusters of rosettes
dec = desialylated erythrocytes
rpm = rat peritonial macrophages

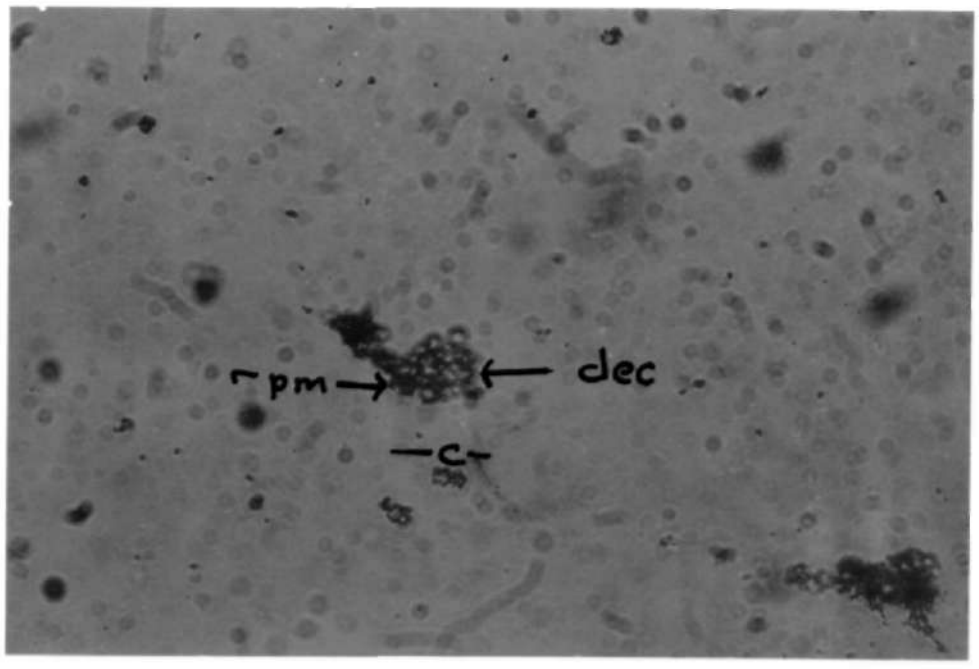


Plate 3: Rosette formation by rat peritoneal macrophages with desialylated erythrocytes from *T. vivax* infected calves in the presence of 25mM D-galactose x 400
-C- = clusters of rosettes
dec = desialylated erythrocytes
rpm = rat peritoneal macrophages

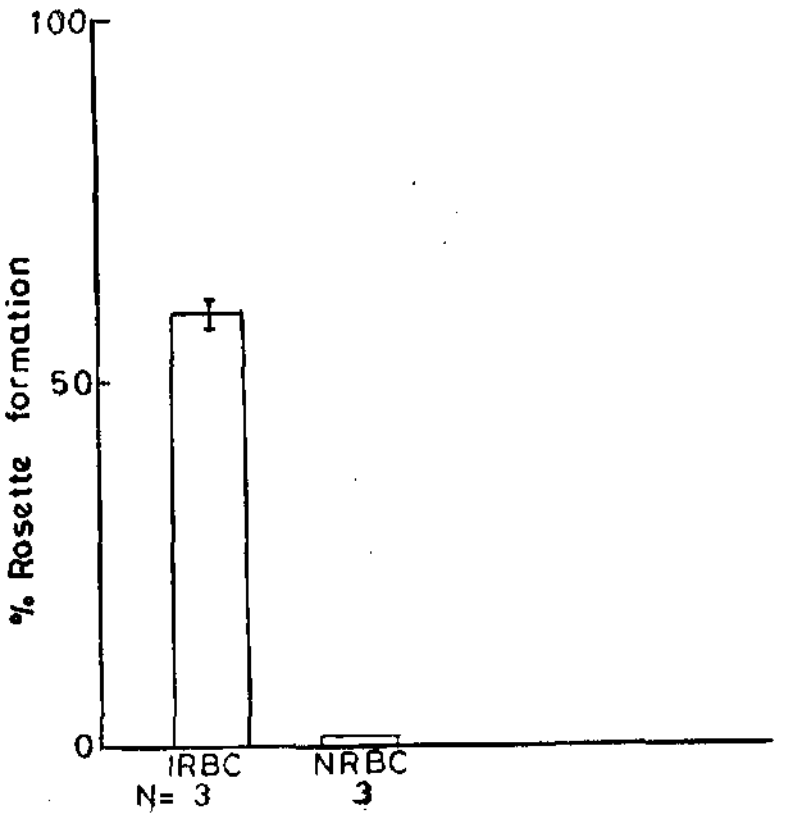


Fig. 2: **Binding between isolated rat peritoneal macrophages and erythrocytes from non-infected and infected calves at the first peak of parasitaemia (22.0 ± 6.0 x 10^6 parasites) which occurred on day 5 post infection. Erythrocytes adhere spontaneously to macrophages when incubated in the binding assay at 7°C for 1hr. The percentage (± SD) of macrophages that bind 3 or more erythrocytes is shown (IRBC) RBC from infected calves, (NRBC) RBC from non-infected calves, N, the number of experiments.**

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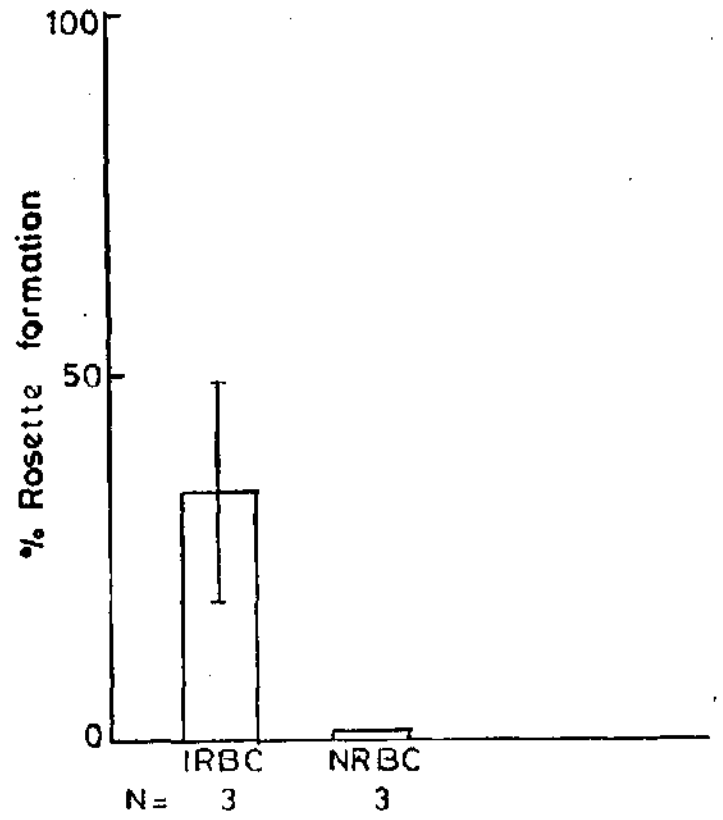


Fig. 3: **Binding between isolated rat peritoneal macrophages and erythrocytes from non-infected and infected calves at the second peak of parasitaemial ($8.25 \pm 2.3 \times 10^6$ parasites) which occurred on day 13 post infection. Erythrocytes adhere spontaneously to macrophages when incubated in the binding assay at 7°C for 1hr. The percentage ($\pm$ SD) of macrophages that bind 3 or more erythrocytes is shown (IRBC) RBC from infected calves, (NRBC) RBC from non-infected calves, N, number of experiments.**

Table 5: Inhibition of cell contacts between erythrocytes from infected calves and rat peritoneal macrophages.

Inhibitors	Percent inhibition of cell contacts between rat peritoneal macrophages and erythrocytes from infected calves.
Monosaccharides	
D-Galactose (25mM)	64.86 ± 5.07^{a}
D-Glucose (25mM)	$29.53 \pm 7.03^{b,c}$
D-Mannose (25mM)	$21.33 \pm 7.01^{b,c}$
Oligosaccharides	
Lactose (Gal β-1, 4-Glu) (25mM)	$56.71 \pm 3.64^{b,d}$
Maltose (Gluα-1,4-Glu) (25mM)	$35.09 \pm 4.76^{b,c}$

Values are expressed as Mean ± SD.
a, b, c, d = values with different superscripts are significantly ($P<0.01$) different.

Peritoneal macrophages were incubated with inhibitors for 5 min. at 4°C before addition of erythrocytes obtained from infected calves when percent binding was very high. Sixty five percent of the rat peritoneal macrophages bound bovine RBC at the peak of binding which occurred on day 4 p.i. (see Table 6 in appendix).

4.2 *IN VIVO* EXPERIMENT.

HAEMATOLOGICAL CHANGES

Results of the haematological changes obtained in the *in vivo* experiments are presented in Table 7 (in the appendix). All the four infected calves developed trapanosomiasis. The prepatent period following needle infection with *T. vivax* varied between 2 and 3 days. The progress of the disease was mild. The initial mean packed cell volume (PCV) was 25.0 $\pm$ 1.63% which dropped significantly ($P<0.05$) to 20.5 $\pm$ 2.08% on day 6 p.i. Following lactose injection in this group of infected calves on day 6, the mean PCV stabilized partially with values of 20.8 $\pm$ 3.50, 18.8 $\pm$ 2.06 and 20.0 $\pm$ 1.83 percents on days 7, 8 and 9 respectively. The mean PCV fell to 16.5 $\pm$ 0.58 per cent on day 13 p.i. (Table 7 in the appendix).

PARASITAEMIA

Results of the parasitaemia during the *in vivo* experiments are presented in Fig. 4 and Table 8 (in the appendix). Soon after establishment of patent infection, the parasitaemia continued to rise and reached a peak 5 days after infection (Fig. 4). This was followed by a sharp decline over the next 2 days. The number of circulating trypanosomes remained low until day 10 p.i. Circulating trypanosomes increased again between days 11 and 13 after infection (Fig. 4, Table 8).

PRODUCTION OF SERUM SIALIC ACIDS

The changes in free serum sialic acids during the infection and post infection periods in the infected calves that were subsequently injected with lactose are presented in Fig. 4.

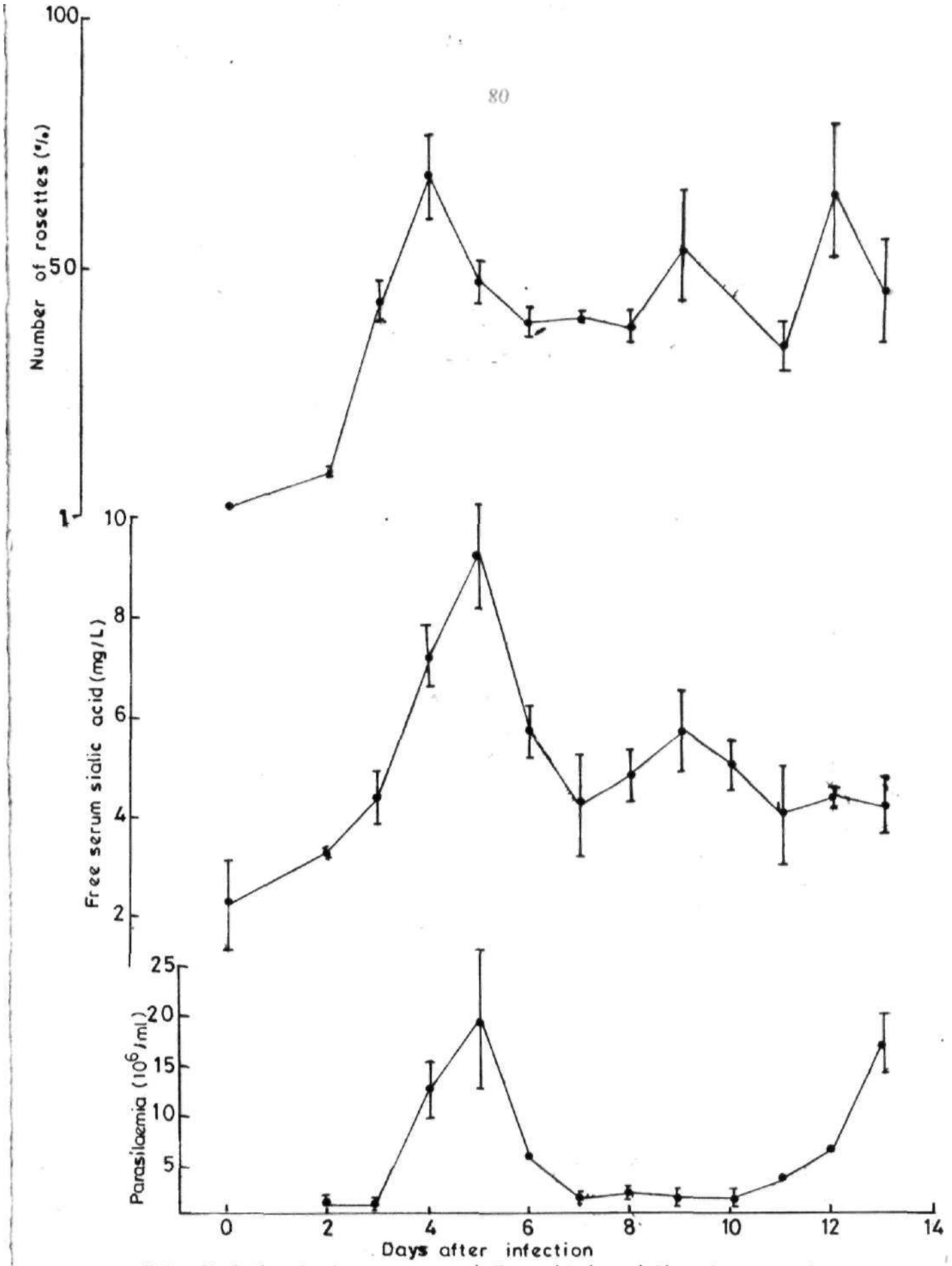


FIG. 4: Variation in free serum sialic acid in relation to percentage rosette formation and parasitaemia during experimental T. vivax infection in calves with subsequent lactose infusion

Free serum sialic acids increased gradually reaching a peak 5 days p.i There was a decline on days 6 and 7, followed with an increase in values between days 8 and 10 and another decline on day 11 p.i. Thereafter, a gradual increase occurred until the study was terminated (Fig. 4, Table 3 in the appendix).

BINDING OF BOVINE ERYTHROCYTES WITH RAT PERITONEAL MACROPHAGES.

Binding of erythrocytes obtained from the infected calves with freshly isolated rat peritoneal macrophages are presented in Plate 4. Endogenously desialylated erythrocytes from infected calves bound spontaneously to freshly isolated rat peritoneal macrophages when incubated in the cell binding assay (Plate 4). A sharp increase in rosette formation was observed from day 2 and peaked on day 4 p.i., with a decline on day 5 p.i. The values stabilized following lactose infusion on day 6 p.i., but peaked again on day 9 p.i., the last day of lactose infusion. A sharp decline followed on day 11 p.i. before an appreciation in values on day 12 p.i. (Fig. 5).

Forty seven percent (47%) of rat peritoneal macrophages bound 3 or more erythrocytes at the first peak of parasitaemia which occurred on day 5 p.i. in the infected calves before lactose injection, while 45% of rat peritoneal macrophages bound 3 or more erythrocytes at the second peak of parasitaemia which occurred on day 13 p.i. after lactose injection (Fig. 4, Table 4 in the appendix).

IN VITRO AND *IN VIVO* EXPERIMENTS - COMPARATIVE STUDIES OF THE BINDING OF ERYTHROCYTES WITH PERITONEAL MACROPHAGES

Forty seven percent (47%) and 59 % of rat peritoneal macrophages bound 3 or more erythrocytes at the first peak of parasitaemia which occurred on day 5 p.i. in the

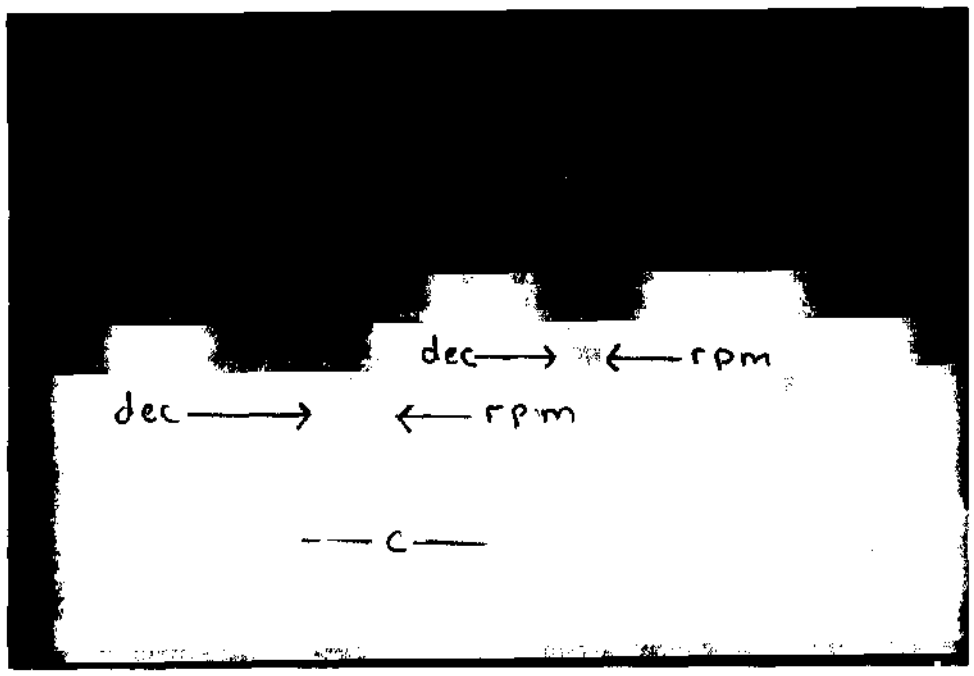


Plate 4: **Rosette formation by rat peritoneal macrophages with desialated erythrocytes from *T. vivax* infected calves with lactose injection x 400.**
-c- = Clusters of rosettes
dec. = desialylated erythrocytes
rpm = rat peritoneal macrophages

infected calves in the *in vivo* and *in vitro* inhibition experiments respectively. There was a significant ($P<0.05$) difference in binding between the 2 groups. Forty five percent (45%) and 34% of rat peritoneal macrophages bound 3 or more erythrocytes in the *in vivo* and *in vitro* inhibition experiments respectively at the second peak of parasitaemia which occurred on day 13 p.i. after lactose injection in the *in vivo* experiment (Fig. 5, Table 4 in the appendix). There was no significant ($P>0.05$) difference in binding between the 2 groups at the second peak parasitaemia.

Following lactose injection in the group of calves in the *in vivo* experiment on day 6 p.i., the number of binding increased significantly ($P<0.05$) on days 7 to 9 p.i. in this group of infected calves compared to the infected calves that were not injected with lactose in the *in vitro* experiment (Fig. 6, Table 9 in the appendix).

Percent inhibition of binding in the *in vitro* and *in vivo* experiments are presented in Fig. 7. Percent inhibition in the *in vitro* studies were 39.71 ± 9.41, 32.09 ± 18.52, 39.23 ± 14.87 and 49.45 ± 14.19 on days 6, 7, 8 and 9 p.i. respectively (Table 10 in the appendix). There was no significant ($P>0.05$) difference in the percent inhibition. Significantly ($P<0.01$) increased number of binding was observed in the *in vivo* studies throughout the periods of lactose injection (Fig. 7, Table 11 in the appendix).

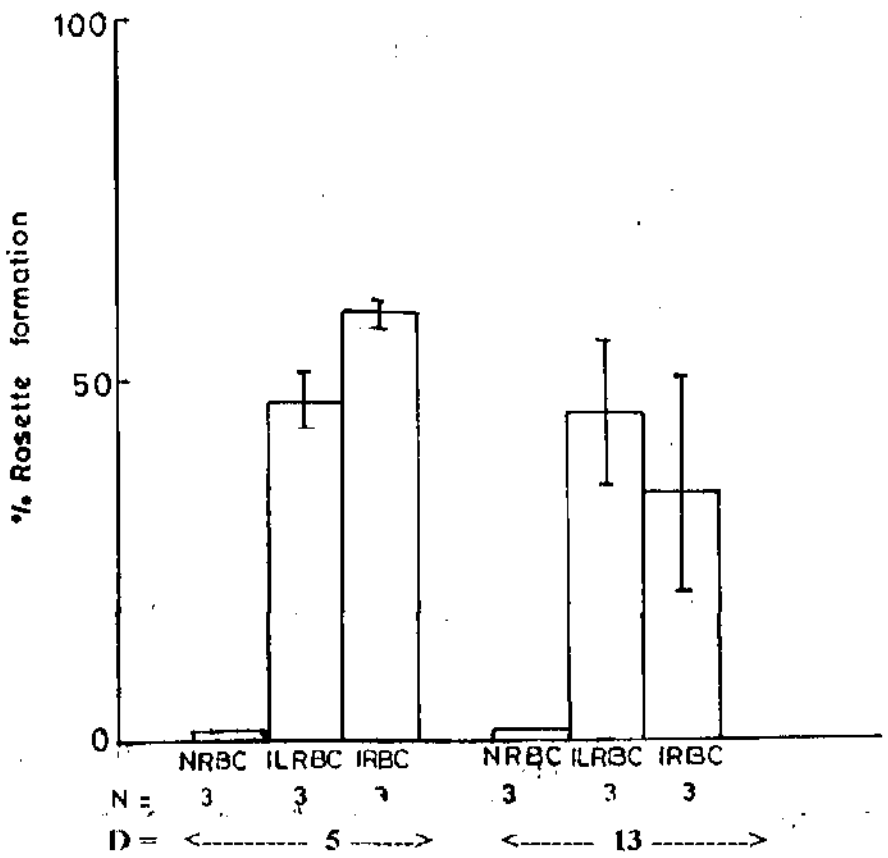


Fig. 5: **Binding between isolated rat peritoneal macrophages and erythrocytes from non-infected calves, infected calves and infected calves with lactose injection at the first and second peaks of parasitaemia which occurred on days 5 and 13 post infection**
The percentage (± SD) of macrophages that bind 3 or more erythrocytes is shown. (NRBC) from non-infected calves, (ILRBC) RBC from infected calves with lactose injection, (IRBC) RBC from infected calves ; N, number of experiments, D, days after infection.

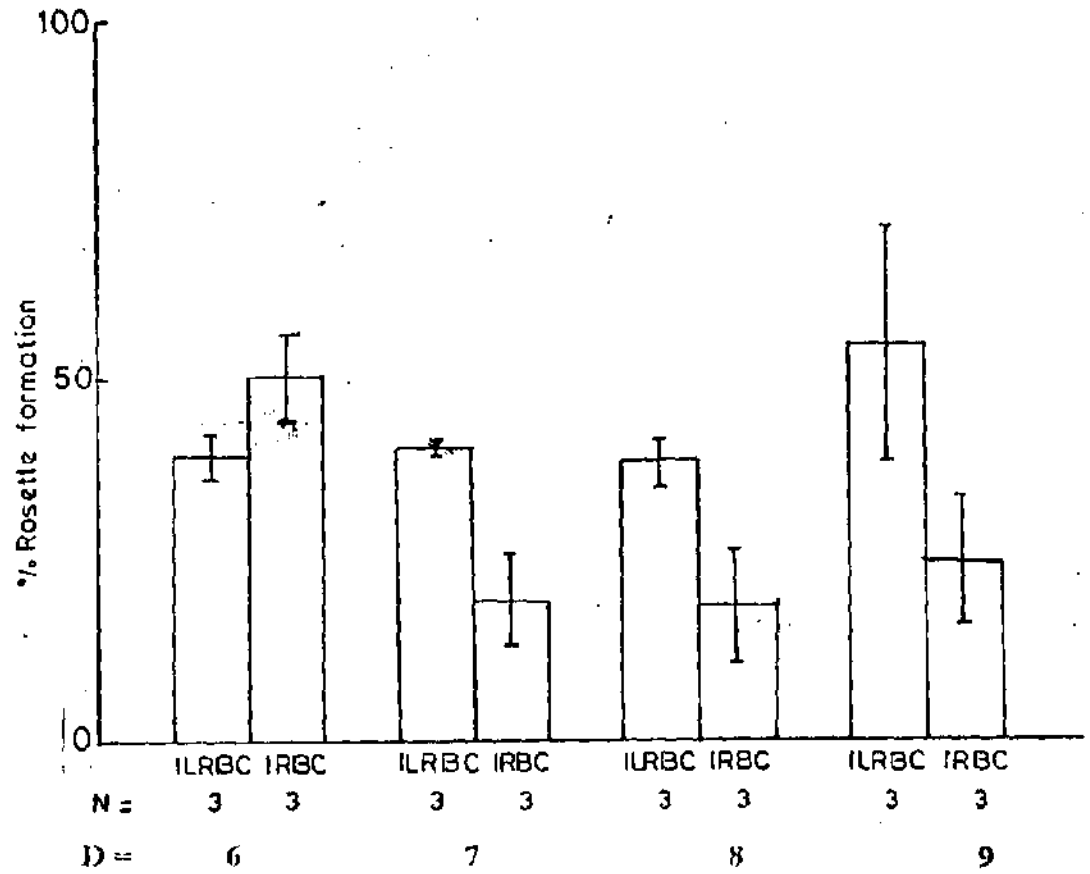


Fig. 6: **Binding between isolated rat peritoneal macrophages and erythrocytes from infected calves and infected calves with lactose injection during the period of lactose injection from days 6 to 9 post infection. The percentage (± SD) of macrophages that bind 3 or more erythrocytes is shown. (ILRBC) RBC from infected calves with lactose injection, (IRBC) RBC from infected calves; N, number of experiments, D, days after infection.**

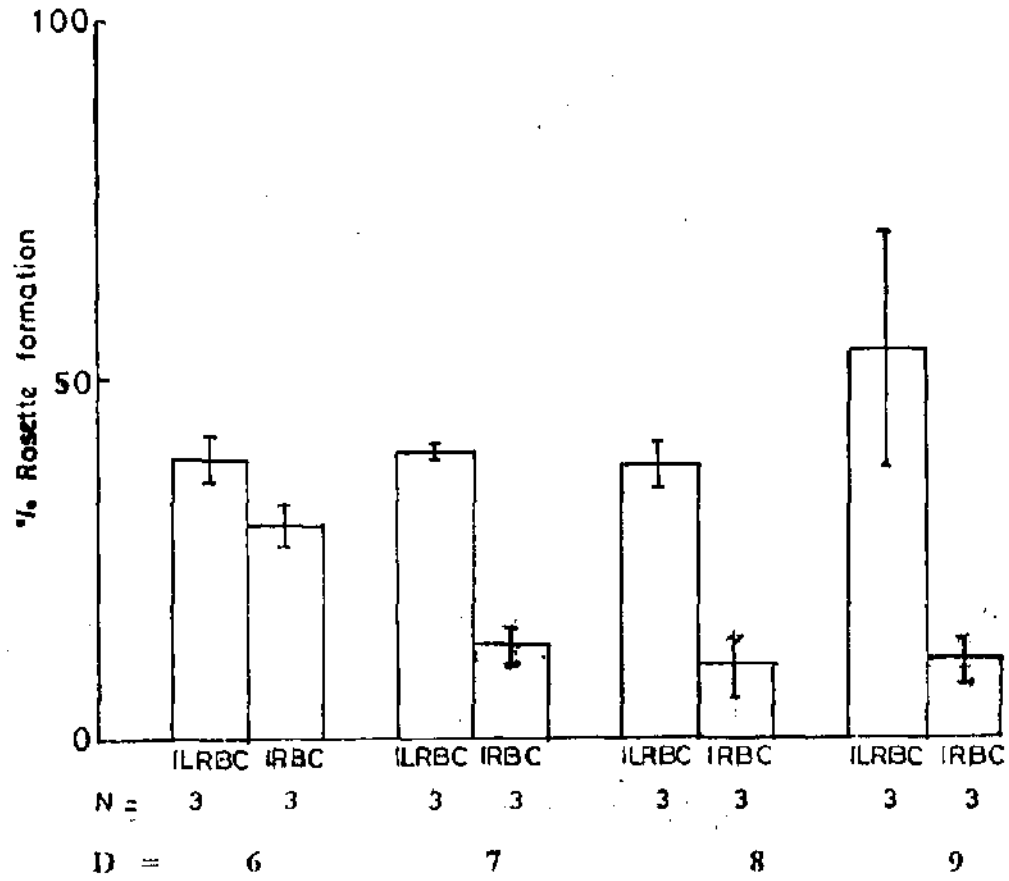


Fig. 7: **Binding between isolated rat peritoneal macrophages and erythrocytes from infected calves in the presence of lactose as inhibitor and erythrocytes from infected calves with lactose injection from days 6 to 9 post infection. *In vitro* and *in vivo* lactose inhibition studies. The percentage (± SD) of macrophages that bind 3 or more erythrocytes in the *in vivo* and *in vitro* lactose inhibition studies is shown. (ILRBC) RBC from *in vivo* lactose inhibition, (IRBC) RBC from *in vitro* lactose inhibition studies; N, number of experiments, D, days after infection.**

CHAPTER 5

DISCUSSION

The results obtained from this study are discussed under the *in vitro* experiment, the *in vivo* experiment and general discussion respectively.

IN VITRO EXPERIMENT

The results in the present study indicated that the infected calves developed trypanosomiasis with the parasites appearing in the peripheral blood as early as the second day post infection. The infected animals soon became lethargic and dehyrdated with mean PCV falling to about 14.5%. With the establishment of the infection, the parasitaemia rose steadily and reached a peak 5 days post infection.

Increase in serum sialic acid tended to coincide with the appearance of the trypanosome in the circulation with the highest serum sialic acid concentrations being recorded during the period of the first peak of parasitaemia. *T. vivax* produces sialidase, the activity of which is related to the number of the parasites (Esievo, 1979, 1983). The latter supports the finding of highest serum sialic acid concentrations during peak parasitaemia in the present study and agrees with earlier experimental *T. vivax* infection in cattle during which the highest concentration of free serum sialic acids coincided with peak parasitaemia, significant reduction of erythrocyte surface sialic acids and development of anaemia (Esievo *et al.*, 1982a). It was then postulated that *T. vivax* sialidase cleaved bovine erythrocytes surface sialic acids producing physico-chemical damages on the erythrocytes rendering them more prone to phagocytosis and removal from the peripheral circulation by the reticuloendothelial system (Esievo *et al.*, 1982a). It was suggested that this sialic acid cleavage contributed, partly to the erythrophagocytosis and the reduction of erythrocytes life-span, which are consistent

characteristic features in the early anaemia of bovine trypanosomiasis (Mackenzie and Cruickshank, 1973; Valli *et al.*, 1978; Dargie *et al.*, 1979a; Esievo *et al.*, 1982a). The increase and variations of serum sialic acids concentrations in relation to the parasitaemia, in the present study further support the observation that the severity of the anaemia of bovine trypanosomiasis is related to the intensity and duration of the parasitaemia (Dargie *et al.*, 1979a; Esievo *et al.*, 1982a).

Recognition of foreign structures is the first step in several macrophage functions (Mohr *et al.*, 1987). Also recognition of opsonized particles can be mediated by Fc and C3 receptors and followed by phagocytosis (Griffin, 1981) or lysis (Johnson *et al.*, 1984). The latter suggests an immunological basis for the phagocytosis or lysis of the foreign structures or opsonized particles. However, carbohydrate-specific recognition appears to be an alternative or additional mechanism; sugar-specific receptor activity (Lectins) is expressed on different macrophages (Kolb-Bachofen, 1986). β-galactose specific lectins have been reported on peritoneal macrophages (Kuster and Schauer, 1981; Muller *et al.*, 1983a, Kelm *et al.*, 1986a) and liver macrophages (Kolb-Bachofen *et al.*, 1982; Mohr *et al.*, 1987).

Cleavage of erythrocyte surface sialic acid by commercially-produced bacterial sialidase resulted in the exposure of subterminal galactosyl residues which then bound to the β-D-galactose specific lectin on the macrophages (Muller and Schauer, 1983; Kelm *et al.*, 1986a). The amount of released sialic acids is a decisive factor for binding and phagocytosis of desialylated erythrocytes (Muller *et al.*, 1983a,b). It may be pertinent to suggest that the "foreign structures" recognized on the erythrocytes as a first step of several macrophage functions (Mohr *et al.*, 1987) may include the newly exposed galactose on erythrocyte membrane during *T. vivax* infections. It is therefore, being

surmised that the physico-chemical damages produced on erythrocyte membranes following cleavage of erythrocytes surface sialic acids by trypanosome sialidase rendering desialylated erythrocytes more prone to erythrophagocytosis, in *T. vivax* infected cattle (Esievo *et al.*, 1982a) is the galactose exposed on such erythrocytes. Results from the present study therefore indicates that the early anaemia observed within the first 7 days of experimental *T. vivax* infection in calves may be mediated through the alternate or additional mechanism which involves carbohydrate-specific, or galactose-specific recognition, since during this period immune mediated recognition and lysis may not have been fully operative. Jennings *et al.* (1974) long suggested direct or indirect trypanosomes effects in the rapid onset of anaemia of animal trypanosomiasis. Thus the Fc and C_3 receptors role in the phagocytosis and lysis may be operative after the first 10 to 14 days when immune mechanism may have become fully operative.

Erythrocytes from infected calves bound strongly to the rat peritoneal macrophages. The binding (estimated by the number of rosettes) increased gradually from the period of the appearance of trypanosomes in circulation and the highest numbers of bound erythrocytes was recorded during the periods of initial and second peaks of parasitaemias. These periods also coincided with the highest values of free serum sialic acids concentrations which implies a greatest loss of erythrocyte surface sialic acids at these periods. Similar increased parasitaemia, significant decrease in erythrocyte surface sialic acids and increased free serum sialic acid concentrations were reported in experimental *T. vivax* infection in cattle (Esievo *et al.*, 1982a). Since binding of erythrocytes to macrophages is enhanced by the removal of 10-20% membrane sialic acids while phagocytosis is enhanced by the removal of 30-40% membrane sialic acid (Muller *et al.*, 1983b), the observation of the highest number of bound erythrocytes

(number of rosettes) during peak parasitaemias, highest serum sialic acids, concentrations, thence during significant loss of erythrocytes surface sialic acids, may be readily explained.

Inhibition tests experiments with erythrocytes from the *T. vivax* infected calves showed that D-galactose is the most inhibitory saccharide. Thus, the recognition and binding of the desialylated erythrocytes as observed by the number of rosettes might have occurred by a galactosyl-specific lectin on the surface of the rat peritoneal macrophages. This is indicated by the inhibition by β-D-galactosyl related saccharides. The inhibition pattern corresponds to the one found for contacts mediated by the Gal Nac/Gal particle receptor on rat liver macrophages (Mohr *et al.*, 1987). These observations indicate that binding, due to numerous interactions of demasked β-galactose residues on the red cells, with specific lectin molecules on the macrophages is a pre-requisite for phagocytosis. The findings reported herein give direct evidence and supports for erythrophagocytosis as a contributing mechanism for the anaemia of bovine trypanosomiasis. This report appears to be the first implicating *T. vivax* sialidase in the exposure of subterminal galactosyl residues on erythrocytes as a pre-requisite in the early onset of anaemia of bovine trypanosomiasis.

It is therefore, reasonable to postulate that the very early anaemia produced in experimental *T. vivax* infection in calves is achieved by the sialidase produced as the trypanosomes increase in numbers in peripheral circulation which cleaved off erythrocyte surface sialic acid, thus exposing β-D-galactosyl residues as the subterminal sugar. The exposed galactose residues may then be bound to the β-D-galactose specific lectins of the host macrophages (as sh own in the *in vivo* experiment) and removal from circulation and sequestration by the reticuloendothelial system.

IN VIVO EXPERIMENT

The relationship between increasing trypanosome parasitaemia with increasing free serum sialic acid production and concomitantly increasing rosettes formation by rat peritoneal macrophages, observed in the infected calves with lactose injection agrees with the results in non-lactose injected infected calves. This suggests a trypanosome sialidase induced removal of erythrocytes sialic acids and exposure of membrane subterminal residues of β-galactosyl moiety in both infected calves and calves infected with lactose injection.

Binding of desialylated erythrocytes from infected calves used for the *in vivo* experiments by rat peritoneal macrophages was about 22% lower than those from the infected calves used for the *in vitro* experiments, but following repeated lactose infusion in the former group, binding increased sharply by 109% on day 7 p.i., with the marked increase in binding remaining throughout the period of lactose injection, which also coincided with the period of marked increase of free serum sialic acids production. A plausible explanation for this is the presence of sustainable parasitaemias during the period of lactose injection in the infected calves. A sustainable trypanosome parasitaemia will readily source a continuous sialidase production, erythrocytes sialic acids cleavage, serum free sialic acid production, exposure of membrane subterminal residues of β-galactosyl moieties, that supports an enhanced rosetting by the rat peritoneal macrophages, over the non-lactose infused infected calves. Indeed, sialidase activity is related to the number of trypanosome parasites (Esievo, 1979, 1983).

The sustained parasitaemia in the infected calves during the period of lactose injection may be explained from the point of energy requirements of trypanosomes. Trypanosomes stop respiring and become immobile within one minute if there is no

supply of exogenous carbohydrate, because of their low energy reserves (Opperdoes *et al.*, 1976). Energy is derived from glucose, but in ageing trypanosomes, with falling parasitaemia, 2-oxoglutarate can replace glucose to some extent (Bowman and Flynn, 1977). Glucose catabolism occurs via a glycolytic pathway which terminates in pyruvate, the glycolytic enzymes being located in microbody-like organelles called glycosomes (Opperdoes and Borst, 1977). In addition to the macrophage inhibitory role of lactose, this disaccharide of glucose and galactose may serve as further energy source for the trypanosomes.

In vitro lactose inhibition study with erythrocytes from infected calves that were not injected with lactose showed relatively lower binding by rat peritoneal macrophages. This may indicate some degree of clearance and sequestration of desialylated erythrocytes by an already enhanced macrophage phagocytic system of the host. It is therefore reasonable to surmise that lactose injection into the infected calves inhibits the galactosyl specific lectin on the host macrophages which may thus contribute partly to some degree of prolongation of erythrocytes life-span. Therefore, the role of lactose in the control of trypanosomiasis by prolonging erythrocytes life-span through inhibition of erythrophagocytosis, may be limited to a base and requires its combination with a trypanocidal agent.

4.3 GENERAL DISCUSSION.

In the two experiments reported, all the infected calves developed trypanosomiasis. The prepatent period following needle infection with *T. vivax* varied between 2 and 3 days in both the *in vitro* and *in vivo* studies.

Soon after establishment of patent infection, the parasitaemia continued to rise

and reached a peak 5 days after infection and this was followed by a gradual decline over the next 2 days. In the non-lactose-injected infected calves, by day 8 after infection, trypanosomes were too scanty to be counted. However, following repeated lactose injection on day 6 in the infected calves with lactose injection, trypanosomes flourished and in numbers large enough to be counted. Circulating trypanosomes increased again between days 9 and 13 after infection in both groups.

There were 2 peaks in the development of parasitaemia with the 2 peaks occurring on days 5 and 13 post infection. The observed peaks of parasitaemia is in accordance with different parasitaemic waves in the early stages of trypanosome infection reported by Esievo *et al.* (1982a). In established infections, parasitaemia has been reported to fluctuate considerably with the trypanosomes appearing and disappearing from the blood at irregular intervals. Generally, there is a period of parasitaemia lasting up to several months, after which the trypanosomes are undetectable in the blood, or alternating periods of crises and relapses follow (Hoare, 1972). In the early stages of infection, *T. vivax* have been reported to invade the lymph glands (Hornby, 1952). It is possible that in relapses following the disappearance of the parasites from the circulation the blood is restocked by trypanosomes sheltering in the lymph glands (Hornby, 1952; Hoare, 1972).

This could also be due to antigenic variation in which individual parasites have the capacity to present different antigenic guises to the host (Ritz, 1916; Esievo and Saror, 1991). The phenomenon of antigenic variation of trypanosomes is possibly essential for the survival of the trypanosomes in the mammalian host (Doyle *et al.*, 1978; Vickerman and Tetley, 1978; Esievo and Saror, 1991) so that as one parasite population is cleared by the host's immune system, another springs up. This evasion mechanism of

the trypanosome is achieved by changing the glycoprotein pattern of the cell surface which may be related to sialic acid activity (Schauer, 1983; Esievo and Saror, 1991). Therefore, the successive waves of parasitaemia represent antigenically distinct populations.

Following repeated lactose injection in the infected and lactose injected animals, trypanosomes remained in circulation throughout the experimental period and also in numbers large enough to be counted, while they disappeared completely in the infected calves not injected with lactose between days 7 and 8 p.i. (Fig. 8 in the appendix). The sustained parasitaemia in the infected calves during the period of lactose injections may be explained from the energy requirement of trypanosomes. In addition to the macrophage inhibitory role of lactose, this disaccharide of glucose and galactose may partly serve as a further energy source for the trypanosomes. Glucose is a weak inhibitor of erythrophagocytosis while galactose is a good inhibitor at relatively high concentrations (Kolb and Kolb-Bachofen, 1978; Muller *et al.*, 1981, 1983a; Mohr *et al.*, 1987).

The development of anaemia as determined by packed cell volume (PCV) started from day 3 p.i. with a general decrease in the PCV in all the infected calves in the course of the infection (Appendix 1). In the infected calves that were not injected with lactose the PCV decreased gradually from days 3 p.i. with very low values of 14.5 $\pm$ 3.54 and 16.0 $\pm$ 1.41%, occurring on days 8 and 13 p.i. respectively. In the group of calves infected and followed with repeated lactose injection, the PCV decreased gradually from day 2 p.i. with very low PCV values of 18.75 $\pm$ 2.06, 18.00 $\pm$ 1.83 and 16.50 $\pm$ 0.58 percents on days 8, 11 and 13 respectively. The periods of low PCV levels in both group of animals fell between the first and second parasitaemic waves. The

mean PCV values were significantly ($P<0.02$) different on day 4 between the two groups of calves.

The degree of anaemia is related to the drop in total red cell mass and hence PCV (Dargie and Alesandro, 1978). The progressive decrease in PCV values observed during the course of the infection supports the earlier observation that anaemia is one of the major pathological and clinical features of trypanosomiasis (Fiennes, 1954; Mackenzie and Cruickshank, 1973; Mamo and Holmes, 1975; Esievo *et al.*, 1982a). Indeed, despite the large number of clinical, biochemical and haematological manifestations of trypanosomiasis, the PCV is probably the most practical indicator of the severity of the disease (Dargie and Alesandro, 1978). However, the rate of decrease in PCV varied in all the infected calves and this could be due to differences in the extent of susceptibility to infection.

Increase in serum sialic acid tended to coincide with the appearance of the trypanosomes in the circulation with the highest serum sialic acid concentrations being recorded during the period of the first peak of parasitaemia in both infected groups of cattle. *T. vivax* produces sialidase, the activity of which is related to the number of the parasites (Esievo, 1979, 1983). The increase and the variations of serum sialic acids concentrations in relation to the parasitaemia, in the present study further agrees and support the observation that the severity of the anaemia of bovine trypanosomiasis is related to the intensity and duration of the parasitaemia (Dargie *et al.*, 1979a; Esievo *et al.*, 1982a).

In both the *in vitro* and *in vivo* experiments binding between desialylated erythrocytes and rat peritoneal macrophages peaked on day 4 post infection though free serum sialic acid concentrations and parasitaemia peaked on day 5 post infection. The

reason for the observed peak binding between desialylated erythrocytes and rat peritoneal macrophages on day 4 rather than day 5 post infection is not immediately known. It is pertinent to note that rat peritoneal macrophages bind and phagocytose sialidase - treated erythrocytes at a rate which is dependent on the amount of sialic acid that has been removed from the cells. Increased binding of erythrocytes is observed after the removal of 10-20% of membrane sialic acids (Muller and Schauer, 1983; Muller *et al.*, 1983a). Removal of 10-20% of sialic acid have been reported to be sufficient to eliminate erythroyctes from the circulation while hydrolysis of 30-40% of sialic acids led to an increase of the number of bound and engulfed erythrocytes (Aminoff *et al*, 1976; Muller *et al*, 1983a). This is because when compared to binding, for phagocytosis a higher number of galactose residues must be unmasked on the erythrocyte membrane thus enabling interaction with a greater amount of lectin molecules on the macrophage to trigger phagocytosis (Muller *et al*, 1983b). In the present study, desialylation of erythroyctes occurred *in vivo* due to the production of sialidase by the trypanosomes. During the first few days of the study with trypanosomes appearing in the peripheral circulation on days 2 and 3 post infection in the *in vivo* and *in vitro* experiments respectively sialidase production may be low with subsequent low sialidase concentration in circulation. This may have resulted in the removal of low quantities of membrane sialic acids (less than 20%). Low sialidase concentrations of up to 0.5mU/ml have been reported not to cause release of appreciable amounts of sialic acid from erythrocytes (Muller *et al*, 1983a, b). In effect, the desialylated erythrocytes will remain in circulation or may subsequently be sequestrated from the blood stream (Aminoff *et al.* 1976; Janeik *et al.*, 1978; Schauer, 1982; Muller *et al.*, 1983a) but this does not lead to phagocytosis (Muller and Schauer, 1983; Kelm *et al.*, 1986a,b). By day 4 post

infection, parasitaemia had increased to 10.5 ± 1.0 x 10^6/ml (Table 2) and 12.6 ± 3.1 x 10^6/ml (Table 8) in the *in vitro* and *in vivo* experiments respectively with subsequent increases in free serum sialic acids to 0.30 ± 0.03 mg/dl and 0.36 ± 0.03 mg/dl (Table 3) in the *in vitro* and *in vivo* experiments respectively. The release of 10-20% of the total sialic acid molecules from the erythroyctes cell surface initiate binding. Using rat erythroyctes, Kuster and Schauer (1981) on the assumption that sialic acid is linked to galactose exclusively, estimated this threshold value of 10-20% to correspond to demasking of about 2 x 10^6 galactosyl residues. However, it is important to determine whether this threshold value corresponds to a certain number and arrangement of lectin molecules on the macrophage surface is required for binding. Macrophages (Kupffer cells and hepatocytes) are involved in the clearance of such desialylated erythrocytes though the route of uptake will depend on the ligand size (Kolb-Bachofen *et al.*, 1982; Kolb-Bachofen, 1988). Jancik *et al.* (1978) observed that sialidase-treated ^{51}Cr labelled erythrocytes were not lyzed in the blood stream but accumulated in spleen and liver of rabbits and they are bound and phagocytozed by macrophages. Similarly, electron microscopy revealed that *in vivo*, both asialolymphocytes (Kolb-Bachofen and Kolb, 1979) and asialo-erythrocytes (Kolb-Bachofen *et al.*, 1984) are found adhering almost exclusively to the liver macrophages shortly after their injection. Therefore the peak binding observed on day 4 may be explained by the cleavage of sialic acid glycosidic bonds. The increased cleavage of sialic acid from the erythrocytes resulting to increased free serum sialic acids may have been sufficient to initiate binding with the host macrophages *in vivo* and not to phagocytosis. This is also reflected in the increased binding between such erythrocytes and rat peritoneal macrophages in the *in vitro* cell binding assay. With further increases in both parasitaemia and free serum sialic acid

concentrations on day 5, binding between the freshly isolated rat peritoneal macrophages and desialylated erythrocytes decreased probably due to the removal of the pre-requisite 30-40% of membrane sialic acids required for phagocytosis with the consequent removal of such desialylated erythrocytes by the host macrophages *in vivo*. Usually, one macrophage is able to engulf between one and ten erythrocytes (Schauer *et al.*, 1984). This is further substantiated by the increased binding between rat peritoneal macrophages and desialylated erythrocytes obtained from calves in the *in vivo* studies particularly during the period of lactose injection (Table 9). The lactose or the product(s) of it's hydrolysis may have prevented *in vivo* erythrophagocytosis by blocking the lectins on the macrophages hence keeping the desialylated erythrocytes longer in circulation.

The peak binding observed on day 4 post infection may also be explained on the basis of fractional receptor occupancy. Desialylated erythrocytes are ligands since they bind with a high degree of specificity to the receptors to form desialylated erythrocyte-receptor complex. Such desialylated erythrocytes may be regarded as full agonists when they bind to the receptors and cause a maximal response (probably phagocytosis) or partial agonists when they evoke a submaximal response (probably just binding) even when all the receptors are filled. The amount of sialic acids removed from the erythrocyte surface may determine the type of agonist to which it will belong since loss of sialic acid alone is responsible for the recognition of erythrocytes by peritoneal macrophages (Kelm *et al.*, 1986a). In some instances, the amount of biological response is directly proportional to the number of receptors occupied and the intrinsic activity of the ligand. In some cases a maximal biological response may be achieved when only a fraction of the receptors is occupied by the ligand while in other cases a significant

fraction of receptors may need to be occupied before a ligand produces a measurable biological effect. In this latter case, there is a threshold level of receptor occupancy necessary for response (Smith *et al.*, 1985b). This may be related to the threshold estimated by Kuster and Schauer (1981) and to the number of galactosyl residues exposed on the erythrocyte surface (Kuster and Schauer, 1981; Muller *et al.*, 1983a,b; Kolb-Bachofen, 1988). On day 4 post infection, the massive exposure of galactosyl residues on the erythrocytes due to the removal of large quantities of membrane sialic acid with subsequent increase in free serum sialic acid, the threshold level of receptor occupancy may have been attained thus resulting in peak binding between the desialylated erythrocytes and rat peritoneal macrophages. Though only the amount of released sialic acids is the decisive factor for binding and phagocytosis of sialidase-treated erythroycles (Muller *et al.*, 1983a,b) clustering of ligands (desialylated erythrocytets) leads to a strong increase in binding and this may be due to simultaneous binding of more than one galactose residue (Kelm *et al.*, 1986a) for peritoneal macrophages. This is in agreement with the observation of peak binding on day 4 post infection in the present study. Similarly, clustering of receptors may also be of importance in the interaction between kupffer cell lectins with multivalent particles (Kolb-Bachofen *et al.*, 1982). Preclustered arrangement of galactose-specific binding sites on liver sinusoidal cells is indeed an absolute requirement for effective recognition and uptake of large particulate ligands (Kolb-Bachofen, 1988; Dini and Kolb-Bachofen, 1989). The signal for uptake of ligand is only triggered when more than a few receptors per cluster are occupied, a condition that is fulfilled best by particles with multiple binding sites but not by soluble glycoproteins (Kolb-Bachofen *et al.*, 1982). Extensive desialylation of erythrocytes from infected calves in the present study provides signals

that will lead to the occupation of more than a few receptors per cluster thus resulting in a biological response. However, whether or not the observed peak binding between rat peritoneal macrophages and desialylated erythrocytes on day 4 post infection is due to any or all of the factors above remains to be determined. But a clearer understanding of the ligand-receptor interaction may be obtained by radioligand studies particularly to determine the number of receptor binding sites and their affinity for the ligands. The relationship between increasing trypanosome parasitaemia, with increasing free serum sialic acids production and a concomitantly increasing rosettes formation by rat peritoneal macrophages observed in the lactose-infused infected calves agrees with the result in non-lactose-infused infected calves. This suggests a trypanosome sialidase induced removal of erythrocytes sialic acids and exposure of membrane subterminal residues of β-galactosyl moiety in both non-lactose and lactose-infused infected calves. In fact, correlation studies in both the non-lactose and lactose-infused infected calves showed a strong positive association ($P<0.01$) between serum sialic acids and rosette formation.

From the results obtained in the two studies above, it is pertinent to hypothesize that the early anaemia observed within the first few days of experimental *T. vivax* infection in cattle may be mediated partly through the alternate or additional mechanism of carbohydrate-specific, hence galactose-specific recognition, a period during which immune mediated recognition and lysis may not have been fully operative. Though, Jennings *et al.* (1974) long suggested direct or indirect trypanosomes effects in the rapid onset of anaemia of animal trypanosomiasis, there is insufficient evidence that antigen-antibody complexes demonstrable on red cells play a significant role in the anaemia (Dargie and Alesandro, 1978).

CONCLUSION AND RECOMMENDATION.

Anaemia, a clinical feature in both human and animal trypanosomiasis has long been established as a major pathological feature of animal trypanosomiasis and particularly in livestock infected with *Trypanosoma congolense* and *Trypanosoma vivax*. The cause and mechanism of this anaemia is yet to be clearly defined. Emphasis has been given to erythrophagocytosis and the reduction of erythrocyte life-span due to the effects of surface sialic acid on the red cells and the trypanosomes, and the production of sialidase by trypanosomes.

From the results obtained in this study, it is reasonable to postulate that the very early anaemia produced in experimental *T. vivax* infection in calves is partly achieved by the sialidase produced as the trypanosomes increase in numbers in peripheral circulation which cleaved off erythrocyte surface sialic acids thus exposing β-D-galactosyl residues as the subterminal sugar. The exposed galactose residues may then be bound to the β-D-galactose specific lectins of the host macrophages leading to their removal from circulation and their subsequent sequestration by the reticulo-endothelial system. Similarly, lactose infusion into cattle infected with *T. vivax* does not prevent exposure of penultimate β-galactose residues on erythrocytes, but rather, has its inhibitory role targeted on the host macrophage phagocytic system. Lactose, perhaps, through the energy requirement of the parasites, sustained detectable parasitaemia, more exposure of membrane galactosyl residues and hence higher *in vitro* rosetting of desialylated erythrocytes by rat peritoneal macrophages. Therefore, the role of lactose in the control of trypanosomiasis by prolonging erythrocytes life-span through inhibition of erythrophagocytosis, may be limited to a base and may require its combination with a trypanocidal agent.

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It will be important to extend the present investigation to purely *in vivo* studies involving the host macrophage system and the desialylated erythrocytes *in vivo* to establish and confirm the participatory roles of desialylation *in vivo*, galactose exposure on erythrocyte surface and host macrophages in the early anaemia obser ved in bovine trypanosomiasis. Similarly the role of lactose as a possible inhibitor of erythrophagocytosis needs further investigation. For instance it will be important to investigate why parasitaemia was sustained in the presence of lactose. Is it possible that lactose or its products of hydrolysis served as an alternative source of energy for the trypanosomes? Could it be that either the host or the parasites reacted to the injection of lactose by secreting an enzyme for example lactase to hydrolyse the lactose to glucose (as an added source of energy) and galactose as an inhibitor of erythrophagocytosis by combining with the β-galactose specific lectins on the host macrophages?. This information will be necessary for lactose to be used effectively as an inhibitor of erythrophagocytosis.

REFERENCES

Akol, G.W.O. and Murray, M. (1981). Early events in the pathogenesis of African trypanosomiasis in cattle, localised skin reactions (chancres) which follow the bites of *Glossina morsitans* infected with *Trypanosoma congolense, T. brucei* and *T. vivax*. Proceedings of 17th Mtg. Of the International Scientific Council, Arusha, Tanzania, October, pp. 230-233.

Allan, D. and Crumpton, M.J. (1971). Fractionation of the phytohaemaglutini of *Phaseolu vulgaris* by polyacrylamide Gel Electrophoresis in sodium Dodecyl sulphate. **Biochemical Biophysical Research Communications**. 44:1143-1148.

Aminoff, D. (1961). Methods for quantitative estimation of N-acetyl-neuramic acid and their application to hydrolysis of sialo-mucoids. **Biochemical Journal**, 81:384-392.

Aminoff, D., Bell, W.C., Fulton, I. and Ingebrigtsen, N. (1976). Effect of sialidase on the viability of erythrocytes in circulation. **American Journal of Hematology**, 1:419-432.

Aminoff, D., William, F., Bruegge, V., Bell, W.C., Sarpolis, K. and Williams, R. (1977). Role of sialic acid in survival of erythrocytes in the circulation. Interaction of neuraminidase-treated and untreated erythrocytes with spleen and liver at the cellular level. **Proceedings of the National Academy of Science, USA** 74:1521-1524.

Anosa, V.O. (1980). Studies on the parasitaemia, plasma volumes, leucocyte and bone marrow counts and the moribund state in *T. brucei* infection of splenectomised and intact mice. **Zentralbl. Veterinaevmed [B]** 27:169-180.

Anosa, V.O. (1981). Applications of Radioisotope Techniques to the study of Trypanosomiasis in Domestic Ruminants and Laboratory Animals. In: "Isotopes and Radiation in Parasitology IV, I.A.E.A., Vienna, pp. 53-57.

Anosa, V.O. (1983). Diseases produced by *Trypanosoma vivax* in ruminants, horses and rodents. **Zent. Fur. Vet.** 717-741.

Anosa, V.O., and Isoun, T.T. (1980). Haematological studies on *Trypanosoma vivax* infection of goats and intact and splenectomized sheep. **Journal of Comparative Pathology**. 90:155-168.

Anosa, V.O., Jennings, F.W., and Urquhart, G.M. (1977). The effect of splenectomy on anaemia of *T. brucei* infection. **Journal of Comparative Pathology**. 87:569-579.

Anosa, V.O. and Kaneko, J.J. (1983a). Pathogenesis of *Trypanosoma brucei* infection in deer mice (*Peromyscus maniculatus*) V. Marophage ultrastructure and function. **Veterinary Pathology.** 20:617-631.

Anosa, V.O. and Kaneko, J.J. (1983b). Pathogenesis of *Trypanosoma brucei* infection in deer mice (*Peromyscus maniculatus*). Light and electron microscopic studies on erythrocyte pathologic changes and phagocytosis. **American Journal of Veterinary Research.** 44:645-6451.

Apted, F.J.C. (1970). Clinical manifestation and diagnosis of sleeping sickness. In: "African Trypanosomiasis", (Mulligan, H.W. ed.) Allan and Unwin Ltd., London, pp. 661-683.

Ashwell, G. and Harford, J. (1982). Carbohydrate-specific receptors of the liver. **Annual Review of Biochemistry.** 51:531-554.

Ashwell, G. and Morell, A.G. (1974). The role of surface carbohydrates in the hepatic recognition of circulating glycoproteins. In: "Advanceds in Enzymology, Vol. 41. (Meister, A. ed.) John Wiley and Sons, New York, London, Sydney, Toronto. pp. 99-128.

Ashwell, G. and Morell, A.G. (1978). In: "the Glycoconjugates", Vol. II (Horowitz, M.I. and Pigman, W. Eds.). Academic Press, New York, San Francisco, London, pp. 231-234.

Benacceraf, B., Biozzi, G., Halpern, B.N. and Stiffel, C. (1975). In: "Physiopathology of the Reticuloendothelial system". (Halpern, B.N. ed.) Charles C. Thomas, Springfield, IL., pp. 52-79.

Bezhorovainy, A., Grohlich, D. and Pinter, J.K. (1970). The carbohydrate Moiety of Bovine Colostrum Whey M-I Glycoprotein and its relationship to casein. **Journal of Agriculture and Food Chemistry.** 18:624-628.

Boch, J., Weiland, T., Bachtaler, E., Geisselbrecht, R.H., Meinrich, H.D., Willberg, G. and Reiter, I. (1984). Parasitological, clinical and serological findings in Dahomey cattle experimentally infected with *Trypanosoma congolense* and *T. brucei*. **Berl. Und. Munch. Tier Woch.,** 97:398-404.

Borst, P., Bernards, A., Van der Ploeg, L.A.T., Michels, P.A.M., Liu, A.Y.C., De Lange, T. and Kooter, J.M. (1983). The control of variant surface antigen synthesis in trypanosomes. **European Journal of Biochemistry.** 137:383-389.

Bowman, A.B.R. and Flynn, I.W. (1977). Oxidative metabolism of ktrypanosomes. In: "Biology of the kinetoplastida I. Academic Press, London, New York.

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

Brand, T.V. (1951). Metabolism of Trypanosomidae and Bodonidae. In: "Biochemistry and Physiology of Protozoa. (Lwoff, A. Ed.) Vol. I, pp. 177.

Brand, T.V. (1966). In: "Biochchemistry of Parasites, New York, London.

Brown, B. (1980). Routine hematology procedures. In: "Hematology, principles and procedures, 3rd Ed., Lea and Febiger. Philadelphia. pp. 29-70.

Brun, R., Baeniswyl, S., and Kunz, C. (1989). *In vitro* drug sensitivity of *Trapanosoma gambiense* isolates. **Acta Tropica.** 46:369-376.

Carr, I. and Deams, W.T. (1980). The macrophage. In: "The reticuloendothelial system", Vol. I, (Carr, I. And Deams, W.T. eds). Plenum Press, New York. pp. 1-17.

Carroll, S.M., Higa, H.H. and Paulson, J.C. (1981). Different cell-surface receptor determinants of antigenetically similar influenza virus Hemagglutinins. **Journal of Biological Chemistry.** 256:8357-8363.

Chandler, R.L. (1952). Comparative tolerance of West African N'dama cattle to trypanosomiasis. **Annals of Tropical Medicine Parasitology.** 46:127.

Chandler, R.L. (1958). Studies on the tolerance of N'dama cattle to trypanosomiasis. **Journal of Comparative Pathology.** 68:253.

Chatfield, C. (1983). Statistics for Technology. A course. In: Applied Statistics. 3rd ed., Chapman and Hall, London. pp. 134-148.

Clarkson, M.J. (1976). Immunoglobulin M. In trypanosomiasis. In: Pathophysiology of parasitic infection, (Soulsby, E.J.L. ed.), Academic Press, New York, San Francisco, London. pp. 171-182.

Corfield, T. (1992). Bacterial sialidases-roles in pathogenicity and nutrition. **Glycobiology.** 2:509-521.

Corfield, A.P. and Schauer, R. (1982). Occurrence of sialic acids. In: "Sialic Acids". (Schauer, R. Ed.) Springer, New York. pp. 5-55.

Corfield, A.P., Higa, H., Paulson, J.C. and Shauer, R. (1983). The specificity of viral and bacterial sialidases for ∝(2-3)- and ∝(2-6)- linked sialic acids in glycoproteins. Biochemica Biophysica Acta. 744:121-126.

Corsini, A.C., Clayton, C., Askonas, B.A., Oglivie, B. M. (1977). Suppressor cells and loss of B cell potential in mice infected with *T. brucei*. **Clinical Experimental Immunology.** 29:122-131.

Cross, G.A.M. and Takle, G.B. (1993). The surface trans-sialidase family of *Trypanosoma cruzi*. **Annual Review of Microbiology.** 47:385-411.

Dacie, J.V. and Lewis, S.M. (1975). In: Practical Haematology, fifth ed. Churchill Livingstone, Edinburg, London and New York. pp. 21-67.

Dargie, J.D. and Alcsandro, P.A.D. (1978). Blood and Hematopoietic Tissue Responses. Discussion summary. In: pathogenicity of trypanosomes. Proceedings of a Workshop held at Nairobi, Kenya, 20-23 November, 1978. (Losos, G. and Chouinard, A. Ed). pp. 149-150.

Dargie, J.D. Murray, P.K., Murray, M., Grimshaw, W. And McIntyre, W.I.M. (1979). Bovine trypanosomiasis, the red cell kinetics of N'dama and Zebu cattle infected with *Trypanosoma congolense*. **Parasitology** (England). 78:271-286.

Dargie, J.D., Murray, P.K., Murray, M., McIntyre, W.I.M. (1979b). The blood volumes and erythrokinetics of N'dama and Zebu cattle experimentally infected with *Trypanosoma brucei*. **Research in Veterinary Science.** 26:245-347.,

Danon, D. (1966). Eleventh Congress of the International Society of Hematology. Papers presented at the plenary session of the congress. pp. 394-405.

Davis, B.S. (1952). Studies on the trypanosomes of some Califonia mammals. University of California Publication on Zoology. 57(3):145.

de-Raadt, P. and Seed, J.R. (1977). Trypanosomes causing disease in man in Africa. In: Parasitic Protozoa 1. (Kreier, J.P. ed). New York Academic Press. pp. 175-237.

Desowitz, R.S. (1959). Studies on immunity and host-parasite relationships 1. The immunological response of resistant and susceptible breeds of cattle to trapanosomal challenge. **Annals of Tropcal Medicine and Parasitology.** 53:293-313.

Dini, L. and Kolb-Bachofen, V. (1989). Preclustered receptor arrangement is a prerequisite for galactose-specific clearance of large particulate ligands in rat liver. **Experimental Cell Research.** 184:235-240.

Dixon, H. (1966). Blood platelets as a source of enzyme activity in washed trypanosome suspensions. **Nature.** 210:428.

Dizeniek, R. (1973). Substrate specificity of neuraminidases. **Histochemistry Journal.** 5:271-290.

Doyle, J.J., Hirumi, H. and De Gee, A.L.W. (1978). Mechanisms of antigenic variation in salivarian trypanosomes. In: "Pathogenicity of trypanosomes". Proceedings of a Workshop held at Nairobi, Kenya, November, 20-23. (Losos, G. And Chouinard, A.J. eds). pp. 44-45.

Dreyfus, H., Louis, J.C., Harth, S., Durand, M. and Massarelli, R. (1983). In: "Neural Transmission". Learning and Memory (Caputto, R. and Ajmone, Marsan, C. eds). Raven Press, New York. pp. 21-23.

Durocher, J.R., Payne, R.C. and Conrad, M.E. (1975). Role of sialic acid in erythrocyte survival, **Blood.** 45:11-20.

Engstler, M., Reuter, G. and Schauer, R. (1992a). Identification of a trans-sialidase activity in procyclic *Trypanosoma brucei*. **Biological Chemistry, Hoppe-Seyler**. 373:843.

Engstler, M., Reuter, G. and Schauer, R. (1992b). Purification and characterization of a novel sialidase found in procyclic culture forms of *Trypanosoma brucei*. **Molecular and Biochemical Parasitology.** 54:21-30.

Engstler, M. and Schauer, R. (1993). Sialidases from African Trypanosomes. **Parasitology Today.** 9:222-225.

476583

Engstler, M., Reuter, G. and Schauer, R. (1993). The developmentally regulated trans-sialidase from *Trypanosoma brucei* sialylates the procyclic acidic repetitive protein. **Molecular Biochemical Parasitology.** 61:1-4.

Engstler, M., Schauer, R. and Brun, R. (1995). Distribution of developmentally regulated trans-sialidases in the kinetoplastida and characterization of a shed-trans-sialidase activity from procyclic *Trypanosoma congolense*. **Acta Tropica.** 59:117-129.

Esievo, K.A.N. (1979). *In vitro* production of neuraminidase (sialidase) by *Trypanosoma vivax*. Proceedings of the 16th Meeting of the OAU/STRC. Trypanosomiasis Research Control, Yaounde, Cameroun, October 29 to November 3. pp. 205-210.

Esievo, K.A.N. (1980). Studies on an aspect of anaemia of bovine trypanosomiasis (*Trypanosoma vivax*). Ph.D. Thesis, Ahmadu Bello University, Zaria.

Esievo, K.A.N. (1983). *Trypanosoma vivax*, stock V953: Inhibitory effect of type A influenza virus anti HAV8 serum on *in vitro* neuraminidase (sialidase) activity. **Journal of Parasitology.** 69:941-945.

Esievo, K.A.N., Saror, D.I., Ilemobade, A.A. and Hallaway, M.H. (1982a). Variation in erythrocyte surface and free serum sialic acid concentrations during experimental *Trypanosoma vivax* infection in cattle. **Research in Veterinary Science.** 32:1-5.

Esievo, K.A.N., Moore, W.E. and Muller, R. (1982b). Scanning electron microscopy of erythrocytes from *Trypanosoma vivax* infected cattle. Departments of Laboratory Medicine and Pathology, Kansas State University, Manhattan, U.S.A. (Unpublished).

Esievo, K.A.N., Saror, D.I., Kolo, M.N. and Eduvie, L.O. (1986). Erythrocyte surface sialic acid concentrations in N'dama and Zebu cattle. **Journal of Comparative Pathology,** 96:95-100.

Esievo, K.A.N. and Saror, D.I. (1991). Immunochemistry and Immunopathology of animal traypanosomiasis. **Protozoological Abstracts.** 15:337-348.

Eylar, E.H., Madoff, M.A., Brody, O.V. and Oncley, J.L. (1962). The contribution of sialic acid to the surface charge of the erythrocyte. **Journal of Biological Chemistry.** 237:1992-2000.

Ezepchuk, Y.V., Vertier, Y.V. and Kostyukova, N.N. (1973). Neuraminidase of *Corynebacterium diphtheniae* as a factor of pathogenicity with a spreading function. **Byull. Eksperiment Biol. Med.** 75:63-65 (In Russian).

Fiennes, R.N.T.W. (1954). Haematological studies in trypanosomiasis of cattle. **Veterinary Record.** 66:423-434.

Fiennes, R.N.T.W. (1970). Pathogenesis and pathology of animal trypanosomiasis. In: "the African trypanosomiasis (Mulligan, H.W. ed.). Allen and Unwin Ltd., London. pp. 729-746.

Ford, J. (1971). Patterns of tsetse distribution and evolution. In: "The role of the trypanosomiasis in African Ecology. A Study of Tsetse-fly problem". Clarendon Press, Oxford. pp. 34-58.

Furth, R., Van, Cohn, Z.A., Hirsch, J.C., Humphrey, J.H., Spector, W.G. and Lange-Voort, H.L. (1972). The mononuclear phagocyte system: a new classification of macrophages, monocytes and their precursor cells. **Bulletin of World Health Organization (WHO).** 46:845-852.

Gabriel, O., Heeb, M.J. and Hinrichs, M. (1984). Interaction of the surface adhesin of the oral Actinomyces ssp. With mammalian cells. In: "Molecular basis of oral microbial adhesion. Proceedings of an ASM Workshop in Philadelphia. pp. 45-52.

Godoy, V.G., Miller Dallas, M., Russo, T.A. and Malamy, M.H. (1993). A role for *Bacteroides fragilis* neuraminidase in bacterial growth in two model systems. **Infection Immunology.** 61:4415-4426.

Goodwin, L.G. (1970). The pathology of African trypanosomiasis. Transactions of the **Royal Society of Tropical Medicine and Hygiene.** 64:797-812.

Grant, P.T. and Fulton, J.D. (1957). The catabolism of glucose by strains of *Trypanosoma rhodesiense*. **The Biochemical Journal** (England). 66:242-250.

Gregoriadis, G., Putman, D., Louis, L. and Neerunjun, D. (1974). Comparative effect and fate of non-entrapped and liposome-entrapped neuraminidase injected into rat. **Biochemical Journal.** 140:323-330.

Griffin, F.M. Jr. (1981). Roles of macrophage Fc and C3b receptors in phagocytosis of immunologically coated *cryptococcus neoformans*. Proceedings of the National Academy of Science, USA. 78:3853-3857.

Gutteridge, W.E. and Coombs, G.H. (1977). Catabolism and the generation of energy 1 stages parasitic in vertebrates. In: "Biochemistry of Parasitic Protozoa". MacMillan Press Ltd., London and Basingstoke. pp. 32-58.

Gutteridge, W.E. and Rogerson, G.W. (1979). Biochemical aspects of the biology of *T. cruzi*. In: Biology of the Kinetoplastida 2. (Lumsden, W.H.R. and Evans, D. eds.) Academic Press, London, New York. pp. 619-652.

Guyton, A.C. (1981). Resistance of the body to infection - the leukocytes, the tissue macrophage system and inflamation. In: "Textbook of Medical Physiology", 6th edition. W.B. Saunders Company Philadelphia, London, Toronto. pp. 65-73.

Hall, B.F., Webster, P., Ma, A.K., Joiner, K.A. and Andrews, N.W. (1992). Desialylation of lysosomal membrane glycoprotein by *Trypanosoma cruzi* - a role for the surface neuraminidase in facilitating parasite entry into the host cell cytoplasm. **Journal of Experimental Medicine.** 176:315-325.

Hammond, D.J., Gutteridge, W.E. and Opperdoes, F.R. (1981). A novel location for two enzyme of *de novo* pyrimidine biosynthesis in trypanosomes and leishmania. **FEBS Letters.** 128:27-29.

Hansson, H.A., Holmgren, J. and Svenner holm, L. (1977). Ultra-structural localization of cell membrane GMI ganglioside by cholera toxin. **Proceedings of the National Academy of Science, USA.** 74:3782-3786.

Herrler, G., Reuter, G., Rott, R., Klenk, H.D. and Schauer, R. (1987). N-Acetyl-9-0-acetylneuraminic acid, the receptor determinant for influenza C virus, is a differentiation marker on chicken enrythrocytes. **Biological Chemistry, Hoppe-Seyler.** 368:451-454.

Hill, G.C. (1976). Characterization of electron transport systems present during the life-cycle of African trypanosomes. In: "Biochemistry of Parasites and Host Parasite relationships". (Van Den Bossche, H. Ed.) Elsevier/North Holland, Amsterdam. pp. 31-50.

Hoare, C.A. (1972). The trypanosomes of mammals. A Zoological Monograph. Blackwell Scientific Publications, Oxford and Edinburg.

Hornby, H.E. (1952). Animal trypanosomiasis in Eastern Africa 1949. H.M. Stationery Office.

Howe, C. and Lee, L.T. (1972). Virus - erythrocyte interactions. **Advances in Virus Research.** 17:1-50.

Ikede, B.O., Elhassan, E. and Akpavie, S.O. (1988). Reproductive disorders in trypanosomiasis. A review. **Acta Tropica.** 45:5-10.

Ilemobade, A.A. and Balogun, T.G. (1981). Pig trypanosomiasis effects of infection on feed intake, liveweight gain and carcass traits. **Tropical Animal Health and Production.** 13:128-136.

ILRAD (1986). Annual Report of the International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

ILRAD (1989a). The control of haemoprotozoan diseases to improve livestock productivity in Africa. In: "ILRAD Reports", October, 1989. Nairobi, Kenya.

ILRAD (1989b). Highlights 1989. Nairobi, Kenya.

Jacobsen, F. (1982). Increase of the *in vitro* complement - dependent cytotoxicity against autologous invasive human bladder tumor cells by neuraminidase treatment, **Acta Pathology Microbiology and Immunology Scandinavian** Sect. C. 90:187-192.

Jancik, J. and Schauer, R. (1974). Sialic acid, a determinant of the life-time of rabbit erythrocytes. **Hoppe-Seylers' Zeitschrift fur Physiologisch Chemie.** 355:395-400.

Jancik, J., Schauer, R., Streicher, H.J. (1975). Influence of membrane bound N-acetylneuraminic acid on trhe survival of erythrocytes in man. **Hoppe-Seyler's Zeitschrift fur Physiologisch Chemie.** 356:1329-1331.

Jancik, J.M., Schauer, R., Andres, K.H. and Von During, M. (1978). Sequestration of neuraminidase-treated erythrocytes: Studies on its topographic morphologic and immunologic aspects. **Cell and Tissue Research.** 186:209-226.

Jandl, J.H., Files, N.M., Barnett, S.B. and MacDoland, R.A. (1965). Proliferative response of the spleen and liver to haemolysis. **Journal of Experimental Medicine (USA).** 122:299-325.

Jennings, F.W. (1976). The anaemia of parasitic infections (Soulsby, E.J.L. ed.) Academic Press, New York, San Francisco, London. pp. 41-67.

Jennings, F.W., Murray, P.K., Murray, M. and Urguhart, G.M. (1974). Anaemia in trypanosomiasis: Studies in rats and mice infected with *Trypanosoma brucei*. **Research in Veterinary Science.** 16:70-76.

Johnson, W.J., Bolognesi, D.P. and Adams, D.O. (1984). Antibody-dependent cytolysis (ADCC) of tumor cells by activated murine macrophages is a two-step process: Quantification of target binding and subsequent target lysis. **Cell Immunology.** 83:170-180.

Jourdian, G.W., Dean, L. and Roseman, S. (1971). The sialic acids. XI: A periodate resorcinol method for the quantitative estimation of free sialic acids and their glycosides. **Journal of Biological Chemistry.** 246(3):430-435.

Kaufmann, S.H.E., Schauer, R. and Hahn, H. (1981).Carbohydrate surface constituents of T cells mediating delayed hypersensitivity that control entry into sites of antigen deposition. **Immunobiology.** 160:184-195.

Kawasaki, T. and Ashwell, G. (1977). Isolation and characterization of an Avian hepatic binding protein specific for N-acetylglucosamine-terminated glycoproteins. **Journal of Biological Chemistry.** 252:6536-6543.

Kelm, S. and Schauer, R. (1986). The galactose-recognizing system of rat penitoneal macrophages. Receptor-mediated binding and uptake of glycoproteins. **Biological Chemistry, Hoppe-Seyler.** 367:989-998.

Kelm, S., Jibril, S. ad-D., Lee, H., Yoshino, T. and Schauer, R. (1986a). The galactose receptor of rat peritoneal macrophages. Binding of sialidase-treated blood cells and glycoproteins. Lectins V (Bog-Hansen, T.C. and Driesscher, E. Van, eds.) Walter de Gruyter and Co., Berlin, New York. pp. 221-228.

Kelm, S., Shukla, A.K., Paulson, J.C. and Schauer, R. (1986b). Reconstitution of the masking effect of sialic acid groups on sialidase-treated erythrocytes by the action of sialyltransferase. **Carbohydrate Research.** 149:59-64.

Kempka, G. and Kolb-Bachofen, V. (1985). Galactose-specific receptors on liver cells: 1. Heptaocyte and liver macrophage receptors differ in their membrane anchorage. **Biochemical and Biophysical Acta.** 847:108-114.

Kempka, G., Roos, P.H. and Kolb-Bachofen, V. (1990). A membrane-associated form of c-reactive protein is the Galactose-specific particle receptor on rat liver macrophages. **The journal of Immunology.** 144:1004-1009.

Khansari, N. and Fudenberg, H.H. (1983). Immune elimination of autologous senescent erythrocytes by kupffer cells *in* vivo. **Cell immunology.** 80:420-430.

Klotz, F.W., Orlandi, P.A., Reuter, G., Cohen, S.J., Haynes, J.D., Schauer, R., Howard, R.J., Palese, P. and Miller, L.H. (1992). Binding of plasmodium *falciparum* 175 *kilodalton* erythrocyte binding antigen and invasion of murine erythrocyte requires N-acetylneuraminic acid but not its 0-acetylated form. **Moleclar Biochemistry and Parasitology.** 51:49-54.

Kobayashi, A., Tizard, I.R. and Woo, P.T.K. (1976). Studies on the anaemia in experimental African trypanosomiasis II. The pathogenesis of the anaemia in calves infected with *Trypanosoma congolense*. **American Journal of Tropical Medicine and Hygiene.** 25:401-406.

Kolb, H. and Kolb-Bachofen, V. (1978). A lectin-like receptor on mammalian macrophages. **Biochemical and Biophysical Research Communications.** 85:678-683.

Kolb, H., Friedrich, E. and Suss, R. (1981). Lectin mediates homing of sialidase-treated erythrocytes of liver as revealed by scintigraphy. **Hoppe-Seyler's Zeuitschrift fur Physiologisch Chemie.** 362:1609-1614.

Kolb, H., Kolb-Bachofen, V. and Schlepper-Schafer, J. (1979). Cell contacts mediated by D-galactose-specific lectins on liver cells. **Biology of Cell.** 36:301-308.

Kolb, H., Vogt, D., Herbertz, L., Corfield, A., Schauer, R. and Schlepper-Schafer, J. (1980). The galactose-specific lectins on rat hepatocytes and kupffer cells have identical binding characteristics. **Hoppe-Seyler's Zeitschrift fur Physiologisch Chemie.** 361:1747-1750.

Kolb-Bachofen, V. (1986). Mammalian lectins and their function - a review. In Lectins-Biology, Biochemistry, Clinical Biochemsitry (Bog-Hansen, T.C. and Van Driessche, E. Eds). Walter de Gruyter and Co., Berlin, Vol. V. pp. 197-206.

Kolb-Bachofen, V. (1988). The galactose - particle receptor on liver macrophages: Biological function and implications for clearance of particulates material. In: Targeting of drugs. (Gregoriadis, G. and Poste, G. eds.) Plenum Publishing Corporation. pp. 45-51.

Kolb-Bachofen, V. and Kolb, H. (1979). Auto-immune reactions against liver cells by syngeneic neuraminidase-treated lymphocytes. **Journal of Immunology.** 123:2830-2834.

Kolb-Bachofen, V., Schlepper-Schafer, J. and Vogel, W. (1982). Electron microscopic evidence for an asialoglycoprotein receptor on kupffer cells. Localization of lectin-mediated endocytosis. **Cell.** 29:859-866.

Kolb-Bachofen, V., Schlepper-Schafer, J. and Kolb, H. (1983). Receptor mediated particle uptake by liver macrophages. The galactose particle receptor mediates uptake via coated and also non-coated structures. **Experimental Cell Research.** 148:173-182.

Kolb-Bachofen, V., Schlepper-Schafer, J., Roos, P., Hulsmann, D. and Kolb, H. (1984). GalMAc/Gal-specific rat liver lectins: their role in cellular recognition. **Biology of the Cell.** 51:219-226.

Krauss, J.H., Reuter, G., Schauer, R., Weckesser, J. and Mayer, H. (1988). Sialic acid-containing lipopolysaccharide of purple non-sulfur bacteria. **Archives of Microbiology.** 150:584-589.

Kuster, J.M. and Schauer, R. (1981). Phagocytosis of sialidase-treated rat erythrocytes; evidence for a two-step mechanism. **Hoppe-Seyler's Zeitschrift fur Physiologisch Chemie.** 362:1507-1514.

Langreth, S.G. and Balber, A.E. (1975). Protein uptake and digestion in bloodstream and culture forms of *Trypanosoma brucei*. **Journal of Protozoology, (USA).** 22:40-

Leeflang, P. (1978). Bovine Trypanosomiasis in Northern Nigeria: A contribution to the epidemiology, host-specificity and drug-sensitivity of *T. vivax*. Ph.D. Thesis, University of Utrecht.

Lis, H., Sharon, N. and Katchalski, E. (1969). Identification of the carbohydrate-protein linking group in soybean hemagglutinin. **Biochemical and Biophysical Acta.** 192:364-366.

Liu, D.Y., Petsohek, K.D., Remold, H.G. and David, J.R. (1982). Isolation of a guinea pig macrophage glycolipid with the properties of the putative migration inhibitory factor receptor. **Journal of Biological Chemistry.** 275:159-162.

Losos, G.J. and Ikede, B.O. (1972). Review of pathology of the diseases in domestic and laboratory animals caused by *Trypanosoma congolense, T. brucei, T. rhodesiense* and *T. Gambiense*. **Veterinary Pathology.** 9 (supplement). 1-71.

Losos, G.J., Paris, J., Wilson, A.J. and Dar, F.K. (1973). Pathology of the disease in cattle caused by *Trypanosoma congolense*. **Bulletin of Epizootic Diseases of Africa.** 21:239-248.

Mackenzie, P.K.I. and Cruickshank, J.G. (1973). Phagocytosis of erythrocytes and leukocytes in sheep infected with *Trypanosoma congolense* (Broden, 1904). **Research in Veterinary Science.** 15:256-262.

Mackenzie, P.K.I., Boyt, W.P., Nesham, V.W. and Pirue, E. (1978). The actiology and significance of the phagocytosis of erythrocytes and leukocytes in sheep infected with *Trypanosoma congolense* (Broden 1904). **Research in Veterinary Science.** 24:4-7.

Mamo, E. and Holmes, P. (1975). The erythrokinetics of Zebu cattle chronically infected with *Trypanosoma congolense*. **Research in Veterinary Science.** 18:105-106.

Mekada, E., Uchida, T. and Okada, Y. (1979). Modification of the cell surface with neuraminidase increases the sensitivities of cells to diphtheria toxin and *Pseudomonas aeruginosa* exotoxin. **Experimental Cell Research.** 123:137-146.

Mohr, M., Kolb, H., Kolb-Bachofen, V. and Schlepper-Schafer, J. (1987). Recognition of xenogencic erythrocytes: the GalNAc/Gal-particle receptor of rat liver macrophages mediates or participates in recognition. **Biology of the Cell.** 60:217-224.

Molyneux, D.H. (1970). Developmental patterns in trypanosomes of the subgenus herpetosoma. **Annals of the Society of Belgium Medicine of Tropics.** 50:229.

Montgomery-Bissell, D., Hammaker, L. and Schmid, R. (1972). Liver sinusoidal cells: Identification of a sub-population for erythrocyte catabolism. **The Journal of Cell Biology.** 54:107-119.

Moran, P., Raab, H., Kohr, W.J. and Caras, I. W. (1991). Glycophospholipid membrane anchor, attachment-molecular analysis of cleavage/attachment site. **Journal of Biological Chemistry.** 266:1250-1257.

Morell, A.G., Irvine, R.A., Sternlieb, I., Scheinberg, I.H. and Ashwell, G. (1968). Physical andk chemical studies on ceruloplasmin V. Metabolic studies on sialic acid-free ceruloplamin *in vivo*. **Journal of Biological Chemistry.** 243:155-159.

Muller, E., Franco, M.W. and Schauer, R. (1981). Involvement of membrane galactose in the *in vivo* and *in vitro* sequestration of desialylated erythrocytes. **Hoppe-Seyler's Zeitschrift fur Physiologisch Chemie.** 362:1615-1620.

Muller, E. and Schauer, R. (1983). Factors influencing binding and phagocytosis of sialidase-treated erythrocytes by homologous rat peritoneal macrophages. Glycoconjugates. In "Proceedings of the 7th Symposium on Glycoconjugates Lund-Ronneby, July 17-23, (Chester, M.A., Heingard, D., Lundblad, A. and Sevensson, S. eds.). pp. 667-668.

Muller, E., Schroder, C., france, M.W., Shukla, A.K. and Schauer, R. (1983a).Involvement of β-galactose specific macrophage lectin in binding and phagocytosis of erythrocytes. In "Lectins III (Bog-Hansen, T.C. and Spengler, C.A. eds.) Walter de Gruyter and Co., Berlin, New York. pp. 435-442.

Muller, E., Schroder, C., Schauer, R. and Sharon, N. (1983b). Binding and phagocytosis of sialidase - treated rats erythrocyte by a mechanism independent of opsonins. **Hoppe-Seyler's Zeitschrift fur Physiologisch Chemie.** 364:1419-1429.

Muller, H.E. (1974). Neuraminidases of bacterial and protozoa and their pathogenic role. **Behring Institute of Mitt.** 55:34-56.

Muller, H.E. (1992). Relationships between ecology and pathogenicity of microoganisms. **Bioforum.** 15:16-22.

Murray, M. (1974). The pathology of African trypanosomiasis. In "Progress in immunology 11.4 (Brent, L. and Holboro, J. ed). Ansterdam, North Holland Publishing Company. pp. 181-192.

Murray, M. (1978). Anaemia of bovine African trypanosomiasis: an overview. In "Pathogenecity of trypanosomes". Proceedings of a Workshop held at Nairobi, Kenya, November 20-30, (Losos, G. and Chouinard, A. eds). pp. 71-81.

Murray, P.K., Murray, M., Wallace, M., Morrison, W.I. and McLyntyre, W.I.M. (1978a). Trypanosomiasis in N'dama and Zebu cattle 1. An experimentalinvestigation of susceptibility to *Trypanosoma brucei, T. Congolense* and mixed infections. International Scientific Council for Trypanosomiasis Research and Control, 15th Meeting. The Gambia, OAU/STRC.

Murray, P.K., Murray, M., Wallace, M., Morrison, W.I. and McLyntyre, W.I.M. (1978b). Trypanosomiasis in N'dama and Zebu cattle II. The influence of weight of infection on the severity of the disease. International Scientific Council for Trypanosomiasis Research and Control, 15th Meeting. The Gambia, OAU/STRC.

Murray, M. and Dexter, T.M. (1988). Anaemia in bovine African trypanosomiasis: a review. **Acta Tropica.** 45:389-432.

Nagamura, Y. and Kolb, H. (1979). In "Glycoconjugates" (Schauer, R., Boer, P., Buddecke, E., Kramer, M.F., Uliegenthart, J.F.G. and Wiegnatt, H. eds). Verlag G. Thieme Struttgart. pp. 490-491.

Naylor, D.C. (1971a). The haematology and histopathology of *Trypanosoma congolense* infection in cattle I. Introduction and histopathology. **Tropical Animal Health and Production.** 3:95-100.

Naylor, D.C. (1971b). The haematology and histopathology of *Trypanosoma congolense* infection in cattle Part II. Haematology (including symptoms). **Tropical Animal Health and Production.** 3:159-168.

Newton, B.A. (1978). In "Pathogenicity of Trypanosomes". Proceedings of a Workshop held at Nairobi, Kenya, 20-23 November, 1978. (Losos, G. and Chouinard, A. eds.). pp. 17-22.

Nohle, U. and Schauer, R. (1981). Uptake, metabolism and excretion of orally and intravenously administered ^{14}C- and ^{3}H-labelled N-acetylneuraminic acid mixture in the mouse and rat. **Hoppe-Seyler's Zeitschrift fur Physiologisch Chemie.** 362:1495-1506.

Nohle, U., Beau, J.M. and Schauer, R. (1982). Uptake metabolism and excretion of orally and intravenously administered double labelled N-glycolylneuraminic acid and single-labelled 2-deoxy-2,3-dehydro-N-acetylneuraminic acid in mouse and rat. **European Journal of Biochemistry.** 126:543-548.

Nuru, S. (1982). Problems and prospects of the Nigerian beef industry in "Beef production in Nigeria" (Osinowo, A.O., Ikhatua, U.J. and Ehoche, O.W. eds). Proceedings of the National Conference on Beef Production held at Kaduna, Nigeria, 27-30 July. 1982, p.12.

Oldfield, M.L. (1983). Trypanosomiasis and meat production. **Science,** 219(4580):8-10.

Opperdoes, F.R. and Borst, P. (1977). Localization of nine glycolytic enzymes in a microbody-like organelle in *T. Brucei*. The glycosome. **FEBS Letters.** 80:360-364.

Opperdoes, F.R. and Cottem, D. (1982). Involvement of the glycosome of *Trypanosoma brucei* in carbon dioxide fixation. **FEBS Letters.** 143:60-64.

Opperdoes, F.R., Borst, P. and Fonck, K. (1976). The potential use of inhibitors of glycerol phosphate oxidase for the chemotherapy of African trypanosomiasis. **FEBS Letters.** 6169-172.

Opperdoes, F.R., Markos, A. and Steiger, R.F. (1981). Localization of malate dehydrogenase (EC 1.1.1.37) adenylate kinase (EC 2.7.4.3) and glycolytic enzymes in glycosomes and the threonine pathway in the mitochondrion of cultured procyclic trypomastigotes of *Trypanosoma brucei*. **Molecular Biochemistry and Parasitology.** 4:291-310.

Pardoe, G.I. and Uhlenbruck, G. (1970). Characteristics of antigenic determinants of intact cell surfaces. **Journal of Medical Laboratory Technology.** 23:249-263.

Paulson, J.C. and Colley, K.J. (1989). Glycosyltransferases: Structure, localization and control of cell type-specific glycosylation. **Journal of Biological Chemistry.** 264:17615-17618.

Pereira, M.E.A. (1983). A developmentally regulated neuraminidase activity in *Trypanosoma cruzi*. **Science.** 219:1444-1446.

Peruzzi, M. (1928). Pathologico-anatomical and serological observations on trypanosomiasis. In "Final Report of League of Nations International Committee on Human Trypanosomiasis. Geneva, League of Nations. 3:245.

Pigman, W. (1977). In "The Glycoconjugates Vol. 1" (Honowitz, M.I. and Pigman, W. eds.) Academic Press, New York, San Francisco, London. pp. 1-11.

Pirofsky, B. (1969). Auto-immunization and the auto-immune haemolytic anaemias. First edition. Williams and Williams Co., Baltimore, USA. pp. 418-430.

Popoff, N.R. and Dodin, A. (1985). Survey of neuraminidase production by *Clostridium butyricum, clostridium beijerinckii* and *Clostridium difficile* strains from clinical and non-clinical sources. **Journal of Clinical Microbiology.** 22:873-876.

Rahmann, H., Hillbig, R., Marx, J., Beitinger, H. and Mehlfield, R. (1987). Brain gangliosides and hibernation. **Journal of Thermal Biology.** 12:18-85.

Rautenberg, P., Reinwald, E. and Risse, H.J. (1981). Sialic acids are responsible for the charge heterogeneity of the variant surface glycoprotein of *Trypanosoma congolense*. **Molecular and Biochemical Parasitology.** 4:129-138.

Reuter, G., Pfeil, R., Stoll, S., Schauer, R., Kamerling, J.P., Versluis, C. and Vliegenthart, J.F.G. (1983). Identification of new sialic acids derived from glycoprotein of bovine submandibular gland. **European Journal of Biochemistry.** 134:139-143.

Reuter, G., Schauer, R., Prioli, R.P. and Pereira, M. E.A. (1987). Isolation and properties of a sialidase from *Trypanosoma rangeli*. **Glycoconjugate Journal.** 4:339-348.

Reuter, G. and Schauer, R. (1988). Nomenclature of sialic acids. **Glycoconjugate Journal.** 5:133-135.

Ritz, H. (1916). On relapses in experimental trypanosomiasis: Second communication. Archives of Schiffs. **Tropical Hygiene.** 20:397-420.

Roeder, P.L., Scott, J.M. and Pegram, R.G. (1984). Acute *Trypanosoma vivax* infection of Ethiopian cattle in the apparent absence of tsetse. **Tropical Animal Health and Production.** 16:141-147.

Roggentin, P. and Schauer, R. (1992). In "Genetics and Molecular Biology of anaerobes". (Sebald, M. ed.) Springer-Verlag, New York. pp. 285-295.

Roggentin, P., Gutschker-Gdanies, G., Schauer, R. and Hobrecht, R. (1985). Correlative properties for a differentiation of two *Clostridium sordelli* phenotypes and their distinction from *Clostridium bifermentans*. **Zbl. Backt. Hyg. A.** 260:319-328.

Roggentin, P., Rothe, B., Kaper, J.B., Galen, J., Lawrisuk, L., Vimr, E.R. and Schauer, R. (1989). Conserved sequences in bacterial and viral sialidase. **Glycoconjugate Journal,** 6:349-353.

Roggentin, P., Schauer, R., Hoyer, L. and Vimr, E.R. (1993). Microreview. The sialidase superfamily and its spread by horizontal gene transfer. **Molecular Microbiology.** 9:915-921.

Roggentin, P., Kleineidam, R.G. and Schauer, R. (1995). Diversity in the properties of two sialidase isoenzymes produced by *Clostridium perfrintges ssp.* **Biological Chemistry. Hoppe-Seyler.** 376:569-575.

Roos, P.H., Hartmann, H.-J., Schlepper-Schafer, J., Kolb, H. and Kolb-Bachofen, V. (1985). Galactose-specific receptors of liver macrophages. 11 characterization of the purified receptor from macrophages reveals no structural relationship to the hepatocytes receptor. **Biochemica Biophysica Acta.** 847:115-121.

Rosenberg, A. and Schengrund, C.-L. (1976). Sialidases. In "Biological roles of sialic acid". (Rosenberg, A. and Schengrund, C. -L. eds.) Plenum Press, New York. pp. 295-359.

476583

Rosenberg, I., Prioli, R.P., Ortega-Barria, E. and Pereira, M.E.A. (1991). State-specific phospholipase C-mediated release of *Trypanosoma cruzi* neuraminidase. **Molecular Biochemistry and Parasitology.** 46:303-306.

Roth, J., Kempf, A., Reuter, G., Schauer, R. and Gehring, W.J. (1992). Occurrence of sialic acids in *Drosophila melanogaster*. **Science.** 256:673-675.

Sadler, J.E., Reakick, J.I., Paulson, J.C. and Hill, R.I. (1979). Purification to Homogeneity of a ∝-Galactoside ∝2 --->3 sialyltransferase and partial purification of an ∝-N-acetylgalactosaminide ∝2 ---> 6 sialyltransferase from porcine submaxillary glands. **Journal of Biological Chemistry.** 254:4434-4443.

Saror, D.I. (1980). Observations on the course and pathology of *Trypanosoma vivax* in red Sokoto goats. **Research in Veterinary Science.** 28:36-38.

Schauer, R. (1978). Characterization of sialic acids. **Methods in Enzymology.** 50:64-89.

Schauer, R. (1982a). Sialic acids: Chemistry, metabolism and function (Schauer, R. ed.) Cell Biology Monographs, Vol. 10, Springer, New York.

Schauer, R. (1982b). In "Advances in carbohydrate Chemistry and Biochemistry (Tipson, R. -S, and Horton, D. ed.) Vol. 40. Academic Press, New York.

Schauer, R. (1983). Sialic acids as potential determinants on differentiation antigens. Biochemical Society Transactions, Vol. II, 602nd Meeting, London. pp. 270-271.

Schauer, R. (1985). Sialic acids and their roles as biological masks. **Trends in Biochemical Science.** 10:357-360.

Schauer, R., Reuter, G., Muhlpfordt, H., Andrade, A.F. and Pereira, M.E.A. (1983). The occurrence of N-acetyl and N-glycolylneuraminic acid in *Trypanosoma cruzi*. **Hoppe-Seyler's Zeitschrift fur Physiologisch Chemie.** 364:1053-1057.

Schauer, R., Shukla, A.K., Schroder, C. and Muller, E. (1984). The anti-recognition function of sialic acids: Studies with erythrocytes and macrophages. **Pure and Applied Chemistry.** 56:907-921.

Schauer, R., Stoll, S. and Reuter, G. (1991). Differences in the amount N-acetyl and N-glycolyneuraminic acid, as well as O-acetylated sialic acids of fetal and adult bovine tissues. **Carbohydrate Research.** 213:353-359.

Schauer, R., Kelm, S., Reuter, G., Roggentin, P. and Shaw, L. (1995). Biochemistry and role of sialic acids. In "Biology of Sialic Acids (Rosenberg, A. ed.) Plenum Press, New York. pp. 7-67.

Schenkman, S. and Eichinger, D. (1993). *Trypanosoma cruzi* trans-sialidase and cell invasion. **Parasitology Today.** 9:218-221.

Schenkman, S., Jiang, M.-S., Hart, G.W., and Nussenzweigh, V. (1991). A novel cell surface trans-sialidase of *Trypanosoma cruzi* generates a stage-specific epitope required for invasion of mammalian cells. **Cells.** 65:1117-1125.

Schenkman, S. de Carvallo, L.P. and Nussenzweig, V. (1992). *Trypanosoma cruzi* trans-sialidase and neuraminidase activities can be mediated by the same enzymes. **Journal of Experimental Medicine.** 175:567-575.

Schlepper-Schafer, J. and Kolb-Bachofen, V. (1988). Red cell aging results in a change of cell surface carbohydrate epitopes allowing for recognition by galactose-specific receptors of rat liver macrophages. **Blood Cells.** 14:259-269.

Schlepper-Schafer, J., Kolb-Bachofen, V. and Kolb, H. (1983). Identification of a receptor for senescent erythrocytes on live macrophages. **Biochemical and Biophysical Research Communication.** 115:551-559.

Schlepper-Schaffer, J., Kolb-Bachofen, V. and Kolb, H. (1980). Analysis of lectin-dependent recognition of asialo-erythrocytes by kupffer cells. **Biochemistry Journal.** 186:827-831.

Schlepper-Schaffer, J., Mohr, M. and Kolb, H. (1986a). Receptors involved in recognition of xenogeneic erythrocytes by isolated rat kupffer cells. In "Cells of the Hepatic sinusoids. (Kirn, A., Knook, D. and Wisse, E. eds.) The Kupffer Cell Foundation Rijswijk. pp. 107-111.

Schlepper-Schaffer, J., Hulsmann, D., Djoukar, A., Meyer, H.E., Herbertz, L., Kolb, H. and Kolb-Bachofen, V. (1986b). Endocytosis via galactose receptors *in vivo* ligand size directs uptake by hepatocytes and/or liver macrophages. **Experimental Cell Research.** 165:494-508.

Schuloff, R.S., Fernandes, G., Good, R.A. and Gupta, S. (1980). Neuraminidase treatment of human T lymphocytes effect on Fc receptor phenotype and function. **Clinical and Experimental Immunology.** 40:611-619.

Scott, J.F. and Deane, H.W. (1966). In "Histology. (Greep, R.O. ed.) McGraw Hill, New York. Pp. 531-553.

Sekoni, V.O. (1994). Reproductive disorders caused by animal Trypanosomiasis: A review. **Theriogenology.** 42:557-570.

Sekoni, V.O., Saror, D.I., Njoku, C.O., Kumi-Diaka, J., and Opaluwa, G.I. (1990). Comparative haematological changes following *Trypanosoma vivax* and *Trypanosoma congolense* infections in Zebu bull. **Veterinary Parasitology.** 35:11-19.

Sempere, J.M., Ganeedo, C. and Asensio, C. (1965). Determination of galactosamine and N-acetyl-galactosamine in the presence of other hexosamines with galactose oxidaes. **Analytical Biochemistry.** 12:509-515.

Sharon, N. and Lis, H. (1972). Lectins, cell-agglutinating and sugar specific proteins. **Science** (New York). 177:949-959.

Shehu, K., Esievo, K.A.N. and Njoku, C.O. (1991). *In vitro* magnesium and calcium induced sialidase activity in caprine intestinal mucosa cells. **Journal of Comparative Pathology.** 104:435-437.

Shugaba, A., Umar, I., Omage, J., Ibrahim, N.D.G., Andrew, J.N., Ukoha, A.I., Saror, D.I. and Esievo, K.A.N. (1994). Biochemical differences (0-acetyl and glycolyl groups) in erythrocyte surface sialic acids of Trypanotolerant N'dama and Trypanosusceptible Zebu cattle. **Journal of Comparative Pathology.** 110:91-95.

Silver, R.P. and Vimr, E.R. (1990). In molecular basis of bacterial pathogenesis (Iglewski, B. and Miller, V. eds.) The Bacteria Vol. II. New York Academic Press Inc. pp. 39-60.

Smedsrod, B., and Aminoff, D. (1985). Use of ^{75}Se-labelled Methionine to study the sequestration of senescent red blood cells. **American Journal of Hematology.** 18:31-40.

Smith, E.L., Hill, R.L., Lehman, I.R., Lefkowitz, R.J., Handler, P. and White, A. (1985a). Carbohydrate Metabolism II. In "Principles of Biochemistry General Aspects, seventh edition. McGraw-Hill Book Company, Auckland, London, Paris. pp. 428-477.

Smith, E.L., Hill, R.L., Lehman, I.R., Lefkowitz, R.J., Handler, P. and White, A. (1985b). Receptors and transport In "Principles of Biochemistry General Aspects, seventh edition. McGraw-Hill Book Company, Auckland, London, Paris. pp. 289-315.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. The English Language Book Society and Bailhere Tin dall, London. pp. 526.

Stephen, L.E. (1970). Clinical manifestations of the trypanosomiasis in livestock and other domestic animals. In: The African Trypanosomiasis (Mulligan, H.W. ed.) Allen and Unwin Ltd., London. pp. 775-792.

Tabel, H. (1978). Serum protein changes in bovine trypanosomiasis: a review. In: "Pathogenicity of Trypanosomiasis. Proceedings of a Workshop held at Nairobi, Kenya, 20-23 November, 1978. (Losos, G. and Chouinard, A. eds). pp. 151-153.

Tachibana, H., Yoshihara, E., Kaneda, Y. and Nakae, T. (1988). *In vitro* lysis of the bloodstream forms of *Trypanosoma brucei gambiense* by stearylamine-bearing liposomes. Antimicrobial Agents and Chemotherapy. pp. 966-970.

Town, C. and Stanford, E. (1979). An oligosaccharide containing factor that induces cell differentiation in *Dictyostelium discoideum*. **Proceedings of the National Academy of Science, USA.** 76:308-312.

Trail, J.C.M. (1984). Productivity of representative breeds of important cattle groups in Africa. In "Evaluation of large ruminants for the tropics. (Copland, J.W. ed). Proceedings of an International Workshop held at CSIRO, Rockhampton Queensland, Australia, 19-33 March, 1984. pp. 101.

Tropical Diseases (1990). World Health Organization Division of Control of Tropical Diseases. pp. 10-11.

Tsagumis, J., Kolb, H., Heiniger, K. and Schlepper-Schafer, J. (1985). Lack of evidence for liver lectins functioning as IgM or IgG-receptors. **Biochemical and Biophysical Research Communication.** 130:1002-1009.

Turner, M.J. (1982) Biochemistry of the variant surface glycoproteins of salivarian trypanosomes. **Advances in Parasitology.** 21:69-153.

Ueer, U., and Engel, W. (1981). The effects of neuraminidase and gangliosides on ovarian LH/hCG receptors during rat development. **Differentiation.** 20:162-167.

Umar, I.A., Omage, J.J., Shugaba, A., Igbokwe, I.O., Ibrahim, N.D.G., Kadima, K.B., Ameh, D.A., Kwanashie, H.O., Agbede, R.I.S., Saror, D.I., and Esievo, K.A.N. (1998). Effects of acute bovine trypanosomiasis (*Trypanosoma vivax*) on plasma kinetics of intravenously administered lactose. **Veterinary Parasitology.** 78:173-178.

Unsworth, K. (1953). Studies on *Trypanosoma vivax* VIII. Observations on the incidence and pathogenicity of *T. vivax* in Zebu cattle in Nigeria. **Annals of Tropical Medicine and Parasitology.** 47:361.

Valli, V.E.O., Forsberg, C.M. and McSherry, B.J. (1978). The pathogenesis of *T. congolense* infection in calves II. Anaemia and erythroid response. **Veterinary Pathology.** 15:732-745.

Valli, V.E.O. and Forsberg, C.M. (1979). The pathogenesis of *T. congolense* infection in calves V. Quantitative histological changes. **Veterinary Pathology.** 16:334-368.

Van den Eijnden, D.H. and Joziasse, D.H. (1993). Enzymes associated with glycosylation. **Current Opinion on Structural Biology.** 3:711-721.

Van Hoof, L., Henrard, C. and Peel, E. (1948). Quelques observations sur les trypanosoma des grand mammiferes an Congo. **Belge Acta Tropica.** 5:327.

Varki, A. (1992). Diversity in the sialic acids. **Glycobiology.** 2:24-40.

Varki, A. and Higa, H.H. (1993). *Studies of the 0-acetylation and (in) stability of* polysialic acid. In "Polysialic acid (Roth, J., Rutishauser, U. and Troy, F.A. eds). Birkhauser Verlag, Basel. pp. 165-170.

Veh, R.W., Sander, M., Haverkamp, J. and Schauer, R. (1979). In "Glycoconjugate Research", vol. I. (Gregory, J.D. and Jeanloz, R.W. eds.) Academic Press. New York, San Francisco, London. pp. 557-559.

Vickerman, K. and Tetley, L. (1978). Biology and ultrastructure of trypanosomes in relation to pathogenesis. In "Pathogenicity of trypanosomes. Proceedings of a Workshop held at Nairobi, Kenya, November 20-23. (Losos, G. and Chouinard, A.J. eds.). pp. 23-31.

Vomel, T. and Platt, D. (1982). Age-dependent red cell phagocytosis and activity of the rabbit reticulohistiocytic system. **Gerontology.** 28:149-155.

Waldstein, S.S., Dubin, A., Newcomer, A. and McKenna, C.H. (1964). Determination of exogenous galactose in blood using "specially purified glucose oxidase". **Clinical Chemistry.** 10:381-390.

Warren, L. (1959). The thiobarbituric acid assay of sialic acids. **Journal of Biological Chemistry.** 234:1971-1975.

Wellhausen, S.R. and Mansfield, J.M. (1979). Lymphocyte function in experimental African trypanosomiasis II. Splenic suppressor cell activitgy. **Immunology.** 122:818-824.

Willet, K.C. (1970). Epizootiology of trypanosomiasis in livestock in East and Central Africa. In: The African Trypanosomiasis. (Mulligan, H.W. ed.) London. pp. 766.

Woo, P.T.K. (1969). The haematocrit centrifuge technique for detection of trypanosomes in blood. **Canadian Journal of Zoology.** 47:921-923.

Woodruff, J.J. and Gesner, B.M. (1969). The effect of neuraminidase on the fate of transfused lymphocytes. **Journal of Experimental Medicine.** 129:551-567.

Woodruff, A.W., Ziegler, J.L., Hathaway, A. and Gwata, T. (1973). Anaemia in trypanosomiasis and "Big spleen Disease" in Uganda. **Transactions of the Royal Society of Tropical Medicine and Hygiene.** 67:329-337.

Woodruff, J.J. and Woodruff, J.F. (1976). Influence A virus interaction with murine lymphocytes I. Then influence of influenza virus A/Japan 305 (H_2N_2) on the pattern of migration of recirculating lymphocytes. **Journal of Immunology.** 117:852-858.

World Health Organization (WHO) (1969). Comparative studies on American and African trypanosomiasis. Report of a WHO scientific group. WHO Technical Report Series. Number 411. pp. 1.

Yaari, A. (1969). Mobility of human red blood cells of different age groups in an electric field. **Blood.** 33:159-163.

APPENDIX

Preparation of some specific reagents.

(a) **Barbital buffered saline:**

Bartibal buffered saline (147mM NaCl, 36mM EDTA, 9mM sodium diethylbartiturate, 27mM diethylbarbituric acid and 0.1% gelatine, pH 7.2) was prepared by dissolving 8.591g of NaCl, 13.401g of EDTA, 1.856g of sodium diethylbarbiturate, 4.973g of diethylbarbiturate and 1.0g of gelatine in 1 Litre of distilled water. The pH of the solution was then adjusted to 7.2

(b) **Phosphate buffered saline:**

Phosphate buffered saline (NaCl-P-buffer: 137mM NaCl, 2.7mM KCL, 8.1mM Na_2HPO_4 and 1.5mM KH_2PO_4, pH. 7.2) was prepared by dissolving 8.006g of NaCl, 0.201g of KCL, 1.150g of Na_2HPO_4 and 0.204g of KH_2PO_4 in 1 Litre of distilled water. The pH of the solution was adjusted to 7.2.

(c) **Medium 199E:**

One percent solution of Medium 199E was prepared by dissolving 1g of Medium 199E in 100ml of distilled water.

(d) **Trypanosome Counting Stain**

The counting stain was prepared by dissolving 3.2g of KI, 1.0g of NaCL, 5.0g of Na_2SO_4 and 0.29g of I_2 in distilled water and the volume was made up to 200ml with distilled water.

Table 1: Packed cell volume (PCV) of infected calves and non-infected calves in the *in vitro* experiment.

Day	Packed Cell Volume (%)	
	Non-infected calves (n = 3)	Infected calves (n = 2)
0	21.0 ± 0.20	19.5 ± 0.71
2	22.0 ± 0.30	21.0 ± 1.41
3	21.5 ± 0.50	20.0 ± 0.00
4	21.5 ± 0.28	19.5 ± 0.71
5	21.5 ± 0.20	19.5 ± 2.12
6	21.0 ± 0.40	17.5 ± 2.12
7	21.5 ± 0.30	16.5 ± 3.54
8	21.0 ± 0.30	14.5 ± 3.54
9	21.0 ± 0.50	16.5 ± 3.54
10	21.0 ± 0.40	16.5 ± 2.12
11	21.5 ± 0.50	16.5 ± 2.13
12	21.5 ± 0.40	19.5 ± 0.71
13	22.0 ± 0.50	16.0 ± 1.41

Values are expressed as mean ± SD.

Table 2: Parasitaemia in the infected calves in the *in vitro* experiment.

Day	Parasitaemia (x 10^6/ml) (n = 2)
0	0
2	0
3	2.0 ± 0
4	10.5 ± 1.0
5	22.0 ± 6.0
6	4.5 ± 0.8
7	0
8	0
9	1.0 ± 0
10	2.0 ± 1.0
11	2.3 ± 0.3
12	6.4 ± 0.1
13	8.3 ± 2.0

Values are expressed as mean ± SD.

Table 3: Sialic acid concentrations of infected calves (*in vitro* experiment) and infected calves with lactose injection (*in vivo* experiment).

Day	Sialic acid concentration (mg/dl)	
	Infected calves (*In vitro* experiment) (n = 2)	Infected calves with lactose injection (*In vivo* experiment) (n = 4)
0	0.13 ± 0.03	0.11 ± 0.05
2	0.17 ± 0.01	0.18 ± 0
3	0.23 ± 0.02	0.22 ± 0.03
4	0.30 ± 0.03	0.36 ± 0.03
5	0.35 ± 0.04	0.46 ± 0.06
6	0.25 ± 0	0.29 ± 0.08
7	0.14 ± 0.01	0.19 ± 0.05
8	0.19 ± 0.03	0.24 ± 0.03
9	0.20 ± 0.02[a]	0.29 ± 0.04[b]
10	0.14 ± 0.02	0.25 ± 0.08
11	0.17 ± 0.03	0.20 ± 0.06
12	0.17 ± 0.04	0.22 ± 0.01
13	0.18 ± 0.07	0.18 ± 0.06

Values are expressed as mean ± SD.

a, b = Values with different superscripts in horizontal rows for each day are significantly ($P<0.05$) different.

Table 4: *Binding between isolated rat peritoneal macrophages and erythrocytes from infected calves (in vitro experiment) and infected calves with lactose injection (in vivo experiment).*

Day	Percent binding (Rosettes formation)	
	Infected calves (*In vitro* experiment) (n = 2)	Infected calves with lactose injection (*In vivo* experiment) (n = 4)
0	-	-
2	1.0 ± 0	9.0 ± 1.0
3	38.0 ± 2.0	43.0 ± 4.0
4	65.0 ± 1.0	68.0 ± 8.1
5	59.0 ± 2.0[a]	47.0 ± 4.0[b]
6	50.0 ± 6.0[a]	39.0 ± 3.0[b]
7	18.0 ± 7.0[a]	40.0 ± 1.0[b]
8	18.0 ± 10.0[a]	38.0 ± 3.0[b]
9	24.0 ± 9.0[a]	54.0 ± 11.0[b]
10	ND	ND
11	17.0 ± 6.0[a]	34.0 ± 5.0[b]
12	26.0 ± 1.0[a]	65.0 ± 13.0[b]
13	34.0 ± 16.0	45.0 ± 10.0

Values are expressed as mean ± SD.

a, b = Values with different superscripts in horizontal rows for each day are significantly ($P<0.05$) different

ND = Not determined.

Table 6: Inhibition of cell contacts between erythrocytes from infected calves and rat peritoneal macrophages by monosaccharides and oligosaccharides.

	Number of Rosettes	Number of Rosettes Inhibited (BP-BI)	Percent Inhibition BP - BI / BP x 100	Percent Inhibition Mean ±SD
Without inhibitor (BP)	68 59 66 59 64 71			
With inhibitors (BI)				
With D-galactose (25mM)	20 18 21 22 24 21	48 41 45 37 40 50	55.88 69.49 68.18 62.71 62.50 70.42	64.86 ± 5.07[c]
With D-glucose (25mM)	46 44 45 47 48 41	22 15 21 12 16 30	22.35 25.42 31.82 20.34 25.00 42.25	29.53 ± 7.03[a]
With D-Mannose (25mM)	50 55 49 48 48 53	18 4 17 11 16 18	26.47 6.78 25.76 18.64 25.00 25.35	21.33 ± 7.01[a]
With Lactose (25mM)	30 27 28 25 31 26	38 32 38 34 33 45	55.88 54.24 57.58 57.63 51.56 63.38	56.71 ± 3.64[d]
With Maltose (25mM)	42 41 41 43 41 42	26 18 25 16 23 29	38.23 30.51 37.88 27.12 35.94 40.84	35.09 ± 4.76[a]

a, c, d = Values with different superscripts are significantly ($P<0.01$) different

Erythrocytes were isolated from 2 infected calves. Binding between the isolated erythrocytes and rat peritoneal macrophages was done without inhibitor (BP) and in the presence of inhibitors (BI). Binding assay was done in triplicate for samples from each calf.

Table 7: Packed cell volume (PCV) of infected calves with lactose injection and non-infected calves in the *in vivo* experiment.

Day	Packed cell volume (%) Non-infected calves (n = 3)	Infected calves with lactose injection (n = 4)
0	25.0 ± 0.5	25.0 ± 1.6
2	25.0 ± 0.0	24.8 ± 2.9
3	24.5 ± 0.3	23.8 ± 2.2
4	25.5 ± 0.4	25.0 ± 1.6
5	24.6 ± 0.8	23.0 ± 2.0
6	25.0 ± 0.5^a	20.5 ± 2.1^b
7	25.0 ± 0.5^a	20.8 ± 2.5^b
8	24.8 ± 0.2^a	18.8 ± 2.1^b
9	25.0 ± 0.3^a	20.0 ± 1.8^b
10	24.8 ± 0.3^a	19.0 ± 1.4^b
11	24.8 ± 0.3^a	18.0 ± 1.8^b
12	24.5 ± 0.5^a	18.8 ± 1.0^b
13	24.5 ± 0.5^a	16.5 ± 0.6^b

Values are expressed as mean ± SD.

a, b, = Values with different superscripts in horizontal rows for each day are significantly ($P<0.05$) different.

Table 8: Parasitaemia in the infected calves with lactose injection in the *in vivo* experiment.

Day	Parasitaemia (x 10^6/ml) (n = 4)
0	0
2	1.8 ± 0.8
3	1.9 ± 0.9
4	12.6 ± 3.1
5	19.9 ± 6.7
6	5.2 ± 0.2
7	2.4 ± 1.1
8	3.8 ± 0.4
9	3.0 ± 1.0
10	3.3 ± 0.8
11	3.4 ± 0.2
12	6.4 ± 0.2
13	16.1 ± 2.7

Values are expressed as mean ± SD.

Table 9: Binding between isolated rat peritoneal macrophages and erythrocytes from the injected calves (*in vitro* experiment) and infected calves with lactose injection (*in vivo* experiment during the period of lactose injection from days 6 to 9 post-infection.

Day	Number of Rosettes (Per cent)	
	Infected calves (n = 2)	Infected calves with lactose injection (n = 4)
6	50.0 ± 6.0[a]	39.0 ± 3.0[b]
7	18.0 ± 7.0[a]	40.0 ± 1.0[b]
8	18.0 ± 10.0[a]	38.0 ± 3.0[b]
9	24.0 ± 9.0[a]	54.0 ± 11.0[b]

Values are expressed as mean ± SD.

a, b = Values with different superscripts in horizontal rows for each day are significantly ($P<0.05$) different.

Table 10: Percent inhibition by lactose in the *in vitro* experiment from days 6 to 9 post-infection.

Day	Number of Rosettes Without Inhibitor (BP)						Number of Rosettes With Lactose (30mM)						Number of Rosettes Inhibited (BP - BI)						Percent Inhibition $\frac{BP - BI}{BP} \times 100$						Percent Inhibition (Mean ± SD)
6	50	44	38	45	60	63	25	27	28	32	30	35	25	17	10	13	30	28	50.0	38.6	26.3	28.9	50.0	44.4	39.71 ± 9.41
7	12	15	13	22	27	25	6	7	7	21	22	20	6	8	6	1	5	5	50.0	53.3	46.2	4.6	18.5	20.0	32.09 ± 18.52
8	8	7	9	28	29	27	3	5	7	20	18	12	5	2	2	8	11	15	62.5	28.6	22.2	28.6	37.9	55.6	39.23 ± 14.87
9	15	12	19	33	30	35	10	8	7	18	15	10	5	4	12	15	15	25	33.3	33.3	63.2	45.5	50.0	71.4	49.45 ± 14.19

Erythrocytes were isolated from 2 infected calves. Binding between the isolated erythrocytes and rat peritoneal macrophages was done without inhibitor (BP) and in the presence of lactose (30mM) as inhibitor (BI). Binding assay was done in triplicates for samples from each of the calves.

Table 11: Binding between isolated rat peritoneal macrophages and erythrocytes from infected calves (*in vitro* experiment) in the presence of lactose as inhibitor and erythrocytes from infected calves with lactose injection (*in vivo* experiment) during the period of lactose injection from days 6 to 9 post-infection.

Day	Number of Rosettes (Percent binding) Infected calves *in vitro* lactose inhibition (n = 2)	Infected calves *in vivo* lactose inhibition (n = 4)
6	30.0 ± 3.0[a]	39.0 ± 3.0[b]
7	13.0 ± 3.0[a]	40.0 ± 1.0[b]
8	10.0 ± 4.0[a]	38.0 ± 3.0[b]
9	11.0 ± 3.0[a]	54.0 ± 16.0[b]

Values are expressed as mean ± SD.

a, b = Values with different superscripts in horizontal rows for each day are significantly ($P<0.01$) different.

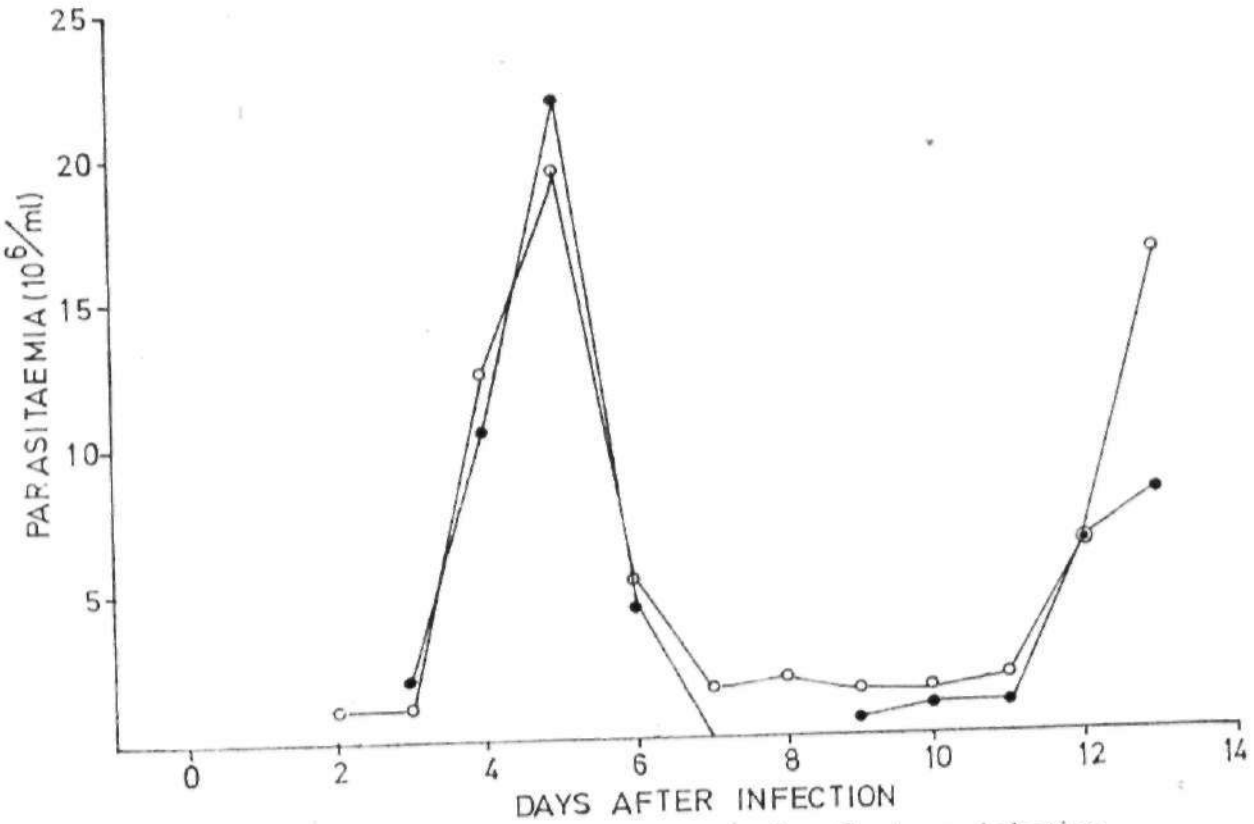


FIG.8: Variation in parasitaemia during T. vivax infection in infected calves • and infected calves with lactose injection o