EFFECT OF ACUTE TRYPANOSOMA VIVAX INFECTION ON PLASMA KINETICS OF INTRAVENOUSLY ADMINISTERED LACTOSE AND THE EFFECT OF THE LACTOSE ON TRYPANOSOMAL ANAEMIA IN ZEBU CATTLE

BY
ISMAILA ALHAJI UMAR
B.Sc (BIOCHEM) UN1MAID., MAIDUGURI,
M.Sc. (BIOCHEM) UNILAG., LAGOS

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Faculty of Science Ahmadu Bello University
Samaru - Zaria

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DECLARATION

I hereby declare that the content herein is a record of my original research and has not been submitted for the award of a degree at any other university. Due credit has been given information and excerpts from both published and unpublished works of other researchers.

Umar I. A.
Department of Biochemistry
A. B. U.

CERTIFICATION

This thesis titled "Effect of acute Trypanosoma vivax infection on plasma kinetics of intravenously administered lactose and the effect of the lactose on trypanosomal anaemia in Zebu cattle", by Umar Alhaji Ismaila meets the regulations governing the award of the degree of Ph.D. in Biochemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

K.A.N. Esievo, Professor — **Date** 7/1/98
Main - Supervisor
Dept. Vet. Pathology and Microbiology

D. A. Ameh, Ph. D — **Date** 7/1/98
Co-Supervisor
Dept. of Biochemistry

H. O Kwanashie, Ph.D — **Date** 7/1/98
Co-Supervisor
Dept of Pharmacology & Clinical Pharmacy

D. A. Ameh, Ph.D — **Date** 7/1/98
Head Department
Dept. of Biochemistry

Dean Postgraduate School, — **Date** 16/9/98
A.B.U. Zaria.

DEDICATION

TO:

My father, Alhaji A. Umar, for his infinite patience and understanding;

My late mother, Mrs Juliana Akharame, for the courage and dignity with which she endured the heavy hands of fate;

and all the warm-hearted who continually strive to make this world a better place.

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ABSTRACT

The plasma lactose concentrations in apparently normal Zebu adults and calves were determined to establish normal values. The adult Zebu had a mean plasma lactose concentration of 0.211 ± 0.021 mM which was significantly ($P<0.05$) lower than that recorded in calves (1.440 ± 0.120 mM). In an experiment to establish its pharmacokinetics, it was shown that intravenously administered lactose was very rapidly eliminated from the body and did not accumulate in the body of the normal calves even upon repeated dosing. The $t_{1/2}$ for lactose in Zebu calves was calculated to be 5.140 ± 0.290 h and mean Ke was 0.138 ± 0.008 h^{-1}. The V_d and C_T for lactose were 179.150 ± 25.230 ml/Kg and 24.750 ± 4.090 ml/Kg/h, respectively.

The effect of Trypanosoma vivax infection on the pharmacokinetics of lactose in the Zebu cattle was further investigated. T. vivax infection in Zebu calves significantly ($p<0.005$) increased the mean $t_{1/2}$ and reduced the mean Ke to 7.600 ± 0.150 hr and 0.091 ± 0.002 h^{-1}, respectively, resulting to the accumulation of lactose in infected animals upon repeated dosing. The V_d and C_T were not significantly affected by the infection.

The T. vivax (Samaru strain) used in this experiment caused a mild infection. The pre-patent period lasted between 2 and 3 days post-infection (pi) and parasitemia peaked between days 4 and 5, pi. Lactose infusion, somehow, sustained the parasites in the blood of the lactose-infused infected animals such that parasites were still detectable in their blood even on days when the trypanosomes disappeared from the blood of the infected uninfused group. Lactose therapy however, ameliorated the T vivax - induced anaemia

in infected calves. During the period of lactose infusion the packed cell volume (PCV) of the lactose-treated infected group recovered by a mean percentage of 0.010 ± 3.200% which was significantly ($p<0.025$) different from the 7.500± 2.900% drop in PCV recorded in the infected uninfused group during the same period. A similar response was observed with the total erythrocyte counts of both these two infected groups in the same period.

T. vivax - induced leucopenia was also ameliorated in lactose-infused infected calves during the period of infusion. The lactose-infused infected group showed a recovery of 25.100% while the infected uninfused group recorded a further drop 17.100% in total leucocyte count during this period. A percentage drop in plasma total proteins of 9.75 ± 1.420% was recorded in the infected uninfused group while an increase of 3.750 ± 1.040% was recorded in lactose-treated infected calves. The concentration of serum free sialic acids in the infected, lactose-infused group was consistently higher than pre-infection levels throughout the duration of experiment, while in the uninfused infected group it fell to pre-infection levels on the days parasites could not be detected in the blood stream.

It was thus concluded that lactose ameliorated the T.vivax induced anaemia, leucopenia and hypoproteinemia in infected Zebu calves.

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LIST OF ABBREVIATIONS

bd. wt.	-	Body weight
C_l	-	Total Clearance
CN	-	Cyanide
CNS	-	Central Nervous System
DIC	-	Disseminated Intravascular Coagulopathy
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylene Diamine Tetracetic Acid
FAs	-	Fatty Acids
FFAs	-	Free fatty acids
g	-	Grams
HMP	-	Hexose Monophosphate
h	-	hour
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
Ke	-	Elimination Rate Constant
Kg	-	Kilogram
ml	-	Millilitres
mM	-	Millimolar
MPS	-	Mononuclear Phagocytic System
NADH	-	Reduced Nicotinamide Adenine Dinucleotide
PCV	-	Packed Cell Volume
pi	-	Post Infection
RBCs	-	Red Blood Cells
SEM	-	Standard Error of Means
$t_{½}$	-	Biological Half-life
Trp	-	Tryptophan
Tyr	-	Tyrosine
V_d	-	Apparent Volume of Distribution

CHAPTER ONE

INTRODUCTION

African trypanosoniasis is caused by tse-tse transmitted trypanosomes. The cardinal sign and diseases promoting factor of Africa bovine trypanosomiasis is anaemia (Hornby, 1921, Fiennes, 1954, Murray, 1974) which is usually accompanied by leucopenia (Esievo and Saror 1983., Anosa, 1988, Igbokwe and Anosa, 1989).

In the early stages of the disease the onset and severity of the anaemia are directly related to the appearance and level of the initial parasitaemia (Dargie et al., 1979; Maxie et al., 1979 Esievo et al.,1982; Sekoni et al., ' 1990e). The anaemia in this period is principally haemolytic (Mamo and Holmes, 1975, Holmes, 1976) and erythrocyte destruction is mainly due to phagocytosis by the expanded and active mononuclear phagocytic system (MPS) (Jennings et al., 1974; Saror, 1980; Anosa and Kaneko 1983 a; b). Increased erythrophagocytosis (Mackenzie and Cruickshank, 1973) and, thus reduction of erythrocyte half-life (Mamo and Holmes, 1975; Dargie et al., 1979; Esievo et al., 1982) are therefore important factors in the development of the anaemia observed in the early stages of trypanosomal infections.

Erythrophagocytosis is a two-step mechanism (Kuster and Schauer, 1981; Muller et al., 1983; Kelm and Schauer, 1986; Kelm et al., 1986a) which requires pre-treatment of the red cells with sialidase (EC 3.2.1. 18) (Muller et al., 1983). The sialidase hydrolyses the surface sialic acids of the red cells, thus exposing D-galactoside residues on the surface of the cells. The first step of the erythrophagocytic process involves binding between the exposed D - galactoside residues on the surfaces of the desialylated

red cells and a beta-D-galactose specific lectin on the surface of macrophages. The regulatory factor for this step is the amount of sialic acids released from the erythrocyte surface by the enzyme (Muller et al., 1983; Kelm et al., 1986b). The second step requires that the binding step be completed and appears to need the presence of certain complement subtances of the serum (Kuster and Schauer, 1981).

Thus desialylation of red blood cells in vitro with bacterial sialidase caused increased sequestration of these cells from the blood stream of rabbits by the MPS (Muller et al., 1981) as well as increased their binding to and subsequent phagocytosis in vitro by rat peritoneal macrophages (Muller et al., 1983; Kelm et al., 1986a). Both these processes were inhibited by beta-glycosidically bound beta - D - galactoside residues (Kolb and Kolb- Bachofen, 1978; Nagamura and Kolb, 1979; Schlepper - Schafer et al., 1980; Kolb et al.,1980; Muller et al., 1981). Lactose has been used to successfully inhibit the sequestration of radio-labelled erythrocytes pre-treated with bacterial sialidase from the blood stream of rabbits (Muller et al., 1981).

Sialidases from trypanasomes (Esievo, 1979; 1980; Pereira, 1983; Pereira and Hoff, 1986; Pereira et al., 1991) have been shown to cleave off erythrocyte surface sialic acids in a number of host species (Durocher et al., 1975; Seaman et al., 1977; Esievo et al., 1982). Specifically, it was shown that Trypanosoma vivax produced sialidase (Esievo, 1979; 1980) which removed the sialic acid coat of red cells, thus exposing more D-galactoside residues on the surfaces of these cells (Omage et al., 1995). Which in turn increased their binding to macrophages, especially liver macrophages (Ibrahim et al.,

1995),supporting an increase in the rate of their sequestration from the blood stream (Esievo and Saror, 1991).

The devastating effects of trypanosomiasis on human and animal populations has stimulated intensive research into the control and elimination of the disease. The curative and prophylactic chemotherapy of African trypanosomiasis, however, presents problems of host toxicity and acquired parasite resistance to the drugs in use (Whiteside, 1962). The problem of chemotherapy of trypanosomiasis is further complicated by the similarities in the biochemical pathways and biomolecules between the mammalian host and protozoan parasite (Whiteside, 1962). These problems necessitate a novel approach to the management of trypanosomiasis. Present chemotherapy is mainly directed against the parasite - to reduce parasitaemia to a level that can be handled by the body's immune system. An alternative approach could be to improve the body's ability to defend itself against the invading parasite. It is suggested that if the anemia caused by trypanosomiasis can be slowed down or prevented the body may be better able to fight off the infection.

The present investigation is an attempt at contributing to the rapidly accumulating knowledge on the chemotherapy of African bovine trypanosomiasis. Since anaemia is generally held responsible for the rapid degenarative processes in the disease (Hornby, 1921; Fiennes, 1954; Murray, 1974), retardation of the rate of development of anaemia in the early stages of the disease, using lactose, may concommittantly retard the degenarative processes. Since the binding step of the erythrophagocytic process is the rate-limiting step (Muller et al., 1983b) lactose may be able to reduce the rate of sequestration of desialylated red cells during the infection. Plasma kinetics data are

essential in the formulation of an effective chemotherapeutic regime so the plasma kinetics of lactose in health and during infection will be necessary to determine the treament regime.

Lactose was selected amongst other compounds with similar activity (e.g. asialofetuin) because it is normally synthesised in the mammalian body and being a low molecular weight carbohydrate would not elicit antigenic reactions in the mammalian host. It is hoped that any findings from this work may then be of help in the formulation of future chemotherapeutic agent or principle against the African trypanosomes. The aims of this research may then be outlined as follows:

a). to establish the normal levels and plasma-kinetics of lactose in normal healthy cattle;

b) to establish the effect(s) of T. vivax infection on the plasma-kinetics of lactose in infected cattle; and

c). to establish the effect(s) of lactose infusion on the course of trypanosomal anaemia during T. vivax infection of cattle.

CHAPTER TWO

LITERATURE REVIEW

2.1 African Trypanosomiasis: an Overview

The important trypanosomes that cause African trypanosomiasis in man and animals belong to the subgroup Salivaria (Hoare, 1964) and are transmitted by inoculation into the host through the saliva of the vector, Glossina spp. when feeding (Ford, 1971). The most significant of these include T. vivax, T. congolense, and the trypanosomes in the brucei subgroup; T. brucei, T. gambiense, T. rhodesiense, T. equiperdum and T. evansi (Hoare, 1964; Losos 1979).

Potentially pathogenic infections caused by African trypanosomes start when the vector deposits metacyclic forms in the subcutaneous tissues of a host. The primary reaction at the site of infection is the development of a chancre (Gordon and Willet, 1958; Woodruff, 1974; Losos, 1979). From this site, the trypanosomes are disseminated throughout the host to colonise and multiply in selected tissue micro-environments which include the circulating blood, capillary beds, and the loose connective tissue stroma of various organs and tissues (Luckins and Gray, 1978; Losos, 1979). African trypanosomes have been divided into two broad groups; the hematinic trypanosomes (Losos et al., 1973; Ssenyonga and Adams, 1975; Banks, 1978; Morrison et al., 1979) which are found mainly in the plasma of the host and these include T. congolense and T. vivax, the other group of trypanosomes invade the tissues and thus are found both intra-and extravascularly (Hoare, 1972; Woodruff, 1974; Losos, 1979); members of this group

include T. brucei, T. evansi, T. gambiense, T. rhodesiense and T. equiperdum.

The pathological changes that occur during African trypanosomiasis are dependent on several factors some of which include; the virulence of the strain of parasite, susceptibility of the host, and the period of the infection during which samples were taken (Stephen, 1966; Roberts and Gray, 1978; Morrison et al., 1978; Murray and Morrison, 1979). An interplay of these factors gives rise to various patterns of the disease; thus peracute, acute, sub-acute and chronic infection have been observed. The disease has been divided into three distinct, but overlapping, phases based on the presence or absence of the parasite in the host and on clinical and pathological findings (Murray et al., 1979, Anosa, 1988). The first phase, which may last from few days to several weeks, begins with the presence of trypanosomes in the blood after the incubation period. The parasitaemia is high and fluctuates, in animals that show acute or chronic pattern of infection and is accompanied by rapidly developing pancytopenia and changes in the blood chemistry. Death may occur in acute cases due to the severe pancytopenia and other deleterious changes (Anosa, 1988). This phase represents the period in which the host defence is still not well organised to tackle the infection and the host may die unless the virulence of the parasite is low. The onset of the anaemia in this phase is directly related to the level of parasitaemia (Dargie et al., 1979; Maxie et al., 1979; Esievo et al., 1982). The anaemia is mainly haemolytic (Mamo and Holmes, 1975; Holmes, 1976) and is caused by erythrophagocytosis by the expanded active mononuclear phagocytic system (MPS) (Murray, 1974; Saror, 1980; Esievo et al., 1982; Anosa and Kaneko, 1983 a;b). There is some level of intravascular haemolysis related to production of haemolytic

factors by trypanosomes (Huan et al., 1975; Tizard and Holmes, 1976; Tizard et al., 1978) and also to reduction in the level of serum haptoglobin in acute T. vivax infection in cattle (Esievo et al., 1984).

In the second phase of the disease the host defence mechanism has been mobilised to a level adequate to depress parasitaemia; though not enough to cure the disease. This phase which may last several months, presuming the survival of the host through the first phase, is characterised by transient low grade and scanty parasitaemias and persistent low grade anaemia and leucopenia (Anosa, 1988). With the tissue-invading trypanosomes, extravascular localisation occurs during this stage and the parasites establish in extravascular sites and are less numerous in the blood (Losos, 1979).

The parasites disappear completely from the blood and tissues in the third phase of the disease (Murray et al., 1977). The leucocyte, erythrocyte and thrombocyte levels slowly begin to recover towards pre-infection levels and other pathological changes are slowly reversed (Murray et al., 1979; Anosa, 1988). In this period the host defence mechanism is well and fully established.

2.2 The Trypanosome

2.2.1 General features

Trypanosomes are flagellate protozoa belonging to the class Zoomastigophora, order kinetoplastida (Hoare, 1964). They possess a characteristic kinetoplast, an organelle situated near the base of the flagellum. Some trypanosomes are pathogenic while others are quite harmless (Hoare, 1972). All are haemoflagellates but some invade other tissues

in the vertebrate host (Hoare, 1972; Losos et al., 1973; Losos, 1979). Their transmission is performed by an intermediate invertebrate vector. Trypanosoma equiperdum is however, mainly transmitted by coitus (Hoare, 1972). African trypanosomiasis is caused by the trypanosomes transmitted by the Tse-tse fly (Ford, 1971), these include the trypanosomes of the brucei subgroup; T. congolense and T. vivax. African sleeping sickness is caused by T. rhodesiense and T. gambiense while T. vivax, T. congolense and T. brucei are responsible for bovine trypanosomiasis. Another human trypanosomiasis found in the South and Central Americas is caused by T. cruzi (Hoare, 1972).

The life cycle of trypanosomes comprises of stages of development in the vector and host. In the vector the parasites undergo morphological transformations (Hoare, 1972) in the alimentary canal. The production of metatrypanosomes occurs in the hind gut (e.g. T. cruzi) or involves the migration of the flagellates from the gut to the proboscis and/or salivary glands, where development is completed (e.g. T. rhodesiense, T. gambiense, T. brucei, T. congolense and T. vivax). The former trypanosomes constitute the stercorarians and the latter make up the salivarians. When the metatrypanosomes are transfered to a mammalian host, they also undergo morphological changes with the result that long slender and short stumpy trypanosomes are found in the infected hosts. Trypanosoma cruzi is found in the human host as leishmanial and trypanosomal forms (Hoare, 1972; Woodruff, 1974).

2.2.2 Intermediary metabolism in the trypanosomes

The oxidation of carbohydrates, fatty and amino acids by the genus Trypanosoma is incomplete or absent (Bowman and Flynn, 1976; Hill, 1976). The African salivarian trypanosomes causing human and animal trypanosomiasis are metabolically highly adapted in both their mammalian and insect vector hosts; while the stercorarian T. cruzi appears to be more primitive and less metabolically modified by its host-parasite relationship (Trigg and Gutteridge, 1977). There is, thus considerable diversity among the different subgenera not only in their life-cycle but also in their biochemical properties; for example, in their pathways for terminal oxidation of reducing equivalents.

Electron transport systems: At least two different hydrogen transport systems exist in trypanosomastidae (Bowman, 1974). The blood stream forms of the Trypanozoon species have no detectable cytochromes, their oxygen-uptake is cyanide-insensitive and they possess a unique terminal L-glycerol-3- phosphate oxidase (Grant et al., 1961; Fulton, 1969, Bowman et al., 1972). In contrast, the oxygen-uptake of the blood steam form of T. cruzi is cyanide-sensitive, indicating the presence of a cytochrome system, and lacks an L-glycerol-3-phosphate oxidase of the cytochrome independent type (Von Brand and Tobie, 1948). Thus the blood stream forms of T. rhodesiense, T. gambiense and T. brucei oxidise NADH using the CN-insensitive and cytochrome-independent L-glycerol-3-phosphate oxidase, while T. cruzi utilises a cytochrome system. The culture form of the

salivarian species, however, utilises a cytochrome dependent system (Von Brand and Tobie, 1948; Grant et al., 1961).

Carbohydrate metabolism: All the members of Trypanozoon and Schizotrypanum subgenera are partial aerobic fermenters (Von Brand, 1967). The blood stream form of T. rhodesiense oxidises glucose to pyruvate, which is not further metabolised, and little CO_2 is produced in the process and about 9% of glycerol accumulates (Grant and Fulton, 1957). The short stumpy stage accumulates much less pyruvate from glucose, but significant amounts of CO_2, acetate and succinate are formed (Flynn and Bowman, 1973). This difference has been attributed to the absence in the long slender forms of a functional mitochondrion and tricarboxylic acid (TCA) cycle. Though several enzymes of the TCA cycle have been detected in these forms they lack the essential enzyme, pyruvate decarboxylase (Shaw et al., 1964). In the stumpy form a mitochondrion and the decarboxylases of pyruvate and alpha-oxoglutarate have developed; pyruvate metabolism is, however, still limited probably due to the low activity of citrate synthetase and succinate dehydrogenase (Flynn and Bowman, 1973). The blood - stream forms of T. cruzi are, however capable of oxidising about 50% of glucose to CO_2 with accumulation of acetate, succinate and some lactate (Ryley, 1956). Thus, the metabolism of glucose in T. cruzi is much more complete than in T. rhodesiense. The culture form (insect midgut) of T. rhodesiense converts about half of metabolised glucose to CO_2 and 8% to acetate and succinate. Furthermore, the culture form, unlike the blood-steam form, does not accumulate glycerol, possibly because a cytochrome system has replaced the L-glycero-3-

phosphate oxidase (Gutteridge and Coombs, 1977). The culture form of T. cruzi differs from its blood stream counterpart in producing less CO_2 and about thrice as much succinate.

The major pathway for glucose metabolism in T. rhodesiense and probably other salivarian species, is the classical glycolytic pathway, with little or no hexose mono-phosphate (HMP) pathway contribution (Grant and Fulton, 1957). In the stercorarian species in addition to glycolysis the HMP pathway also plays a significant role (Bowman et al., 1963) contributing between 28% to 40% of the metabolic process (Mancilla and Naquira, 1964).

Lipid metabolism: Acetate and glycerol are incorporated into total lipids and into saturated and unsaturated fatty acids (FAs) by the culture form, but not the blood-stream form of T. rhodesiense (Venkatesan and Ormerod, 1976). Both forms can however absorb, interconvert and esterify preformed C_{16} and C_{18} saturated and unsaturated FAs. Thus, the de novo FA synthesis in the blood stream forms is temporarily suppressed and hence they depend on the host for preformed FAs (Gutteridge and Coombs, 1977). Palmitic acid oxidation is not significant in both the blood stream and culture forms. This is not surprising, especially in the blood-form which lacks a mitochondrion. The culture form oxidises some FA, but this provides little of the energy needs of the parasite. In T. rhodesiense the main sterol in the blood - form is cholesterol and in the culture form it is ergosterol (Threlfall et al., 1965). The culture form of T. cruzi has predominantly cholesterol (Von Brand et al., 1959) with traces of ergosterol. It was suggested that

cholesterol was absorbed, by the blood form of T. rhodesiense, intact from the host blood while ergosterol might be formed endogenously by the culture form.

Amino acid metabolism: Blood stream stages of T. rhodesiense can synthesisze alanine, glycine, serine, aspartic acid and glutamic acid from carbohydrates (Gutteridge and Coombs, 1977). The culture forms of T. cruzi can produce alanine, aspartate, glutamate, glycine, cysteine and threonine from serine (Godfrey, 1976) while the culture form of T. brucei is able to convert proline to alanine and aspartic acid (Evans and Brown, 1972).

The culture form of T. brucei oxidises proline as a major source of energy (Evans and Brown, 1972); while threonine is a major source of acetate for lipid synthesis in the culture forms of T. brucei and T. cruzi (Wood and Schiller, 1975). Neither the long slender nor the stumpy blood stream forms of T. rhodesiense are able to oxidise glutamic and aspartic acids, alanine or proline, however when it adapts to culture conditions, the parasite is characterised by high rates of proline and glutamate supported oxidation (Gutteridge and Coombs, 1977). The blood stream T. gambiense catabolises all the aromatic amino acids, i.e. phenyl alanine, tyrosine and tryptophan (Stibbs and Seed, 1975b). The culture form of T. cruzi oxidizes glutamate, aspartate and alanine to a considerable extent (Zeledon, 1959).

2.3 Distribution and Epidemiology of African Trypanosomiasis

African human trypanosomiasis is restricted to the tse-tse infested areas of Africa, reaching as far as latitude 14° N in West Africa and as far south as the north-east corner

of Botswana, in the Okavango delta (Woodruff, 1974). The T. gambiense infection is found mainly in the western half of tropical Africa, while the T. rhodesiense disease is restricted to the eastern third of the continent (Ady, 1965; Ormerod, 1967; Ford, 1971). Bovine trypanosomiasis exists not only in all areas infested by the tse-tse fly (Ford, 1971) but also in areas outside the fly-belts (Mornet, 1954; Mornet and Morel, 1956). Epidemiological surveys (Unsworth, 1953; Shaw, 1958; Lawrence and Bryson, 1958; Glover, 1965). Indicated a preponderance of T. Congolense infection in East Africa and a T. vivax infection in West Africa.

The two parasites that cause the human disease differ markedly in their epidemiology, largely because of differences in the virulence to mammalian hosts (Woodruff, 1974). The T. gambiense depends essenstially on a man-fly-man cycle (Ford, 1971) because it is unable to maintain adequately high parasitaemia in animals that normally serve as reserviors of infection (Ashcroft, 1959a; b; Desowitz, 1960). The extreme virulence of T. rhodesiense (Hoare, 1972) makes the human patient too ill to leave his home (Woodruff, 1974) that the parasite depends for survival on an ungulate-fly-ungulate cycle with man being usually an adventitious victim (Baker, 1974) when he impinges on this cycle. Glossina palpalis, and G. tachinoides are the species chiefly responsible for transmitting T. gambiense, while G. morsitans, G. pallidipes, and to a lesser extent, G. Swaynnertom transmit T. rhodesiense (Ford, 1971; Davies, 1977).

Animal trypanosomiasis is transmitted principally by the Glossina spp; bovine trypanosomiasis is transmitted mainly by G. brevipalpis, G. longipalpis, G. fusca, G. austeni and the five species mentioned above (Davies, 1977). In the northern Sahelian

Zone horse flies and to a lesser extent, stable flies and keds transmit T. evansi to dromedaries and equines (CAT, 1988); in the rainy season there is concentration of animals and insect swarms along permanent or temporary water-causes or pools and this makes conditions more favourable for mechanical transmission. In tse-tse-free areas mechanical, non cyclical transmission can reduce the incidence of disease. These mechanical vectors can also transmit T. vivax from cattle infected during transhumance in areas subject to flooding by large rivers and lakes (CAT, 1988).

Zebu in the northern Sudanese tropical wooded savannas get exposed to tse-tse of the Nemorhina subgenus at water courses which provide refuge to the flies during the dry season. The animals generally get infected with T. vivax, but when they get exposed to G. m. submorsitans populations the animals may get infected with T. congolense and T. brucei in addition to T. vivax (Davies, 1977). When they return to the sahelian pastures in the rainy season, the infected zebu are a source of T. vivax infections. The trypanosome is maintained through mechanical transmission by the abundant horse flies.

In the tropical wooded southern sudanese savannas, sedentary and transhumant cattle are exposed to G. m. submorsitans in the dry season (Davies, 1977; CAT, 1988). In the rainy season, cattle that graze in the wooded savannas, within the network of riverine forests, encounter G. morsitans that is present throughout the savanna at that time of the year. Further south, the subequatorial savannas and equatorial forest-savanna mosaics, are exclusively infested by the subgenera Nemorhina and Austenina (Ford, 1971) so only trypanotolerant breed can survive in these areas.

2.4 Pathogenesis of African Trypanosomiasis

2.4.1 Invasion of tissues and blood from site of infection

Natural or artificial subcutaneous injection of the T. brucei subgroup cause a chancre-like skin lesion due to the multiplication of the parasites in the connective tissues (Losos, 1979). A similar multiplication of T. congolense has been demonstrated at sites of injections (Luckins and Gray, 1978). There is no available information on the behaviour of T. vivax at the site of infection, but because the metacyclic forms of all species are injected by the vector into the subcutaneous tissues, it has been suggested that the metacyclics and the microenvironment of the subcutaneous tissues interact in establishing infections (Losos, 1979). From the site of infection the trypanosomes invade the blood-stream, either directly or through lymphatics, causing parasitaemia.

Other than at the initial site of infection T. congolense is found only in the blood vessels in domestic and laboratory animals (Losos et al., 1973; Banks, 1978; Ssenyonga and Adam, 1975). T. vivax is also primarily a parasite of the blood, although, occasionally the trypanosome has been found outside blood vessels (Bungener and Mehlitz, 1977). This may have arisen from passive, rather than active, transport of T. vivax into the tissues as a result of haemorrhages (Losos, 1979). The small number of T. vivax localised in the tissues cause much less damage and inflammatory reaction than do organisms of T. brucei subgroup (Losos, 1979). With the T. brucei group there is invasion of a wide range of tissues by large numbers of parasites. Post mortem examinations (Morrison et al., 1979) revealed the presence of parasites in fluid taken from the peritoneal, thoracic and pericardial cavities, sinovial fluid, cerebrospinal fluid,

aqueous humour and subcutaneous edema fluid. The parasites have a pre-dilection for certain tissues and organs and so cause more severe damage in these. Severe lesions have been reported in the heart, CNS, eyes, skin, skeletal muscle, kidney, testicles and pituitary gland (Ikede and Losos, 1972; Murray, 1974; Morrison et al., 1979).

All infections caused by trypanosomes are characterised by fluctuations in the level of parasitaemia, with periodic peaks. In infections caused by all species, the levels of parasitaemia are highest and the peaks most frequent early in infection (Losos, 1979; Esievo et al., 1982; Esievo and Saror, 1983; Sekoni et al., 1990c). A majority of natural infections in domesticated animals are characterised by parasitaemias that last for weeks or months. The levels and patterns of parasitaemia observed depend on the species and strain of trypanosomes and on the species of animal infected. Sekoni et al. (1990c) demonstrated that the mean parasitaemia was higher and more chronic in cattle infected with T. congolense than in cattle infected with T. vivax.

2.4.2 The role of trypanosome species or strain and trypanotolerance in parasitaemia

From series of experiments (Stephen, 1966; Roberts and Gray 1978; Morrison et al., 1978; Murray and Morrison, 1979) it has become clear that the susceptibility of a breed of cattle, of a strain of mouse, or of an individual within a breed or strain is directly related to the capacity to reduce or control the level of parasitemia. Saror and his co-worker (1979) have shown that the more trypanotolerant Ndama breed of cattle has a greater capability to control parasitemia than the susceptible Zebu breed. It has been established that different strains of T. congolense vary in virulence in cattle (Siegmund,

1973; Morrison et al., 1981). There appears to be a correlation between the level and duration of parasitemia and the extent of the pathogenicity of the trypanosome strain (Losos and Ikede, 1972; Losos et al., 1973). Infection due to T. vivax Y58 was relatively milder and more acute than that by T. congolense 2295 which showed a higher and more chronic parasitemia in Zebu bulls (Sekoni et al., 1990c).

2.4.3 Trypanosome metabolism in pathogenesis of African trypanosomiasis

Investigators of the pathogenic mechanisms in trypanosomiasis have implicated the metabolic activity of the trypanosomes (Von Brand, 1973; Stibbs and Seed, 1975a; 1976; Newton, 1979); products from trypanosomes due to either secretion or cytolysis (Leeflang et al., 1976; Tizard et al., 1978b; 1979; Esievo, 1979; 1980) and activation of the release of certain components by the host (Boreham, 1968; 1970; 1979; Zwart and Veenendaal, 1979).

The metabolic activity of a parasite might affect its host in two ways: by depleting essential nutrients and /or producing toxic metabolites. The high motility of trypanosomes in the blood depends upon adequate supplies of glucose and Voorheis (1969) has suggested that the continual demand for glucose by the parasite in acute infections may result in decreased glucose metabolism in the host's peripheral tissues leading to a condition resembling diabetes mellitus. It has however been suggested that depletion of carbohydrate reserves in the host results in the breakdown of liver function and the onset of lethal hypoglycemia; while Von Brand (1973) maintained that the hypoglycemia was more likely due to breakdown in hepatic or endocrine mechanisms

controlling the metabolism of carbohydrate reserves than a direct result of the massive consumption of glucose by the trypanosomes.

Another aspect of the high glucose consumption by blood - stream trypanosomes is the production of pyruvate by the organisms and the accumulation of this metabolite in the blood of experimental animals (Coleman and Von Brand, 1957; Grant and Fulton, 1957) is in direct relation with parasitemia. This condition may lead to depletion of alkali reserves, acidosis and lowered affinity of haemoglobin for oxygen (Newton, 1979). It has been suggested that deficient oxygenation of haemoglobin, coupled with mechanical blockage of the circulation, leads to cellular death by anoxia. Goodwin and Guy (1973) have suggested that high concentrations of pyruvate in tissue fluids may be associated with the observed changes in the structure of connective tissues. The fibroblasts in infected animals contain lipid droplets, cease to produce collagen fibres, and thus may become detached into the surrounding tissue fluid (Goodwin et al., 1973). It seems likely that the high pyruvate concentrations in tissue fluids leads to increased lipid synthesis and accumulation in the fibroblast and this may be responsible for or at least contribute to, the degenerative changes observe in connective tissues of infected rabbits (Goodwin and Guy, 1973).

2.4.4 The role of trypanosome products in pathogenesis of African trypanosomiasis

Haemolytic factors: Tizard et al. (1979) hypothesised that when trypanosomes are lysed by immune cytolysis in vivo they release active phospholipase A1 which acts on endogenous phosphatidyl choline to generate free fatty acids (FFAs), the most significant

of which are linoleic and palmitic acids. Phospoholipase A1 is capable, in conjunction with FFAs, of causing damage to cells and of degranulating mast cells (Condrea et al., 1964). Although FFAs cause haemolysis in vitro (Tizard et al., 1977) they do not do so in vivo because this activity is blocked by serum albumin (Tizard et al., 1978a). Although FFAs bound to serum albumin are not cytolytic, they remain capable of influencing lymphocytes: linoleic acid is a potent immuno suppressive agent (Tizard et al., 1978b; Assoku and Tizard, 1978) and palmitic acid is capable of acting as B-lymphocyte stimulant (Hazlett and Tizard, 1978). Another haemolytic factor produced by T. brucei (Huan et al., 1975) is a protein capable of damaging erythrocytes.

Sialidase (neuraminidase) and sialic acids: It has been shown that trypanosomes produce sialidase in vitro (Esievo, 1979) and in vivo (Esievo, 1980) and that the level of this enzyme is directly proportional to the number of parasites (Esievo, 1983). This enzyme has been shown to cleave off erythrocyte surface sialic acids in a number of host species (Seaman et al., 1975; Esievo et al., 1982). The life span of the desialylated erythrocytes is reduced by their increased binding to, and eventual phagocytosis by liver and peritoneal macropahges in vitro (Kuster and Schauer, 1981; Muller et al., 1983) and in vivo (Durocher et al., 1975; Jancik et al., 1978) thus leading to anaemia in those animals (Esievo et al., 1982). The severity of anaemia in trypanosomiasis has been shown to depend on level and duration of the initial peaks of parasitaemia (Dargie et al., 1979b; Maxie et al., 1979; Esievo et al., 1982; Sekoni et al., 1990c). Proteolytic enzymes are also

known to remove variable fractions of the erythrocyte surface sialic acids in the form of glycopeptides (Cook et al., 1960) and such proteases have been reported to be released by trypanosomes (Tizard et al., 1978).

2.4.5 Host metabolism in African trypanosomiasis

Serum and tissue fluid albumin levels fell steadily during the course of T. brucei infection in rabbits, and levels of non-protein nitrogen rose (Goodwin and Guy, 1973); Newport et al., (1977) observed a significant fall in the levels of 7 (threonine, serine, valine, isoleucine, leucine, tyrosine and tryptophan) out of the 18 amino acids investigated during T. gambiense infection of laboratory animals. Tryptophan (Trp.) and tyrosine (Tyr.) levels were most affected: Try. (50% of control level) and Trp. was almost undetectable. Goodwin (1970) had earlier suggested that catecholamine metabolism was defective in African trypanosomiasis. This was later confirmed by the observation that lowered serum Tyr. resulted in concomitant fall in levels of derivative catecholamine pools in the brain (Wurtman et al., 1974). It is known that depression of catecholamine biosynthesis results in changes in sleep or activity patterns (Jouvet, 1969); body temperature (Svensson, 1971), glycogen and lipid metabolism and possibly cause mental depression (Schildkraut, 1965). It is possible that the observed fall in serum Trp. could result in decreased synthesis of niacin and serotonin by the host, leading to pellagra-like syndrome, changes in sleep patterns and depression (Stibbs and Seed, 1975a).

Transamination of Tyr. yields p-hydroxyphenylpyruvate - a known inhibitor of adrenalin synthesis. High concentrations of this metabolite have been found in urine, but

not in blood, of infected animals (Stibbs and Seed, 1975b; 1976). Similarly, deamination of Trp, yields a variety of pharmacologically active compounds, one of which is indole ethanol (tryptophol). There is evidence that tryptophol can cause sleep, convulsions and death by respiratory depression when injected into mice, rats and cats (Sabella et al., 1969). Tryptophol has also been reported to cause immunodepression in laboratory rodents (Ackerman and Seed, 1976).

Kinins were found to be released in rabbits and cattle infected with T. brucei and humans infected with T. rhodesiense (Boreham, 1968; 1970) while levels of alpha-globin precursor, kininogen, decreased. The enzymes that act on kininogen to form kinins, kallikreins, rise in the plasma and urine of T. brucei infected rabbits (Boreham, 1979) due to activation by trypanosome antigen (Boreham and Wright, 1976a,b).

Changes in Plasma Proteins: There are conflicting reports on the effect of trypanosomiasis on serum levels of total proteins. Decreased levels of total serum proteins have been reported in calves (Fiennes et al., 1946), zebu bulls (Sekoni et al., 1990c) and in sheep (Anosa and Isoun, 1976) infected with T. vivax or T. congolense. Normal levels were reported in sheep infected with T. vivax (Clarkson, 1968) while increases were recorded in three goats infected with T. vivax or T. congolense (Anosa and Isoun, 1976). Reports on the effects of the disease on serum albumin levels are also conflicting (Clarkson, 1968; Anosa and Isoun, 1976; Tabel, 1979). Esievo et al. (1984) reported the almost complete depletion of serum hepatoglobin in bovine T. vivax infection.

Decrease in the level of complement C3 in the serum of humans with sleeping sickness (Greenwood and Whittle, 1976); T. rhodesiense infected monkeys (Nagle et al., 1974), T. congolense infected cattle (Kobayashi and Tizard, 1976) and a "7sr1" protein similar to C3 in T. vivax infected calves (Clarkson et al., 1975) have been reported. The C3 levels in T. vivax infected cattle have correlated with the distinct first and second waves of parasitaemia (Tabel, 1979). A decline in the total hemolytic complement activity in both T. vivax and T. congolense infections has been observed. Reduction of complement activity and serum level of C3 may be partially due to considerable consumption of complement component by immune complexes in fighting the repeated parasitaemias, and/or the activation of complement by trypanosomal material like surface glycoproteins (Musoke and Barbet, 1977; Nielson et al., 1978). The release of the anaphylatoxins C3a and C5a may cause dilatation and may enhance capillary permeability (Mulle-Eberhard, 1968) thus explaining the extensive edema in infected cattle. Hypocomplementaemia potentially deranges the immune system. Tabel (1979) and Rurangirwa et al. (1979) have shown that immunosuppression plays a part in bovine trypanosomiasis.

The IgG antibodies, which are directed against the common antigens, were reported to increase to various degrees during trypanosomiasis in cattle (Desowitz, 1959; Luckins, 1972; Clarkson et al., 1975b). There is however no evidence that this increase was from enhanced synthesis due to polyclonal activation of B-cells as observed in T. brucei infected mice (Hudson et al., 1976). Increases in serum levels of IgM have been reported in natural (Luckins, 1972) and experimental (Clarkson et al., 1975b; Luckins,

1976; Kobayashi and Tizard, 1976) infection of cattle either with T. vivax or T. congolense and in humans suffering from sleeping sickness (Masseyeff et al., 1972). Luckins (1976) however, reported a drop to subnormal levels after the initial rise in two Zebu bulls experimentally infected with T. congolense. There is evidence that some homogenates of trypanosomes contain a mitogen for B-lymphocytes (Esuruoso, 1976; Corsini et al., 1977) and cause a polyclonal synthesis of immunoglobulins; however, T. congolense homogenates lack mitogenic activity for lympocytes of mice, guinea pigs, and rats (Mansfield et al., 1976). The increased level of IgM in T. vivax infection in cattle may be satisfactorily explained by elevated levels of antibodies to T. vivax antigens.

Immunosuppression: Holmes et al. (1974) have studied immune-responsiveness of cattle during trypanosomiasis. Their findings show that there is extensive proliferative activity, in the first 3 - 4 months after infection, in lymphoid organs of cattle experimentally infected with T. congolense or T. brucei. In chronic bovine infections evidence of depletion of lymphoid organs has been found. The lymph nodes of such animals are small, fibrotic and relatively acellular; there is marked reduction in the paracortical areas and the lymphocytic follicles are small and inactive.

The possible immunosuppressive effect of trypanosomiasis was reported by Parkin and Hornby (1930) who observed a loss of resistance to intercurrent infections. Since then many secondary infections have been associated with trypanosome infection (Fiennes, 1954). Holmes et al. (1974) showed that secondary humoral response to polyvalent clostridial vaccine in T. congolense - infected cattle was lower than in

uninfected controls, thus providing a direct evidence of the immunosuppressive effect of trypanosomiasis. The complete humoral unresponsiveness observed in laboratory rodents 3 weeks after infection (Freeman et al., 1974; Mansfield and Bagasra, 1978) has been attributed either to the polyclonal exhaustion of B-cells by a mitogenic factor of trypanosomal origin (Esuruoso, 1976; Assoku and Tizard, 1978) or to depressed suppressor cells and/or suppressed helper T-cells (Jayawardena and Waksman, 1977; Mansfield and Bagasra, 1978). Mansfield and Bagasra (1978) hypothesised that proliferating B-cell clones fail to switch from IgM to IgG synthesis and that the suppressor T-cell function is lost or depressed. Corsini et al. (1977) as well as others proposed that a trypanosomal mitogen from T. brucei is responsible for the unregulated proliferation and IgM synthesis of B-Cell clones and that clonal exhaustion eventually results in the observed suppression of B-cell function. Another theory that has gained popularity is that put forward by Jayawardena and Waksman (1977) and supported by Corsini et al. (1977) which proposes the generation of suppressor cells during trypanosomiasis. They suggested that non-specific suppressor T-cells are stimulated directly by trypanosomes to produce suppressive factors that act through macrophages. The presence of such suppressor macrophages, as well as suppressor T-cells, in the spleens of T. brucei - infected mice has been demonstrated (Eardley and Jayawardena, 1977).

Polyclonal activation resulting in clonal exhaustion (Greenwood, 1974 a,b; Assoku et al., 1977; Askonas et al., 1979) may not be a major mechanism of immunosuppression in bovine trypanosomiasis since it has been shown (Rurangirwa et

al., 1983) that cattle chronically infected with T.congolense responded to vaccination better than those with acute infection. It has been further suggested (Esievo and Saror, 1991) that although the hypergammaglobulinaemia observed in T. brucei (Musoke et al., 1981) and T. congolense (Masake et al., 1983) infections in cattle was due to the production of specific antibodies against the numerous variable antigen types, immunosuppression may be due to antigen competition between the trypanosome and the heterologous test antigens (Nantulya et al., 1982). This hypothesis is supported by the observation that the degree of immunosuppression depends on the level of parasitaemia (Baltz et al., 1981; Rurangirwa et al., 1983).

2.4.6 Pathogenesis of anaemia in African trypanosomiasis

Anaemia has long been established as a significant pathological feature of African trypanosomiasis (Hornby, 1921; Fiennes, 1954; Murray, 1974; Murray, 1979). It is in fact the major disease-promoting factor of bovine trypanosomiasis. The onset of anaemia in trypanosomiasis corresponds with the appearance of the parasite in the blood; its severity depending on the level and duration of the initial peaks of parasitaemia (Dargie et al., 1979; Maxie et al., 1979; Esievo et al., 1982). The anaemia is generally believed to be haemolytic, occurring intravascularly very early in the acute phase, and extensively extravascular in the sub-acute and chronic courses of the disease. The underlying mechanisms of anaemia have been divided into two categories (Murray et al., 1979): one set that operate in the presence of the parasite; and the other category include those that operate in the apparent absence of the trypanosomes.

Direct haemolysis: The mechanisms involved in the first category may include release of haemolytic factors by living or dying trypanosomes. T. brucei produces a protein capable of damaging red blood cells (RBC's) (Huan et al., 1975). Major African trypanosomes; the T. brucei subgroup, T. vivax and T. congolense, are able to produce a haemolytic factor(s) (Murray et al., 1979), while T. congolense autolysates have been shown to produce phospholipase A1 (Tizard and Holmes, 1976; Tizard et al., 1978) which releases haemolytic FFAs from endogenous phospholipid into the blood stream (Nguyen Huan et al., 1975; Tizard et al., 1978a;b). This suggested mechanism has however been questioned (Esievo and Saror, 1991) since FFAs are rapidly bound by serum albumin, thus, serum levels of FFAs have to be enormous in order to both exceed the binding capacity of the albumin and also achieve haemolytic activity (Tizard et al., 1978 a,b). Thus this mechanism of erythrocyte destruction is probably insignificant in bovine trypanosomiasis.

It is also possible that the fever which often attends trypanolytic crisis plays a role in erythrocyte damage and destruction. It has been shown that RBCs exposed to temperatures a few degrees above normal body temperature for a few hours have increased osmotic fragility, undergo accelerated haemolysis in vitro and have a shortened life-span in vivo (Karle, 1969).

Jennings et al. (1974) also agreed that the rapid onset of anaemia in T. brucei - infected mice was due to damage to erythrocytes as a direct or indirect result of trypanosome infection, but they were of the opinion that an immunological mechanism

might have been significant only at the later stages of the infection, when immune response might have been mounted by the host.

Immune-mediated haemolysis: Immunoglobulins and complement substances have been implicated in anaemia of trypanosome-infected laboratory animals (Woo and Kobayashi, 1975), calves (Kobayashi et al., 1976) and human subjects (Zoutendyk and Gear, 1951; Barret-Connor et al., 1973). Erythrocytes from infected rabbits (Woo and Kobayashi, 1975) were lysed when brought in contact with fresh complement, suggesting the presence of antigen-antibody complexes. Antibodies against trypanosomes have been eluted from RBC's of infected rabbits and it has been shown that trypanosome antigens are readily absorbed unto normal rabbit erythrocytes (Woo and Kobayashi, 1975). Immunoglobulins and complement C3 have been demonstrated on RBCs of calves infected with T. congolense (Kobayashi et al., 1976). The presence of complement-dependent trypanosome antigen-antibody complexes on erythrocytes of infected animals likely facilitates phagocytosis by the expanded and active MPS via Fc or complement receptors on the macrophage (Murray, 1979). It has been suggested, however (Esievo and Saror, 1991) that immunoglobolins and complement substances act as opsonins in the phagocytosis of desialated RBCs produced in acute bovine trypanosomiasis (Esievo 1979; Esievo et al., 1982).

Disseminated intravascular coagulopathy: Another mechanism that has been implicated in anaemia, although the effect has been shown to be insignificant (Murray et al., 1979b), is disseminated intravascular coagulation (DIC). DIC has been described in infected humans (Barrett-Connor et al., 1973; Jenkins et al., 1974; Robins-Browne et al., 1975); rabbits (Boreham and Facer, 1974) and cattle (Valli et al., 1978a). In DIC red cells are damaged by widespread fibrin deposition in the microvasculature leading to a form of haemolytic anaemia termed microangiopathic haemolytic anaemia in which the erythrocytes appear as distorted cells which are liable to lysis or phagocytosis (Wintrobe, 1975).

Haemodilution and erythropoietic response: The suggestion of haemodilution as an important factor in causing anaemia in the early stages of trypanosomiasis (Fiennes, 1954; Naylor, 1971; Holmes, 1976; Villi et al., 1978) has been strongly refuted experimentally (Dargie et al., 1979a;b). The contribution of haemopoietic response in the development of trypanosomal anaemia at this early stage of the disease is at best controversial; with reports of depressed haemopoiesis (Boycott and Price-Jones, 1913; Fiennes, 1954; Naylor, 1971b; Losos and Ikede, 1972) and active erythropoiesis (Boreham, 1968; Mackenzie and Criukshank, 1973; Jennings et al., 1974; Murray et al., 1974; Assoku, 1975; Holmes and Jennings, 1976; Ingh et al., 1976; Maxie et al., 1976, 1979; Saror, 1980). Other reports chart a middle course between these two extremes by suggesting that although no obvious evidence of dyshaemopoiesis was observed the erythropoietic response were moderate for the degree of anaemia and the number of cells

being removed (Dargie, 1978), but less than adequate to prevent anaemia due to reticuloendothelial blockage of iron metabolism (Dargie et al., 1979a;b).

Erythrophagocytosis: In the early stages of bovine trypanosomiasis, perhaps the most significant mechanism for anaemia is the one involving the expanded and active mononuclear phagocytic system which is observed in trypanosomiasis. Erythrophagocytosis in animal trypanosomiasis has been observed in T. congolense - infected sheep (Mackenzie and Cruikshank, 1973), T. vivax - infected goats (Saror, 1980) and T. congolense - infected cattle (Fiennes, 1954; Naylor, 1971a; Losos et al., 1973; Valli and Forsberg, 1979). Erythrophagocytosis can be caused by coating of erythrocyte surfaces with trypanosome antigen (Jennings, 1976) leading to interaction with anti-trypanosome antibodies and subsequent sequestration of the affected erythrocytes by erythrophagocytosis. Such antigenic material has been demonstrated on the surfaces of sheep erythrocytes (Mackenzie et al., 1978). Another process has been suggested, which involves the production of sialidase by T. vivax (Esievo, 1979) and the cleavage of erythrocyte surface sialic acids during bovine trypanosomiasis (Esievo et al., 1982). This exposes new antigenic sites against which antibodies may be produced and thus making the affected erythrocytes suitable for phagocytosis.

Sialidase-treated erythrocytes from different species have been found to be rapidly sequestrated from the blood stream by the liver and spleen (Durocher et al., 1975; Aminoff et al., 1977; Jancik et al., 1978) In vitro studies have shown that Kupffer's cells and peritoneal macrophages interact more strongly with desialylated erythrocytes than

with intact ones (Durocher et al., 1975; Janeik et al., 1978; Kolb and Kolb-Bachofen, 1978; Kuster and Schauer, 1981; Muller et al., 1981, 1983). Only recently Omage et al. (1995) showed that more erythrocytes from T. vivax infected Zebu calves bound to rat peritoneal macrophages than did those from un-infected calves.

Trypanosomes produce sialidase in vivo (Esievo, 1979); and in vitro (Esievo, 1980; Pereira, 1983; Pereira and Hoff, 1986; Engstler et al., 1991; Engstler and Schauer, 1993) and this enzyme has been shown to cleave off red cell surface sialic acids in a number of host species (Durocher et al., 1975; Seaman et al., 1977; Esievo et al., 1982), thus making them more prone to erythrophagocytosis.

Erythrophagocytosis is a two-step process (Kuster and Schauer, 1981; Muller et al., 1983; Kelm et al., 1986a, Kelm and Schauer, 1986) and requires pre-treatment of the erythrocytes with sialidase. The first step involves the binding of the desialylated red cell to the macrophage surface via a beta-D-galactose specific lectin on the macrophage surface. The second step requires that the first step be completed and it involves the engulfing of the attached red cell. This latter step appears to require the presence of certain complement substance of the serum (Kuster and Schauer, 1981). The rate limiting step of the overall process is the binding step and the only factor regulating this step appears to be the amount of sialic acids released from the surface of the erythrocytes (Muller et al., 1983; Kelm et al., 1986b). Experiments with rabbits (Muller et al., 1981) and T. vivax - infected Zebu calves (Ibrahim et al., 1995) have shown that a beta-galactosyl-specific lectin, seems to be involved in the mechanism of sequestration of desialylated erythrocytes in vivo. Thus, sialidase exposes penultimate beta-galactose

residues of the erythrocyte membrane in trypanosomiasis (Omage et al., 1995) and interaction of these cells with a beta-galactose-specific lectin on the liver and spleen macrophages (Ibrahim et al., 1995) results in the rapid sequestration of the sialidase - treated erythrocytes from the blood-stream. The involvement of immunoglobulins and complement factors of the classical and alternative pathways have been excluded from the engulfment process (Muller et al., 1983). Thus the phagocytosis of bound desialylated red cells by the macrophage is a function of only their number and binding strength to the macrophages (Muller et al., 1983).

The binding of desialylated erythrocytes to macrophage surfaces is inhibited by beta-glycosidically bound beta-D-galactosyl residues (Kolb and Kolb-Bachofen, 1978; Nagamura and Kolb, 1979; Kolb et al., 1980; Schlepper-Schafer et al., 1980). Galactose and related monosaccharides as well as lactose have been shown to inhibit similar processes in vitro (Kelm and Schauer, 1986). Lactose and asialofetuin prevented the sequestration of bacterial sialidase-treated erythrocytes from the blood-stream of experimental rabbits (Muller et al., 1981).

2.5 Pathology of African Trypanosomiasis

2.5.1 Clinical pathology

Unlike the trypanosomes of the T. brucei group T. congolense (Hornby, 1949; Losos et al., 1973) appears to be a parasite of the plasma. Large numbers of the parasite have been found in the blood vessels of the brain, heart and skeletal muscles (Losos et al., 1973). The changes usually observed are anaemia, emaciation and

polioencephalomalacia, probably caused by the accumulation of trypanosomes in the cerebral vessels (Goodwin, 1974). Other clinical changes are pyrexia, weakness, lethargy, dull rough hair coats, pale mucous membrane (probably due to the severe anaemia), enlarged superficial lymph nodes and jugular pulsations (Murray, 1974; Murray et al., 1979)). The uneven distribution of the parasites, which show a preference for swarming in the smaller blood vessels of selected organs (Goodwin, 1974), probably causes disturbances in the microcirculation.

The pathogenesis of T. vivax infections differ in some respects from that of T. congolense infections (Losos and Ikede, 1972). The organism is fairly evenly distributed throughout the circulatory system. In such animals there is evidence of massive terminal thrombosis in relatively large blood vessels. There is also loss of weight and condition, anaemia and disturbances of plasma proteins (Sekoni, 1990; Sekoni et al., 1990a; 1990b; 1990c). Trypanosomes of the T. brucei subgroup localise in solid tissues particularly in the loose connective tissue stroma (Losos, 1979). Two stages of the disease in man are recognised (Woodruff, 1974): the early or blood-lymphatic stage and the later, meningoencephalitic stage. The early stage is marked by a moderate anaemia, splenomegaly, hepatic dysfunction, myocarditis and reproductive disorders (Woodruff, 1974). In the later stages the CNS is involved and there is marked lethargy and eventual brain damage.

2.5.2 Gross pathology

Increases in the weights of certain organs of T vivax and T. congolense - infected cattle were observed very early in the infection (7 days) (Losos and Mwambu, 1979). Spleen increased by 50%; liver by 25% and kidneys by 60%; and increase in the weight of the heart (15%) was also observed in the T. congolense infection (Losos and Mwambu, 1979). At a later stage of infection (60 days) weights of lymph nodes increased by 50% in T. vivax infection but not in T. congolense infection (in fact a decrease in weight was observed in these cases). Further increases were observed in the weights of the liver (50%) while the kidney attempted a return to normality (increase was only by 25%); the adrenals also increased in weight by 25%.

Calves infected with T. congolense had much less body fat and body flesh and a greater proportion of visceral to carcass weight. There was atrophy of the thymus gland (Valli et al., 1978b) and the heart was more globose and heavier and tended to have less fat around coronary vessels. Lungs from infected calves did not collapse completely and were slightly larger (Valli et al., 1979b). The liver and spleen were grossly enlarged. The kidneys were also slightly heavier. The most prominent change in the haemopoietic system was marked conversion of the fat to haemopoiesis in the femoral marrow with almost all but a small central area of medullary fat converted to haemopoiesis in most animals (Valli et al., 1978b).

2.5.3 Histopathology

Thymic and splenic changes: Thymic lobules were small and there was a prominent reduction in cortical width. Inter-lobular septa were infiltrated with fat and frequently with eosinophils; there was also a noticeable reduction in density of lymphocytes in both the cortex and medullary areas (Valli et al., 1979b).

Morrison and Murray (1979) identified two distinct phases in splenic cellular changes during T. congolense infection of mice. In the early proliferative phase a peak of proliferative activity occurred coincident with the first peak of parasitemia, this phase is characterised by marked increases in plasma cells and a corresponding decrease in the percentage of small and medium lymphocytes, and this occurs in both the white and red pulp of the spleen in the first ten days (Valli et al., 1978b). In the later depletion phase there is further increase in content of erythroid cells and increase in numbers of large macrophages and granulocytes often with prominent hemosiderin accumulation.

Lymphoid changes: Unlike the spleen the lymph nodes of T. congolense infected mice (Morrison and Murray, 1979) show only minor changes before day 20 of infection. Thereafter, however, marked proliferative activity starts in the follicular cortex and numerous plasma cells appear in the medullary cortex. These changes become more pronounced as the infection progresses. The follicles of infected calves were generally larger than those in control animal (Morrison et al., 1979) though the cell density within the germinal centres was lower, and in many cases there was follicular hyalinosis and fewer mitoses in infected animals than in controls. The area normally occupied by

thymic-dependent small lympocytes had a marked increase in stromal collagen and a dense collection of large macrophages, often with prominent hemosiderin accumulations (Morrison and Murray, 1979). Focal plasmacytosis occurred around small vessels in the medullae, and the postcapillary venules were prominent with high vesicular endothelium. In T. brucei infections, during which the organisms enter the tissues and are present in lymph (Lambert and Houba, 1974; Ssenyonga and Adam, 1975), the changes observed in the lymph nodes are much more severe and occur much earlier than with T. congolense (Murray et al., 1974).

Hepatic changes: The liver changes consisted of hepatocellular atrophy with sinusoidal dilation and endothelial and kupffer - cells proliferation, periportal lymphoid accumulation occurs but is not prominent, and no areas of necrosis are usually present (Valli et al., 1979b). In T. rhodesiense infection of man parenchymatous degeneration - which are associated with liver dysfunction are observed (Woodruff, 1974).

Renal changes : Renal changes usually consist of lymphoid aggregates occasionally including germinal - centres around arcuate arterioles and occasionally in juxtaglomerular locations (Valli et al., 1979b). There is a moderate overall atrophy of tubular epithelium without areas of necrosis and a generalised increase in glomerular size and mesengial density (Murray et al., 1975). Hemosiderin may be present in basilar areas of proximal tubules and within the mesengial areas of the glomeruli (Nagle et al., 1974). A diffuse and global increase in glomerular cellularity and a decrease in luminal area, due to

clogging of capillaries by hemosiderin - bearing macrophages, are also observed (Nagle et al., 1974; Lambert and Houba, 1974; Murray et al., 1975).

Adrenal Gland Changes: An increase in cortical width, which is largely due to increase in width of the zona fasiculata, of the adrenal gland is observed in infected animals (Valli et al., 1979b). There is also increased nuclear density of the zona fasiculata indicating that the cells within, although more numerous, are smaller.

Cardiac changes: The heart is one of the most severely affected organs in trypanosomiasis (Ikede and Losos, 1972; 1975; Murray, 1974; Morrison et al., 1979). There is diffuse myocarditis (Morrison et al., 1979) which in most terminal cases is held responsible for death. Lesions are initially found beneath the epicardium and endocardium and are more severe in the atria than in the ventricles. In more advanced cases the entire myocardium is involved; there is marked distortion and degeneration of myocardial fibres and in some sites necrosis is apparent (Morrison et al., 1979). The lymphatics in the epicardium are distended and contain numerous macrophages and polymorphonuclear leukocytes and sometimes lymphocytes and trypanosomes (Murray, 1974; Galvao-Castro et al., 1978). In some cases the lymphatics are completely occluded by thrombi that contain fibrin as well. Furthermore, there often is severe necrosis of both arteries and veins in the epicardium (Morrison et al., 1979). An overall increase in the number of mycocardial nuclei per unit area, with the nuclei enlarged and more vascular, was observed in some cases (Valli et al., 1979b).

Pulmonary changes: Changes in the lungs consists of a marked and consistent increase in width of alveolar walls with increasd numbers of inflammatory cells within the alveolar walls and stainable iron present throughout the alveolar septa (Valli et al., 1979b).

Brain changes: Changes in the brains of animals infected with T. congolense (Valli et al., 1979b) consisted of a mild dilatation of the perivascular spaces and increased number of lymphocytes in these areas. The capillary lumina was uniformly dilated and contained haemosiderin-bearing macrophages (Valli and Forsberg, 1979).

The effects of the T. brucei, subgroup on the brain are much more severe because of their invasion of the CNS in large numbers (Ikede and Losos, 1972; Murray, 1974; Morrison et al., 1979). The invasion of the CNS by the trypanosomes begins and continues in the form of a progressive leptomeningitis, after variable length of time from first infection, until in the later stages irreparable damage is done to the brain tissues (Woodruff, 1974). There is widespread cellular infiltration with mononuclear leukocytes (Valli et al., 1979b; Mwambu and Losos, 1979) and this is more pronounced under the pia matter (Woodruff, 1974). The proliferation progresses until it is general throughout the white matter and focal areas. Permanent and irreversible neuronal degeneration may occur with proliferation of glial cells and sometimes pin-point areas of haemorrhages (Mwambu and Losos, 1979).

Enteric changes: Changes in the enteric tract consist of a thickening of the squamous epithelium of ruminal villi in both the germinal and keratinised layers (Valli et al., 1979b). A greater area of lamina propria in the intestinal villi of infected animals is

occupied by eosinophil and plasma cells (Valli and Forsberg, 1979). In addition there is usually a mild but consistent dilation of the central lymphatics of the intestinal villi. A mild reduction in pancreatic acinar cell size as well as in cellular area occupied by zymogen granules may be seen.

Bone marrow changes: The bone marrow of infected animals is usually much more cellular than that of normal animals (Valli et al., 1979b). The fat cells in infected calf marrows were only half the size of those in controls (Valli et al., 1978a). Lymphoid modules (and occasionally germinal centres) are frequently present in the marrows of infected animal and are never seen in marrows of controls (Valli et al., 1979a). Hemosiderin is increased in the marrow which tends to be larger and more coarse than in controls - indicating a mild degree of myelofibrosis. In infected calves there were approximately three times as many myeloids and about 15 times as many erythroid cells (Morrison et al., 1979). Red blood cells were six times as numerous per unit area in the marrows of infected calves, indicating capillary dilation in this tissue (Valli et al., 1978a).

Reproductive organ changes: Low production trait, especially male sterility is one of the symptoms of bovine trypanosomiasis. Genital lesions have been reported in sheep and goats infected with T. vivax (Isoun and Anosa, 1974; Anosa and Isoun, 1980) and in sheep, rabbit and mice infected with T. brucei (Ikede, 1979; Ikede and Akpavie, 1982; Anosa and Kaneko, 1984) and in cattle infected with T. vivax and T. congolense (Sekoni, 1990, Sekoni et al., 1990a). Trypanosome - induced abortion has also been reported in zebu cattle (Nuru, 1974; Ogwu, 1983). Genital lesions can lead to deterioration of seminal

characteristics and eventually to cessation of spermatogenesis when the testes themselves are involved (Lagerlof, 1936; Rao, 1971; Kumi-Diaka et al., 1980; Ikede et al., 1988, Sekoni et al., 1988; 1990b).

Working with bulls infected with T. vivax and T. congolense, Sekoni et al. (1990a), found testicular degeneration ranging in degree from the mild to the severe; complete destruction of all cellular structures within the interstitial tissues and seminiferous tubules was recorded in one bull. Epididymal, seminal vesicular and prostrate gland degeneration as well as decreases in epididymal sperm reserves were observed. No lesions were observed in the cowper's glands, while only one animal had moderate degeneration of the ampullae. In the same experiment another group of bulls infected with T. congolense showed the same lesions in more severe and extensive forms. These degenerative changes appear to be irreversible after a particular stage since chemotherapy could not result in regeneration of damaged testicular machinery in T. vivax and T. congolense - infected bulls (Sekoni, 1990) and T. brucei - infected rabbits (Ikede and Akpavie, 1982) as well as T. brucei and T. vivax infected sheep (Akpavie, 1987).

Chronic infections with T. vivax and T. congolense caused an increase in reaction time of ejaculation (indicative of reduced libido) decreased semen volumes and sperm concentration in ejaculated semen; as well as increase in morphologically abnormal spermatozoa (Sekoni et al., 1988); the changes were more severe in the T. congolense infection. Abnormalities observed (Sekoni et al., 1990b) in the sperm morphology included sperm head abnormalities, detached heads, proximal cytoplasmic droplets, distal

cytoplasmic droplets, midpiece abnormalities, and sperm-tail abnormalities. Sperm morphological abnormalities have been correlated with infertility and possible sterility (Blom, 1950, Saacke et al., 1968). Reduced libido and impotence has been reported in humans with sleeping sickness (Woodruff, 1974).

Abnormal estrous cycles and degeneration of the female genitalia have been reported in infections by various pathogenic trypanosomes in livestock. T. vivax infection caused silent estrous, irregular estrous, and temporary anestrus in sheep (Adenowo, 1989) and anestrus of up to 6 months in chronically infected Zebu heifers (Ogwu et al., 1984). T. congolense infection of Boran cows (Luckins et al., 1986, Llewelyn et al., 1988) caused anestrus of up to 100 days and irregular or short estrous cycles and anestrus in goats (Mutayoba et al., 1988).

The structure and function of the ovaries are affected by trypanosomiasis. T. vivax - induced cystic degeneration (Vohradsky, 1971) and arrest of ovarian function (Ogwu et al., 1984) have been reported in cattle. Infection with T. congolense caused stoppage of cyclical activity of the ovaries of Boran cows (Luckins et al., 1988) and development of atrophic ovaries with multiple follicular cystic degeneration and total absence of corpora lutea in Zebu heifers (Ogwu and Njoku, 1991). Other effects of bovine trypanosomiasis on the female genitalia include flaccidity and atony of the uterine horns (Ogwu et al., 1984), massive endometrial mononuclear cell infiltration, glandular atrophy, periglandular cell infiltration, myometrial atrophy and fibroplasia in the uterus (Ogwu and Njoku, 1991).

Trypanosome - induced reproductive problems such as abortions during various stages of pregnancy, birth of weaklings, premature births, birth of anaemic offsprings, low birth weight and neonatal calf mortalities have been reported in T. vivax and T. congolense - infected cattle (Ogwu et al., 1986; Ogwu and Njoku, 1991; Anene et al., 1991) and in T. rhodesiense infected women (Woodruff, 1974).

2.6 Chemotherapy of African trypanosomiasis

Treatment of African trypanosomiasis involves the use of therapeutic drugs, injected parenterally into the patient. These treatments primarily aim to limit loses due to these diseases. Although attempts to vaccinate with killed or irridiated trypanosomes (Kligler and Weitzmann, 1926) have given promising results in laboratory animals, in the field this method is unreliable. This is due to the complex host immune reaction and antigenic variation of the parasite (Cross and Johnson, 1976; Cross, 1977; 1978a;b; Barry and Hajduk, 1979). So in the absence of an effective immunisation technique, prophylactic trypanocidal drugs are used to protect human and animal populations (Fiennes, 1952; 1953a;b; 1960).

Development of very effective drugs between 1940 and 1960 (Fiennes, 1960) has led to the wide use of trypanocides to control African trypanosomiasis; however, since 1961 no new trypanocide has passed the experimental stage (CTA, 1989). So the drug treatment of trypanosomiasis is dependent on a limited number of products. Suramin and pentamidine are used for prophylaxis and treatment of early stages of the disease in man; while organic arsenicals such as tryparsamide and melaminyl compounds for advanced

cases, when trypanosomes have invaded the CNS (Whiteside, 1962). The drugs used in veterinary medicine generally fall into four chemical families (CTA, 1989): urea compounds (e.g. Suramin), phenanthridium compounds (Ethidium, Novidium, Prothidium and Trypamidium), quinapyramine compounds (Antrycide) and aromatic amidine compounds (Berenil).

2.6.1 Mechanism of Action of Trypanocides

Study of biochemical modes of action of antimicrobials has led to identification of six key areas of metabolism as targets for drug action (Franklin and Snow, 1975; Gutteridge and Coombs, 1977): energy metabolism, membrane function, cofactor synthesis, protein synthesis and cell wall synthesis. The selectivity of the drugs for the microbial systems is dependent on five factors (Gutteridge and Coombs, 1977) which include: the ability of the drug to selectively permeate or concentrate in the parasite, to attack a target present only in the parasite, to discriminate between isofunctional targets in the parasite and the host, and to block a target that is of greater importance to the parasite than to the host. Another factor is the selective activation of the drug only in the parasite.

Drugs interfering with energy metabolism: Organic arsenicals, such as tryparsamide, act by inactivating sulphydryl groups of proteins and enzymes (Gale et al., 1972; Franklin and Snow, 1975) thereby inactivating the enzymes. It has been suggested that the organic arsenical trypanocides inactivate trypanosomal pyruvate kinase (Newton, 1974) hence inhibiting the glycolytic pathway which is the chief energy source for blood-stream

African trypanosomes (Von Brand, 1967; Voorheis, 1969). The selectivity of the arsenicals appears to rest in part on their greater permeability into the trypanosomes, in part on the greater sensitivity of the trypanosomal pyruvate kinase to inhibition, and in part on the greater dependence of trypanosomes on glycolysis for ATP synthesis (Gutteridge and Coombs, 1977).

The mode of action of Suramin is still obscure, it is, however known to inhibit many enzymes (Coombs, 1976). In trypanosomes the remarkable sensitivity of the unique enzyme L-alpha-glycerophosphate oxidase to Suramin is believed to be responsible for its selective destruction of trypanosomes in the blood of hosts (Gutteridge and Coombs, 1977). This oxidase is responsible for regeneration of NAD^+ during glycolysis in the parasite.

Drugs interfering with nucleic acid synthesis: The trypanocides of the phenanthridine group act by interfering with synthesis of nucleic acids. Ethidium was reported to selectively inhibit trypanosomal DNA and RNA synthesis (Stephen, 1958; Stephen and Williamson, 1958), later reports (Gale et al., 1972; Newton, 1974; Franklin and Snow, 1975; Gutteridge and Coombs, 1977) indicated that DNA and RNA polymerases were inhibited due to intercalation of the drug with DNA. The aromatic diamidines such as Pentamidine and Berenil also act by inhibiting nucleic acid synthesis (Bauer, 1958; Fulton, 1960). The drugs bind, though not by intercalation, to the DNA (Gutteridge and Coombs, 1977).

Though the phenanthridines and aromatic diamidines exert a very specific effect on kinetoplast DNA synthesis at lower drug concentrations (Frankline and Snow, 1975; Borst and Fairlamb, 1976) it is unlikely that this interaction is related to their trypanocidal action (Gutteridge and Coombs, 1977), since it is improbable that kinetoplast DNA is transcribed and translated in the blood forms of the parasites (Borst and Fairlamb, 1976). Further more, the drugs bind to DNA from whatever source (Franklin and Snow, 1975); thus the selectivity of these drugs seems to be a function of permeability (Gutteridge and Coombs, 1977) where the drugs penetrate into the parasite but not into the host cells.

Drugs interfering with protein synthesis: The quinapyramine trypanocides, e.g. Antrycide, act by inhibiting protein synthesis in the parasite (Ormerod, 1951). Antrycide seems to act by displacing Mg^{2+} and polyamines from the cytoplasmic ribosomes of trypanosomes (Newton, 1974; Franklin and Snow, 1975; Gale et al., 1975) and this results in the aggregation and inactivation of the ribosomes thus blocking protein synthesis (Hanas et al., 1975; Gutteridge and Coombs, 1977). The selectivity of the drug depends on selective permeability.

2.6.2 Problems and prospects in chemotherapy of trypanosomiasis

Resistance has been reported to occur against all these drugs and development of resistance to one compound is often accompanied by cross-resistance to others (Whiteside, 1962). T. cruzi infections are characterised by their resistance to all drugs in general use for the treatment of African trypanosomiasis. Tests with a nitrofuran

derivative, however, seemed to be encouraging (Haberkorn et al., 1970). The need for new trypanocides, therefore, is very real and urgent.

The chemist's achievements have been limited by his lack of information about the nature of primary drug-binding sites on sensitive cells and about the essential reactions he should aim to block (Whiteside, 1962). The obvious approach to the problem of developing new drugs include: detailed comparison of homologous enzymes in host and parasite, unique cell components or metabolic pathways in parasites sought. Efforts in these directions have met with little success. Reasons for this failure are many, but in the particular case of chemotherapy of trypanosomiasis the main bottle-neck has been the difficulty of obtaining parasite material in the quantities required for biochemical research (Whiteside, 1962).

Salivarian species of Trypanosoma can only be grown continuously in vitro in the culture form (Weinmann, 1959). The medium generally used is complex and contains blood constituents; however, some progress towards a completely defined medium for growth of T. brucei has been made (Cross and Manning, 1973). Blood stream forms can not be cultured in vitro: the best that has been achieved is an increase in parasite numbers of eight to ten-fold in the presence of L-cells maintained in tissue culture medium. Very limited success has been achieved in growing amastigotes of T. brucei in non-cellular media (Trager, 1959; Lemma and Schiller, 1964; Pan, 1971).

However, in the absence of cultures of trypanomastigotes forms the effects of drugs have been studied on a number of systems (Whiteside, 1962); including non-pathogenic flagellates which can be readily grown on defined media, culture forms of

laboratory strains of pathogens, blood-stream forms either in vitro as washed suspension of non-dividing parasites, and enzymes and other cell constituents isolated from parasites.

There are many angles from which chemotherapy can be attacked. Isoenzymes with different drug sensitivity are now being found in parasites and mammalian cells. Nutritional studies are beginning to reveal metabolic deficiencies in trypanosomes; greater understanding of these deficiencies may lead to the development of new metabolite analogues. The kinetoplast of trypanosomes is regarded as a specialised area of a unique type of mitochondrion which plays a vital role in the developmental cycle of trypanosomes; in the T. brucei group the presence of kinetoplast DNA appears to be mandatory for the development of the parasite in culture and in its insect vector. Inhibition of the synthesis of kinetoplast DNA by acridines, phenanthridines and diamidines gives rise to "dyskinetoplastic" organisms which are unable to infect the insect vector (Newton, 1966).

Another well established characteristic of the trypanosomes is their ability to undergo antigenic variation in the mammalian host (Gray, 1965;). Successful immunisation against African trypanosomiasis has been impossible because of the apparently unlimited capacity of these trypanosomes for changing their antigenic character (Gray, 1965). Detailed knowledge of the chemical nature of these antigens and of factors controlling their synthesis may reveal biochemical mechanisms sufficiently different to those functioning in host cells for them to become targets for chemotherapeutic attack. Restriction of antigenic variation would give host defence

mechanism a better chance of controlling infections and would possibly permit the development of a technique of immunization.

Other biochemical peculiarities of trypanosomes which, on further investigation, may provide sites for the action of selective inhibitors include the branched electron transport pathway (Hill, 1972) and the biosynthetic and biodegradative reactions involved in the changes in lipid and sterol composition of trypanosomes during their developmental cycles (Newton et al., 1973).

2.7 Lactose

2.7.1 Lactose Metabolism in Mammals

Lactose (beta-D-galactopyranosyl (beta-1-4)-D-Glucopy ranoside) is found in the free state in appreciable quantity only in the milk, and it is a major energy source for infants (Smith et al., 1985). Lactose is also found as the carbohydrate components of glycolipids in the membrances of cells where they serve as surface antigens. Lactose is a better nutrient in milk than glycose for infacts because it is slowly hydrolysed in the intestine (Smith et al., 1985) by the mucosal brush-boarder enzyme, Lactase (B-galactosidase), resulting in a steady energy supply and a comparatively constant blood glycose level between feeding (schaafsma and McDonald, 1983). Lactose is also believed to assist in the development of a favourable environment in the intestine resulting in the development of lactric acid flora, which may inhibit the growth of pathogenic flora (Schaafsma and McDonald, 1983). The disaccharide is also believed to accelerate calcium-uptake in the intestine (Allen, 1982).

Lactose can however be toxic in lactose intolerant individuals. Lactose intolerance in infants is due to congenital absence or inadequacy of intestinal lactase, while in adults it is due to the natural decline in Lactase activity as the individual matures (Bowman and Brand, 1985) - especially with reduced intake of dairy products. In Lactose intolerance Lactose is not digested in the small intestine and is passed into the colon. The sugar is not absorbed in the colon and is acted upon by bacterial lactase. The monosaccharide products are partly metabolised, but poorly absorbed, hence the solute content of the colon is increased. This results in osmotic shift of water into the colon with consequent severe and chronic diarrhoea.

Lactose is excreted in the urine and urinary out-put is high in infants and pregnant or lactating women; and increases in adults following a long-term exclusive milk diet (Pillegi and Szustkiewicz, 1974).

Lactose is synthesised in the mammary glands from uridine Diphosphate-galactose and glucose in a reaction catalysed by Lactose synthetase (smith et al., 1985). Lactose synthetase is an enzyme complex containing galactosyl transferase and a modifier sub unit (stryer, 1981). During pregnancy, galactosyl transferase is synthesised and stored in the mammary gland, but there is little modifier submit. At paturition, abrupt changes in the levels of some hormones elicit the synthesis of large amount of modifier submit and the resulting lactose synthetase complex then forms large amounts of lactose (stryer, 1981). Galactosyl transferase is present in most tissues where it participates in the synthesis of the carbohydrate moiety of glycoproteins.

2.7.2 Chemistry and uses of lactose

Lactose occurs as a white powder which is odourless and has a faintly sweet taste (Zadow, 1991). It is stable in air but readily absorbs odours. It is very slightly soluble in alcohol and is insoluble in chloroform and ether. Aqueous solution of the sugar is neutral to litmus paper. Due to the free hydroxyl group at the anomeric carbon, it exists in two forms: The alpha- and beta-forms (Zadow, 1991). This feature makes lactose a reducing sugar and thus it gives positive reaction with fehling's solution.

The disaccharide is extracted from whey. The whey is subjected to various treatments (liming, heating and filtration) to obtain the "Mother liquor" (Zadow, 1991). The mother liquor is concentrated, refiltered and the filtrate allowed to crystallise. The lactose crystals are then centrifuged off.

Lactose has found wide use in baby food, confectionery and pharmaceutical industries. Lactose is a useful carrier for flavours and colours and hence it is used in products such as sachet wafers, seasoning and baked goods; the confectionery industry uses lactose to obtain desired end product properties relying on lactose to alter the crystallisation characteristics of other sugars (Zadow, 1991). In the pharmaceutical industry lactose is used as diluent and binder in tablets and as a placebo in drug trials (Bentley, 1984).

More recently (kolb and kolb-Bachofen, 1978, Nagamura and Kolb, 1979, Kolb et al., 1980; Schlepper-Schafer et al., 1980; Kelm and Schauer, 1986) Lactose and other B-galactoside compounds have been used to inhibit erythrophagocytosis of desialylated RBCS by rat peritomeal macrophages. The rapid sequestration of homologous

desialylated erythrocytes from the blood stream of rabbits has been considerably in hibited by intravenously administered lactose (muller et al., 1981).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This research was designed to establish whether lactose would be able to slow down the rate of development of anaemia during Trypanosoma vivax infection of Zebu calves. Since lactose was to be used as a possible chemotherapeutic agent, it was necessary to establish its plasma kinetics properties in Zebu calves in the presence and absence of T. vivax infection. It was also important to establish the normal level of lactose in healthy Zebu cattle since lactose is normally produced in mammals and is occasionally found in plasma in appreciable quantities.

3.2 Materials

All chemicals used in this experiment were reagent grade (see appendix I). The strain of Saccharomyces carlsbergensis used for the partial fermentation of plasma samples in this work was obtained from the department of Microbiology, Ahmadu Bello University Zaria; and the fermentation specificity confirmed. The T. vivax (Samaru strain) was obtained from a natural infection in a local Zebu cow.

3.3 Experimental Design

The experiment was divided into three distinct phases as follows:

3.3.1 Phase 1: To Establish The Normal Levels of Lactose in Apparently Healthy Zebu Cattle

i) Animals: Thirty-six (36) adult cattle and ten (10) calves, all of the Zebu breed were randomly selected from a herd certified healthy and not previously exposed to the risk of trypanosome infection. The animals were residents of the environment and were, thus, already acclimatized. They were being grazed daily and supplemented with cotton seed and groundnut hay. Salt lick and water were supplied ad libitum.

ii) Collection of samples: Five millilitres of blood were collected from each animal, by jugular venepuncture, into tubes containing Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant. A portion of the collected blood was used for the determination of basic haematological parameters. The plasma was harvested, by immediately centrifuging the remaining samples at 3,000 g for 15 minutes and stored at -20°C until required for analysis.

iii) Analysis of samples:

(a) Percentage packed cell volume (PCV) was determined by the standard microhaematocrit method.

(b) The lactose concentration in the plasma was determined by method of Wahba (1965) (see appendix II) after partial fermentation of plasma samples, to remove interfering substances, using S. carlsbergensis by the method described by Horowitz et al., (1951) (see appendix II).

3.3.2. PHASE II: To Establish the Normal Plasma Kinetics of Lactose in Healthy Zebu Calves

i.) Animals: Seven zebu calves, 12-18 months old, weighing 70 to 80 kg and with no previous record of exposure to trypanosomal infection, were purchased from an area known to be tsetse free. On arrival the animals were acclimatized and vaccinated against anthrax, black quarter, bovine pleuropneumonia and rinderpest. Daily grazing was supplemented with cotton seed and ground nut hay. Water and salt lick were provided ad libitum.

ii) Treatment: The calves were divided into two groups of three and four animals each. The animals in the first group of three calves were each given, intravenously, a single dose of 0.5 g lactose/kg body weight as a solution in physiological saline (0.9% NaCl) at an infusion rate of approximately 9 ml/minute. Five millilitres blood were collected at specified time intervals (30-60 minutes intervals) from the jugular vein on the other side opposite the site of infusion of lactose solution, into EDTA- tubes. A portion of the blood was used for determination of the haemogram. The rest was centrifuged at 3,000 g to harvest the plasma, which was stored at - 20°C.

The animals in the second group of four calves were each given repeated doses of 0.5 g lactose/kg body weight as described above. Three doses at four-hourly intervals were administered per day. Five millilitres of blood samples were collected at specified time intervals as earlier described. Plasma was harvested and stored at -20°C.

iii) Analysis of samples: Plasma lactose concentration was determined for all collected samples as stated earlier. PCV was determined by the haematocrit method; total red blood cell (RBC) and leukocyte counts were done using the new improved Neubauer haemocytometre. Total plasma protein was estimated using the Hand refractometre.

iv) Preparation of lactose solution: Lactose solution for infusion was prepared in physiological saline (0.9% NaCl). A 50% solution was prepared with warming, and sterilised at 15 lbs/sq in. pressure for 5 minutes. The solution was allowed to cool to room temperature slowly. The solution was warmed to 37% just before infusion into the animals.

v) Analysis of data: The plasma lactose profiles for each animal was prepared by plotting the plasma lactose concentrations against the sampling time. Semi-logarithmic plots of the same data were prepared by plotting the concentration values on the logarithmic scale of a semilogarithmic graph sheet the regression line was calculated (Chatfield, 1983) and drawn. The biological half - life ($t_{1/2}$), elimination rate constant (K_e), total clearance (C_l) and apparent volume of distribution (V_d) of lactose in the animals were calculated from the semi-log plots as described by Grahame-Smith and Aronson (1992).

The $t_{1/2}$ was defined here as the time taken for half the concentration of lactose in the plasma at a given time to disappear; while the K_e was the amount of lactose eliminated from the body via all routes per unit time (hr). Total clearance was defined as the volume of body fluid from which the lactose was eliminated per hour, while V_d was

the total volume of plasma in which the administered lactose needed to be dissolved to give a uniform concentration.

3.3.3. PHASE III: Effect of Trypanosoma vivax Infection on Plasma Kinetics of Lactose and Effect of Intravenous Lactose on Course of Infection

i). Animals: Fourteen Zebu calves, 12-18 months old and weighing 80-90 kg were purchased and pretreated as earlier described. The animals were divided into four groups, of which two contained four calves each and the other two contained three calves each; housed and treated separately.

ii). Treatment: The various groups were treated as follows:

a) Group I (normal controls): These three calves were not infected nor were they given any lactose therapy. Five millilitres of blood were collected every morning, at 08:00 hr (local time) for analysis of haematological parameters.

b) Group II (lactose controls): The four animals in this group were not infected but each received repeated doses of 0.5 g lactose/Kg body weight thrice daily at four-hourly intervals (at the same period as when lactose was administered to group iv). Five millilitres of blood samples were collected for haemogram and plasma lactose estimation as earlier described.

c) Group III (infected controls): Each of these three animals was infected by jugular venepuncture with 5 ml of goat blood, infected with T. vivax (samaru strain), containing 11×10^6 trypanosomes. Five millilitres of blood were collected daily at 08:00 hrs (local time), to monitor parasitaemia and other haemograms. Serum for estimation of serum

free sialic acids, was prepared by allowing 5 ml of collected blood to clot and centrifuging at 3,000g.

d) group IV (experimental animals). These four animals were infected with T. vivax as described for (c) above. On day six, post infection (pi), when infection had been established (as indicated by declining PCV), lactose therapy was started and lactose was administered thrice daily as earlier stated. Lactose therapy was continued through to day 10, pi. For the first two days of lactose therapy blood samples were collected at specified (30 minutes - 60 minutes) intervals in between infusions and plasma harvested for the determination of plasma kinetics of lactose in infected animals. Blood for determination of parasitaemia and haemograms was collected in EDTA-tubes, first thing every morning at 08:00 hours (local time) every day throughout the duration of the experiment. Serum was prepared for determination of serum free sialic acids.

The various treatments were stopped on the 13th day, pi.

iii) Analysis of samples: Plasma lactose concentration and all haematological parameters were determined as earlier described. Parasitaemia was estimated by the microhaematocrit method of Woo (1969) at low parasitaemia and by the use of the new improved neubauer haemocytometre when parasitaemia was heavy. The serum free sialic acids were determined by method of Aminoff (1961).

iv) Parasites: Trypanosoma vivax, samaru strain, obtained from a patient cow at the Veterinary Teaching Hospital, A.B.U., Zaria, was identified and purity certified. A goat was infected with 5 ml of infected blood from the patient. Parasitaemia was monitored

and at peak parasitaemia the "infected controls" and "experimental" calves were infected with 5 ml of blood containing 11 x 10^6 trypanosomes, from the donor goat.

v) Analysis of data: The plasma lactose profiles were drawn and pharmacokinetic parameters calculated from semi-logarithmic plots as earlier described. Percentage daily changes in PCV, total RBC and leucocyte counts, and other parameters were calculated for each group before (D0 - D6, pi), during (D6 - D10, pi) and after (D10 - D13, pi) lactose therapy in the groups infused with lactose as follows.

$$\frac{P_{11} - P_{10}}{P_{10}} \times \frac{100}{1} = X\%$$

Where,

P_{11} = Value of parameter on any given day

P_{10} = Value of parameter the previous day

X = Daily percentage change in parameter.

For the "experimental animals the daily percentage changes in the parameters during the period of lactose infusion were corrected for the contribution of lactose, in the absence of infection, to changes in the parameter as follows:

$$Xc = Xe - X_t$$

where

Xc = corrected daily percentage change in the parameter for the experimental group, between two successive days.

X_e = uncorrected daily percentage change in the parameter for the experimental group, within the same period.

X_l = the daily percentage change in the parameter in the "lactose control" group within the same period.

All comparisons were done by the students' t-test for comparing means from two independent groups.

CHAPTER FOUR

RESULTS

4.1 Phase I: Normal Levels of Lactose in Apparently Healthy Zebu Cattle

Tables 4:1:1 and 4:1:2 give the normal plasma lactose concentrations of the thirty six adult Zebu cattle and the ten Zebu calves investigated in this phase of the experiment, respectively. In the adult cattle the plasma lactose ranged from 0.061 to 0.549 mM with a mean of 0.211 ± 0.021 mM. The plasma lactose levels in calves ranged from 0.84 to 2.10 mM; with a mean of 1.44 ± 0.12 mM, which was significantly ($P<0.005$) higher than that observed in the adult Zebu.

4.2 Phase II: Plasma Kinetics of Lactose in Apparently Healthy Zebu Calves

The profiles of plasma lactose concentrations in two of the three calves given a single intravenous dose of 0.5 g lactose per Kg. bd. wt. are presented in Figs. 4:2:1 and 4:2:2. The profiles were prepared from data in Table A2-1 (in appendix III). The plasma lactose rose very rapidly in all the calves soon after the intravenous infusion, reaching a peak 15 minutes later and by the second or third hour post infusion, the levels had fallen by a mean of approximately 58% of the maximum plasma concentration recorded after infusion. The drop in plasma lactose continued gradually thereafter, until the levels fell slightly below the pre-infusion levels though attempt was made later to normalise the levels.

Figures 4:2:3 and 4:2:4 show the plasma lactose profiles of two of the four calves administered 0.5 g lactose per kg. bd. wt. thrice at four-hourly intervals (see Table A2-2

for data). The profiles indicated that lactose did not accumulate in the plasma even with repeated dosing; the plasma lactose invariably fell to exactly or near pre-infusion levels prior to the next infusion. The pattern of peaks and the subsequent drops in the plasma lactose after infusion were comparable with those seen in the calves given single doses.

Figures 4:2:5 and 4:2:6 are semi-log plots of data in Tables A2-1 and A2-2. The plasma kinetic parameters of lactose in the calves were calculated from these plots and are presented in Table 4:2:1. The semi-log plots indicated that the plasma kinetics of lactose in Zebu calves is a biphasic process with a distinct distribution phase and a later elimination phase. The mean biological half-life ($t_{1/2}$) of lactose in Zebu calves was 5.14 $\pm$ 0.29 h with a range of 4.20 to 6.00 h. The elimination rate constant (K_e) ranged from 0.116 to 0.165 h^{-1} with a mean value of 0.138 $\pm$ 0.008 h^{-1}. The mean apparent volume of distribution (V_d) and total clearance (C_l) were, respectively, 179.15 $\pm$ 25.23 ml/kg and 24.75 $\pm$ 4.09 ml/kg/h. The C_l values ranged from 12.25 to 37.36 ml/kg/h while 105.75 to 220.00 ml/kg was the range for the V_d.

4.3 Phase III: The Effect of T. vivax Infections on Plasma Kinetics of Lactose and the Effect of Intravenous Lactose on Trypanosomal Anaemia and Other Blood Parameters in Infected Zebu Calves.

4.3.1 The effect of T. vivax infection on plasma kinetics of lactose in infected Zebu calves.

The plasma lactose profiles of the four lactose control calves (Figs. 4:3:1 and 4:3:2) confirmed the earlier observation in Figs 4:2:3 and 4:2:4, viz., that lactose does not accumulate in the plasma of healthy calves upon repeated dosing intravenously. Figs. 4:3:1 and 4:3:2 were prepared from data in Table A2-3. The plasma lactose profiles of

the infected animals given intravenous lactose infusions (Figs. 4:3:3, 4:3:4, 4:3:5 and 4:3:6) however, indicated that upon repeated intravenous dosing lactose accumulated in the plasma of the infected animals, as in most of the cases the plasma lactose levels did not fall back to the pre-infusion levels and subsequent pre-infusion levels were higher than preceding pre-infusion levels. Infected calf 2 (Fig. 4:3:4), however, was a deviant as it showed a profile similar to those observed in the uninfected, lactose infused control calves (Figs. 4:3:1 and 4:3:2) indicating that in this particular calf lactose did not accumulate as it did in the other three counterparts. The data used in preparing Figs. 4:3:3, 4:3:4, 4:3:5 and 4:3:6 and presented in Table A2-4 which also shows that the plasma lactose levels in calves 1,3 and 4 never fell back to pre-infusion levels throughout the remaining duration of the experiment even when lactose infusion was discontinued. The situation was, however, different in calf 2 of the same group as the plasma lactose in this animal returned to pre-infusion level soon after lactose infusion was stopped.

Semi-logarithmic plots of the data in Tables A2-3 and A2-4 were prepared as earlier described. On Tables 4:3:1 and 4:3:2 are presented the calculated values for the plasma kinetics parameters of lactose in the healthy, uninfected control and T. vivax infected calves, respectively. The mean $t_{\frac{1}{2}}$ for lactose in the control calves was 4.73 $\pm$ 0.19 hs, which confirmed the value observed in an earlier experiment (Table 4:2:1) and was also significantly ($p<0.005$) lower than that recorded in the T. vivax - infected animals (7.60 $\pm$ 0.15 hs). The mean K_e for the infected calves, 0.091 $\pm$ 0.002 h^{-1}, was significantly ($P<0.005$) lower than the 0.148 $\pm$ 0.006 h^{-1} recorded for the uninfected control calves. No significant differences were observed in the V_d and C_l values of the

two groups of calves. The mean V_d for the infected and uninfected groups were, respectively, 170.27 ± 22.92 ml/kg and 135.77 ± 13.66 ml/kg, while the mean C_l were 15.41 ± 1.88 ml/kg/h and 20.18 ± 2.48 ml/kg/h respectively.

4.3.2 Effect of intravenously administered lactose on T vivax - induced anaemia in Zebu calves.

Parasitaemia: Parasites were first detected in the blood of infected calves in the group not treated with lactose on day 3, pi, and the parasitaemia rose to a peak on the fifth day, pi; after which it declined until parasites could not be detected in the blood by day 7, pi through to day 9, pi. The parasites were again detected on the tenth day starting the second parasitaemic wave (Fig. 4: 3: 7).

A similar pattern was observed for the mean parasitaemia of the infected group infused with lactose (Fig. 4:3:7). However, parasites were still detectable on the seventh day, pi, through to day 10, pi, though the parasitaemia was not high enough to be counted with the counting method used in this work. The second parasitaemic wave started on the tenth day, pi. Thus parasites were detectable in this group throughout the duration of the experiment.

The parasitaemia in the uninfused group peaked on day 5,pi, while in the infused group it peaked on day 4. The peak parasitaemia in the uninfused group was significantly ($P<0.005$) higher than that of the infused group. Fig. 4:3:7 was prepared from data in Table A2-5.

Percentage Packed Cell Volume: Figure 4:3:8 shows the profiles of mean PCV for all four groups prepared from data in Tables A2-6 and A2-7. The profiles of PCV for individual calves in the lactose - infused infected group are presented in Figs. 4:3:10, 4:3:11, 4:3:12 and 4:3:13 to show the responses of the PCV in individual animals to lactose therapy. The fluctuations in PCV of normal healthy uninfused calves (Fig. 4:3:8) were within a narrow insignificant range. Though the PCV changes in the healthy group that was infused with lactose were initially insignificant, a mean PCV drop of 4.3 $\pm$ 3.2% (Table 4:3:3) was observed during the period of lactose infusion.

Table 4:3:4 shows the daily mean percentage changes in PCV as well as the mean cumulative percentage changes (over the indicated periods) for the T. vivax - infected groups. The values for the lactose - infused group during the period of infusion were corrected for the contribution of lactose infusion to the changes in PCV as described earlier in chapter three. The drop in mean PCV in both T. vivax - infected groups coincided with the decline in the first parasitaemic wave. Decline in PCV appeared to have started earlier in the lactose - infused group. The PCV in the lactose - infused group by day 6. pi, had dropped by a mean of 19.5 $\pm$ 4.8% which was not significantly different from the 13.3 $\pm$ 5.1% mean drop observed in the infected, uninfused group during the same period.

Lactose infusion was started in the lactose-infused group on day 6.pi. when the disease had established as indicated by declining PCV, and discontinued on day 10. pi. On day 7.pi (a day after lactose infusion commenced) the PCV of the lactose-infused group actually recovered by a mean of 1.7 $\pm$ 4.9% as compared to the value before

infusion started, while in the uninfused group the drop in PCV continued with a further drop of 6.3 ± 3.6%. The difference between these values was significant (P<O.05). The PCV in the infused group showed a slight mean drop of 3.2 ± 3.3% on day 8,pi, which was significantly (P<0.005) smaller than the mean drop of 17.2 ± 4.9% seen in the uninfused group; even though parasites were still detectable in the blood stream of the infused group during this period and were undetectable in the uninfused group. On day 9, pi, the uninfused group showed a mean increase in PCV of 18.6 ± 4.6% while the infused group showed a mean increase of only 5.5 ± 2.6% which was significantly (P<0.005) smaller. It is pertinent, however, to mention here that parasitaemia was still undetectable in the uninfused group on this day while parasites were still detected in the blood of the infused animals. The percentage changes in PCV on day 10, pi, for both groups were not statistically different. The cumulative percentage change in PCV for the groups between days 6 and 10, pi, (period of lactose infusion) indicated that there was a net decrease of 7.5 ± 2.9.% in the uninfused group and a small but significant (P<0.025) increase of 0.012 ± 3.2% in the PCV of the infused group.

After lactose infusion was discontinued the PCV in the infused group continued to drop by a further mean of 5.4 ± 2.3% on day 11, pi, which was significantly (P<0.005) different from the 2.6 ± 2.6% mean increase observed in the uninfused group on the same day. On day 12,pi, the uninfused group showed a further mean increase in PCV of 24.9 ± 9.7%, while the infused group showed a mean increase of only 1.6 ± 2.7% which was significantly (P<0.005) smaller than that observed in the uninfused group.

It should be pointed out that the cumulative change in PCV of calf 2 of the infused group over the period of lactose infusion was similar to those of the infected, uninfused calves (Table A2-14) but quite different from those of its counterparts. It should also be noted that it was in this calf that the infused lactose did not stay in the blood even upon repeated dosing.

4.3.3 Effect of T. vivax infection and Intravenously Administered Lactose on other Blood Parameters.

For the purpose of this work anaemia during bovine trypanosomiasis was established by monitoring the PCV of infected animals. However, to demonstrate that the experimental infection, employed in this investigation, followed the established course of T. vivax - infection in cattle; other blood parameters were also monitored; all the results of which are presented in the appendix; with the exception of the serum free sialic acid concentrations.

Total Red Blood Cell Count: The profiles of the mean total RBC counts for all four groups, for the duration of the experiment, are presented in Fig A2-1 (see appendix). Table A2-16 (in appendix) gives the daily mean percentage changes in total RBC count as well as mean cumulative percentage changes over the specified periods. The results indicate that the total RBC count in all four groups of calves followed a pattern similar to what was observed for the PCV in the corresponding groups.

Total Leucocyte Count: The changes in mean total leucocyte count in all four groups during the course of the experiment are presented in Fig A2-2 (in appendix), prepared

from Tables A2-10 and A2-11. In the T. vivax infected groups there was a steady fall as the infection progressed, with an attempt to normalise at the later stages of the infection. This decrease in total leucocytes was not observed in the other two uninfected groups (see Table A2-20).

During the period before infusion was started the mean total leucocyte count in the infected, uninfused group dropped by 20.2 ± 5.0%, which was, however, not significantly different from the 17.1 ± 16.7% drop recorded for the infected, infused group. During the period of lactose infusion while a 25.1 ± 26.8% recovery in the total leucocyte count was recorded in the lactose-infused, infected group a further drop of 7.3 ± 2.3% was recorded in the other infected group. There was a significant ($P<0.05$) difference in these responses. The recoveries in total leucocytes observed in both infected groups (28.3 ± 14.1% and 13.3 ± 8.6% for uninfused and infused groups respectively, were not significantly different, during the period after lactose was discontinued.

Differential Leucocyte Count: The profiles of the mean absolute lymphocytes, neutrophil, monocyte and eosinophil counts for the two infected groups are presented in Fig. A2-3. The lymphopenia in the infused group (Table A2-20) was significantly ($P<0.005$) more pronounced than in the uninfused group. The neutropenia and eosinopenia observed in the two infected group were at the same level. Monocytosis was however observed in the lactose - infused group but not in the uninfused group. (Table A2-20).

Total Plasma Proteins: Figure A2-4 (in appendix III) shows the profiles of mean plasma total proteins in all four groups; prepared from Table A2-12. In Table A2-20 can be seen that T. vivax - infection caused a significant drop ($P<0.005$) in the plasma total proteins of calves; but lactose infusion not only prevented that fall but actually caused a significant ($P<0.005$) increase in this parameter.

Serum Free Sialic Acids: The profiles of the mean serum free sialic acids for the two infected groups are presented in Fig 4:3:14 (prepared from data in Table A2-13). The increase in serum free sialic acids coincided with the rise in parasitaemia and declined with the parasitaemia. The levels of serum free sialic acids in the uninfused group returned to the pre-infection value on the days when parasites could no longer be detected in the blood. In the lactose-infused group, however, the level never returned to the pre-infection value throughout the period of the experiment.

Table 4:1:1 Normal Levels of Plasma Lactose in Apparently Healthy Adult Zebu Cattle

Animal Number	Plasma Lactose concentrations (mM)	Aninal Number	Plasma Lactose concentration (mM)
1	0.122	19	0.305
2	0.549	20	0.275
3	0.397	21	0.061
4	0.397	22	0.122
5	0.336	23	0.214
6	0.366	24	0.305
7	0.183	25	0.397
8	0.305	26	0.244
9	0.122	27	0.092
10	0.122	28	0.061
11	0.275	29	0.183
12	0.183	30	0.366
13	0.122	31	0.092
14	0.061	32	0.061
15	0.244	33	0.122
16	0.061	34	0.092
17	0.061	35	0.183
18	0.153	36	0.366
MEAN ± SEM		0.211 ± 0.021	

Each value is the average of a triplicate determination.

Table 4:1:2. Normal Levels of Plasma Lactose in Apparently Healthy Zebu Calves

ANIMAL NUMBER	*PLASMA LACTOSE CONCENTRATIONS (mM)*
1	1.25
2	0.93
3	2.10
4	1.57
5	1.32
6	1.50
7	1.62
8	0.84
9	1.74
10	1.53
MEAN ± SEM	1.44 ± 0.12

Each value is the average of a triplicate determination.

Table 4:2:1. Calculated Plasma Kinetics Parameters of Lactose in Apparently Healthy Zebu Calves.

CALF NUMBER	$t_{\frac{1}{2}}$ (h)	ke (h^{-1})	V_d (ml/kg)	C_T (ml/kg/h)
1	6.00	0.116	221.18	25.53
2	5.70	0.122	220.00	26.78
3	6.00	0.116	105.75	12.25
4	4.20	0.165	225.29	37.36
5	4.60	0.151	206.10	30.98
6	5.15	0.135	147.50	19.88
7	4.35	0.159	128.24	20.47
MEAN	5.14	0.138	179.15	24.75
± SEM	± 0.29	± 0.008	± 25.23	± 4.09

Values were calculated from semi-logarithmic plots of data in Tables A2-1 and A2-2.

$t_{\frac{1}{2}}$ = Biological half-life for lactose

K_e = Elimination rate constant for lactose.

V_d = Apparent volume of distributions for lactose

C_T = Total clearance of lactose.

Table 4.3:1. Calculated Plasma Kinetics Parameters for Lactose in Uninfected Control Calves.

CALF NUMBER	$t_{1/2}$ (h)	K_e (h^{-1})	V_d (ml/kg)	C_l (ml/kg/h)
1	4.60	0.151	173.56	26.21
2	5.25	0.132	110.38	14.63
3	4.35	0.159	136.25	21.75
4	4.70	0.148	122.88	18.13
MEAN	4.73	0.148	135.77	20.18
± SEM	± 0.19	± 0.006	± 13.66	± 2.48

Values were calculated from semi-logarithmic plots of data in Table A2-3

Table 4:3:2. Calculated Plasma Kinetics Parameters for Lactose in T. vivax - infected Calves.

CALF NUMBER	$t_{1/2}$ (h)	K_e (h^{-1})	V_d (ml/kg)	C_l (ml/kg/h)
1	7.50	0.092	151.11	14.00
2	7.30	0.095	114.70	10.84
3	7.60	0.091	213.04	19.35
4	8.00	0.087	203.22	17.44
MEAN	7.60	0.091	170.27	15.41
± SEM	± 0.15	± 0.002	± 22.92	± 1.88

Values were calculated from semi-logarithmic plots of data in Table A2-4

Table 4:3:3. The Daily Mean Percentage Changes and Cumulative Mean Percentage Changes in PCV and Total RBC Counts of Healthy, Lactose-Infused Control Zebu Calves over the Period of Lactose Infusion.

	POST - INFUSION TIME (DAYS)				
	1	2	3	4	CUMULATIVEPERCENTAGE CHANGE*
MEAN PERCENTAGE CHANGE IN PCV (n = 4)	-0.75	-5.70	+1.30	-0.70	-4.30 ±3.2
MEAN PERCENTAGE CHANGE IN TOTAL RBC COUNTS (n = 4)	-0.90	-8.10	+4.20	+0.50	-4.20 ± 3.2

Positive and negative signs indicate increases and decreases, respectively.

* These values represent the net percentage changes for all the animals over the period considered.

Table 4:3:4. The Daily Mean Percentage Changes and Mean Cummulative Percentage Changes in PCV of T Vivax-Infected Zebu Calves Before, During and After Lactose Infusion.

	PERCENTAGE CHANGE IN PCV BEFORE LACTOSE INFUSION (MEAN ± SEM)						PERCENTAGE CHANGE IN PCV DURING LACTOSE INFUSION (MEAN ± SEM)					PERCENTAGE CHANGE IN PCV AFTER LACTOSE INFUSION WAS STOPPED (MEAN ± SEM)			
Post-Infection Time (Days)	2	3	4	5	6	Cup-erc	7	8	9	10	Cup-erc	11	12	13	Cuperc
Infected, uninfused Group (n=3)	+8.3 ±1.7[a]	+4.5 ±2.6	-3.3 ±1.6[c]	-3.3 ±4.4	-19.5 ±0.5	-13.3 ±5.1	-6.3 ±3.6[a]	-17.2 ±4.9[c]	+18.6 ±4.6[c]	-1.8 ±4.5	-7.5 ±2.9	-2.6 ±2.6[c]	+24.9 ±9.7	-19.1 ±2.0[c]	+8.4 ±12.0[c]
Infected, Lactose-infused Group (n=4)	-3.0 ±1.0[c] (p<0.0005)	-4.0 ±1.6	+5.6 ±3.4[a] (p<0.005)	-8.0 ±2.3	-10.0 ±2.9	-19.5 ±4.8	+1.7 ±4.9[a] (p<0.05)	-3.2 ±3.3[b] (p<0.005)	+5.5 ±2.6[c] (p<0.005)	-4.0 ±3.2	+0.01 ±3.2[b] (p<0.025)	-5.4 ±2.3[c] (p<0.005)	+1.6 ±2.7[b] (p<0.005)	-9.4 ±2.3[b] (p<0.005)	-13.2 ±2.2[c] (P<0.01)

Cuperc = Mean Cummulative Percentage change in PCV over the period indicated. Positive and negative signs indicate increases and decreases, respectively. Comparison was done down the columns and values with different superscripts are satistically different at the confidence limits indicated.

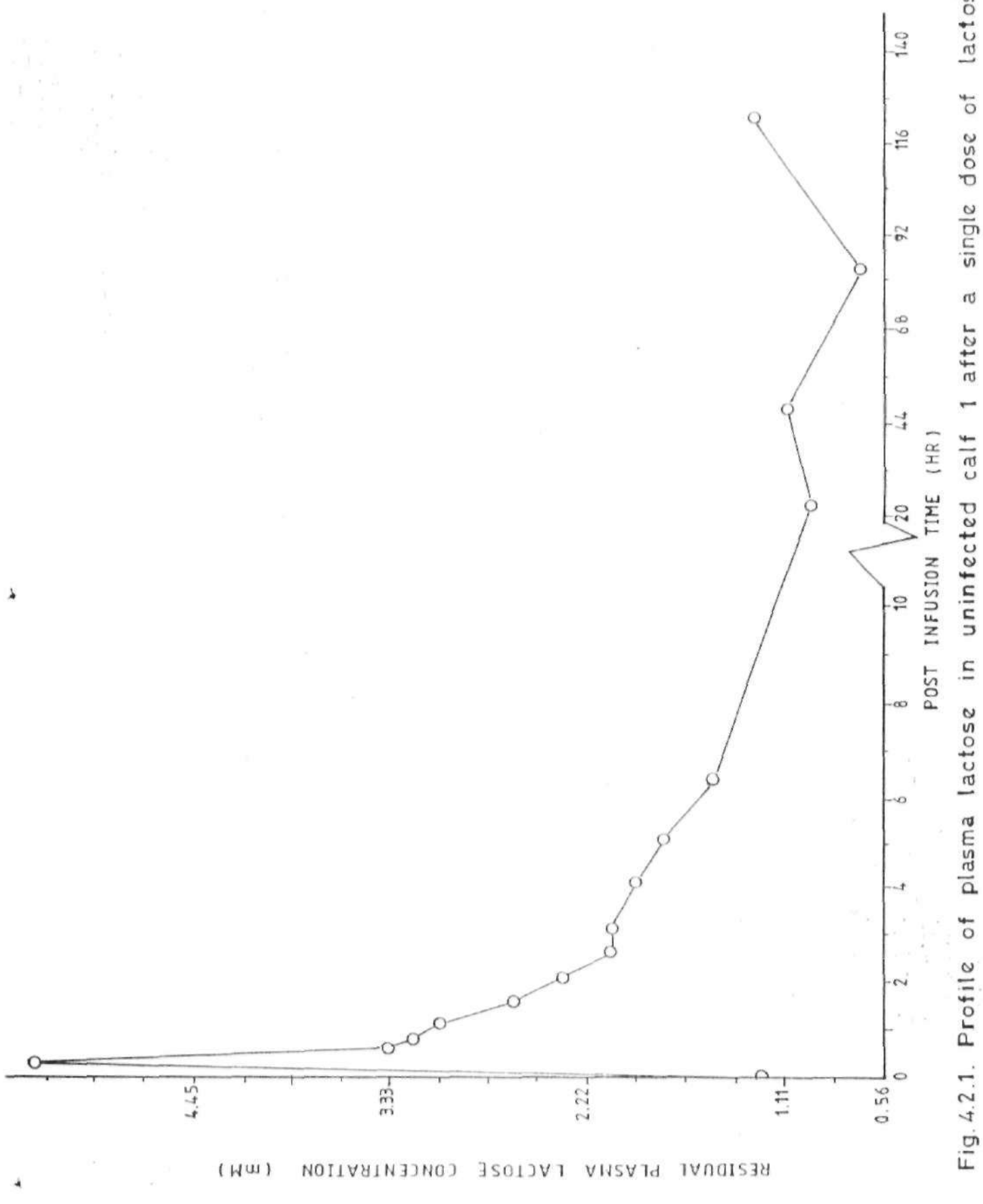


Fig. 4.2.1. Profile of plasma lactose in uninfected c

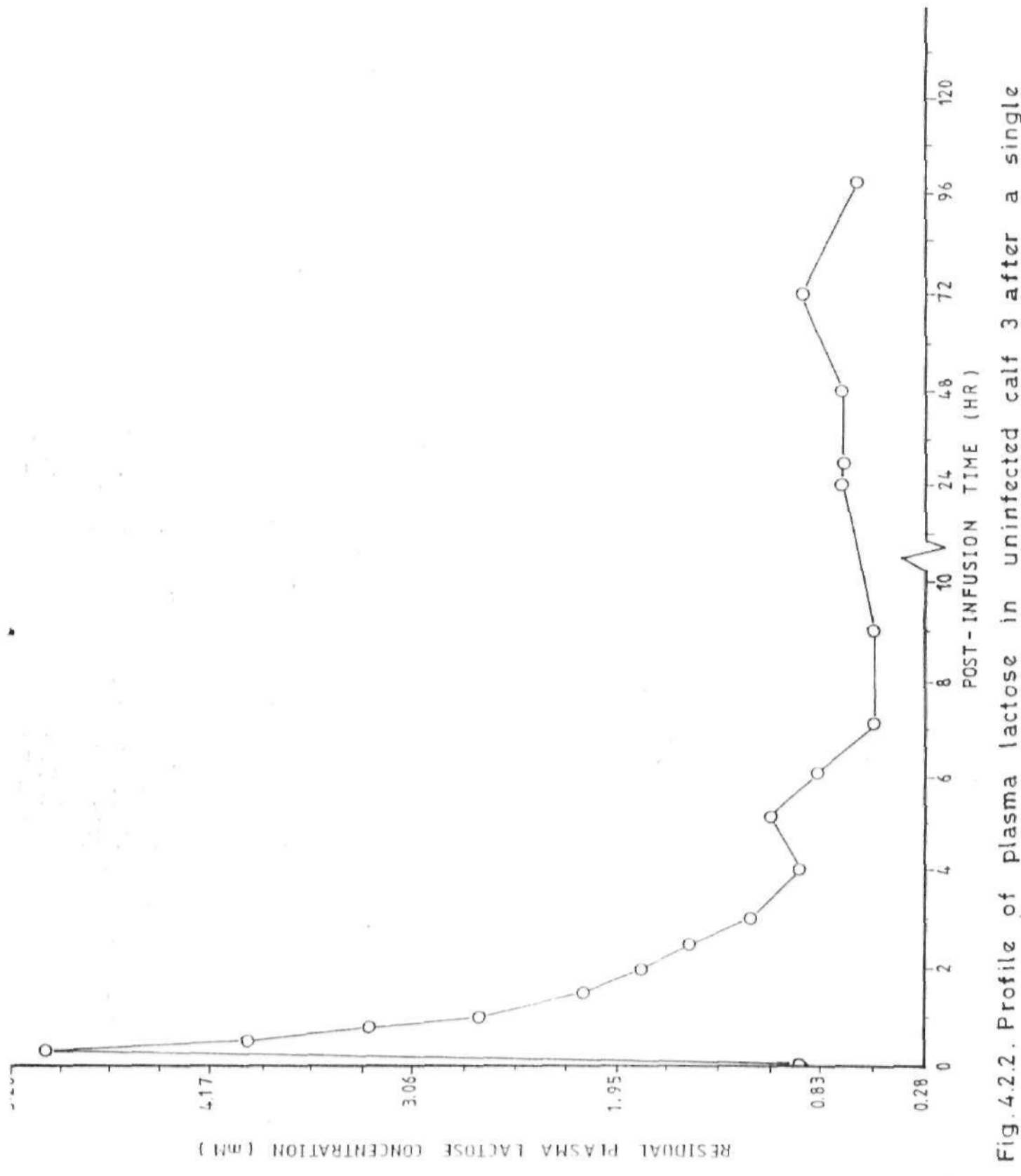


Fig.4.2.2. Profile of plasma lactose in uninfected calf 3 after a single dose of lactose.

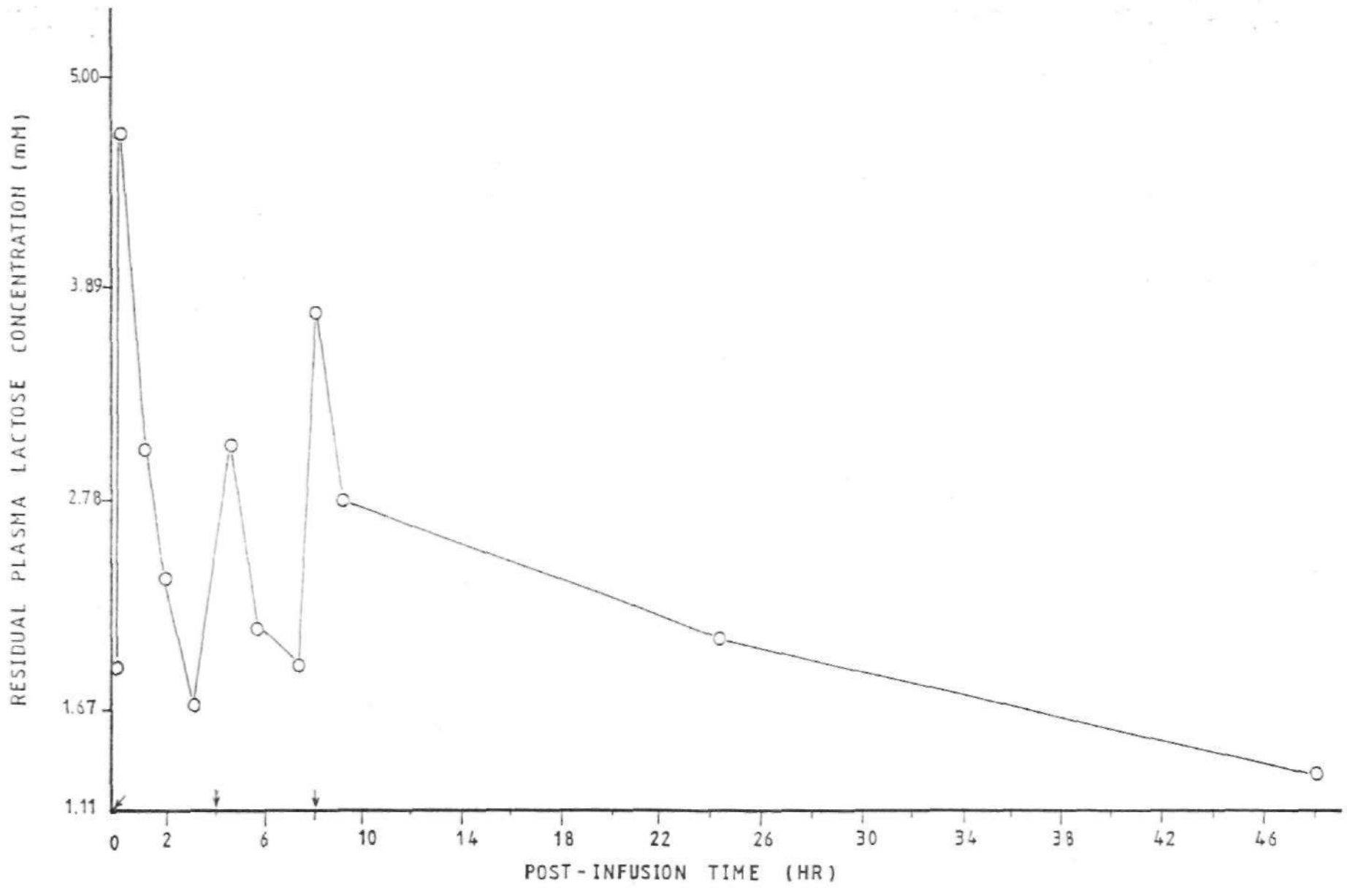


Fig. 4.2.3. Profile of plasma lactose in uninfected calf 2 after three doses of lactose at intervals of four hours.

↑ indicates points of infusions

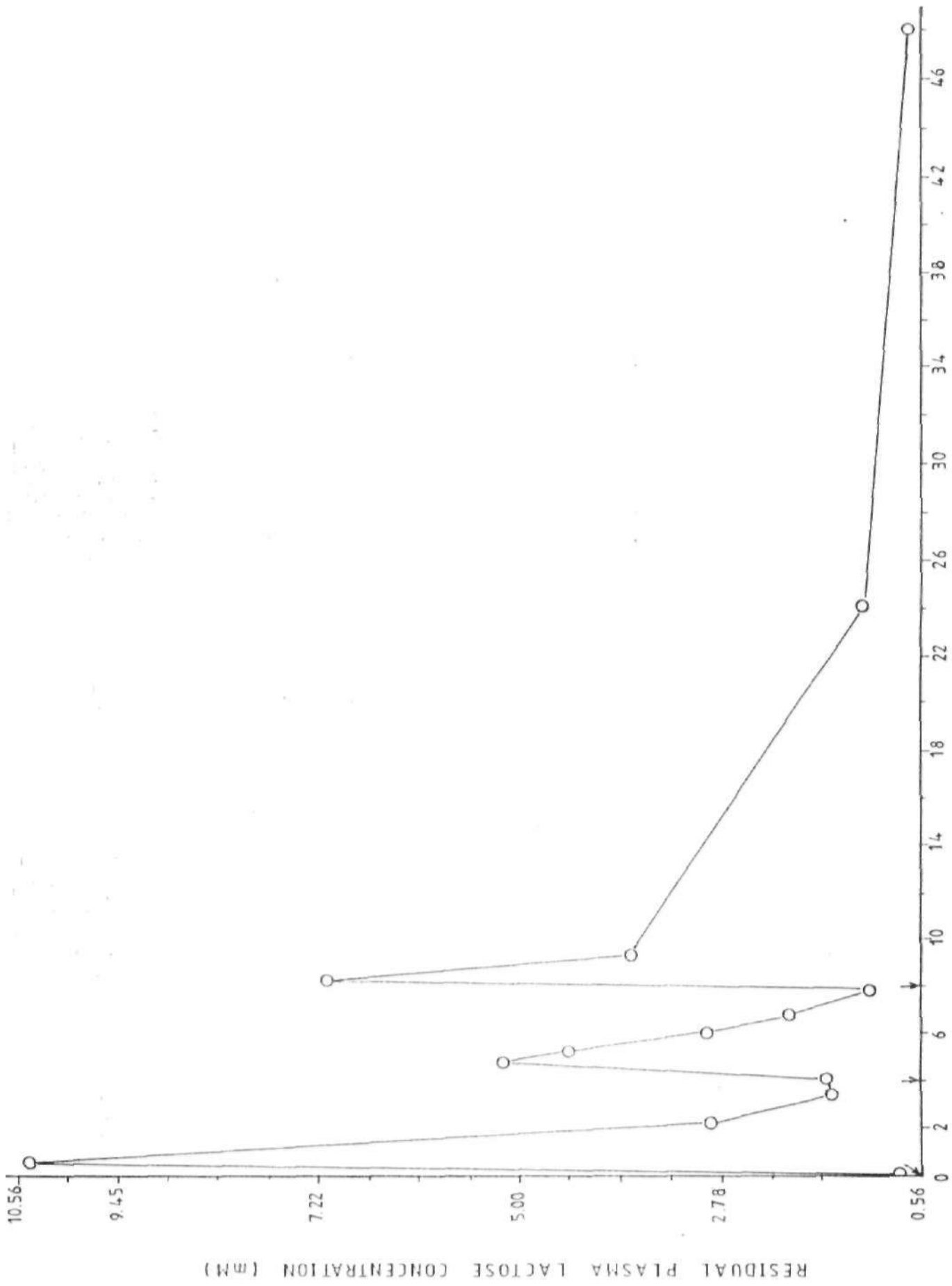


Fig. 4.2.4. Profile of plasma lactose in uninfected calf 4 after three doses of lactose at intervals of four hours.

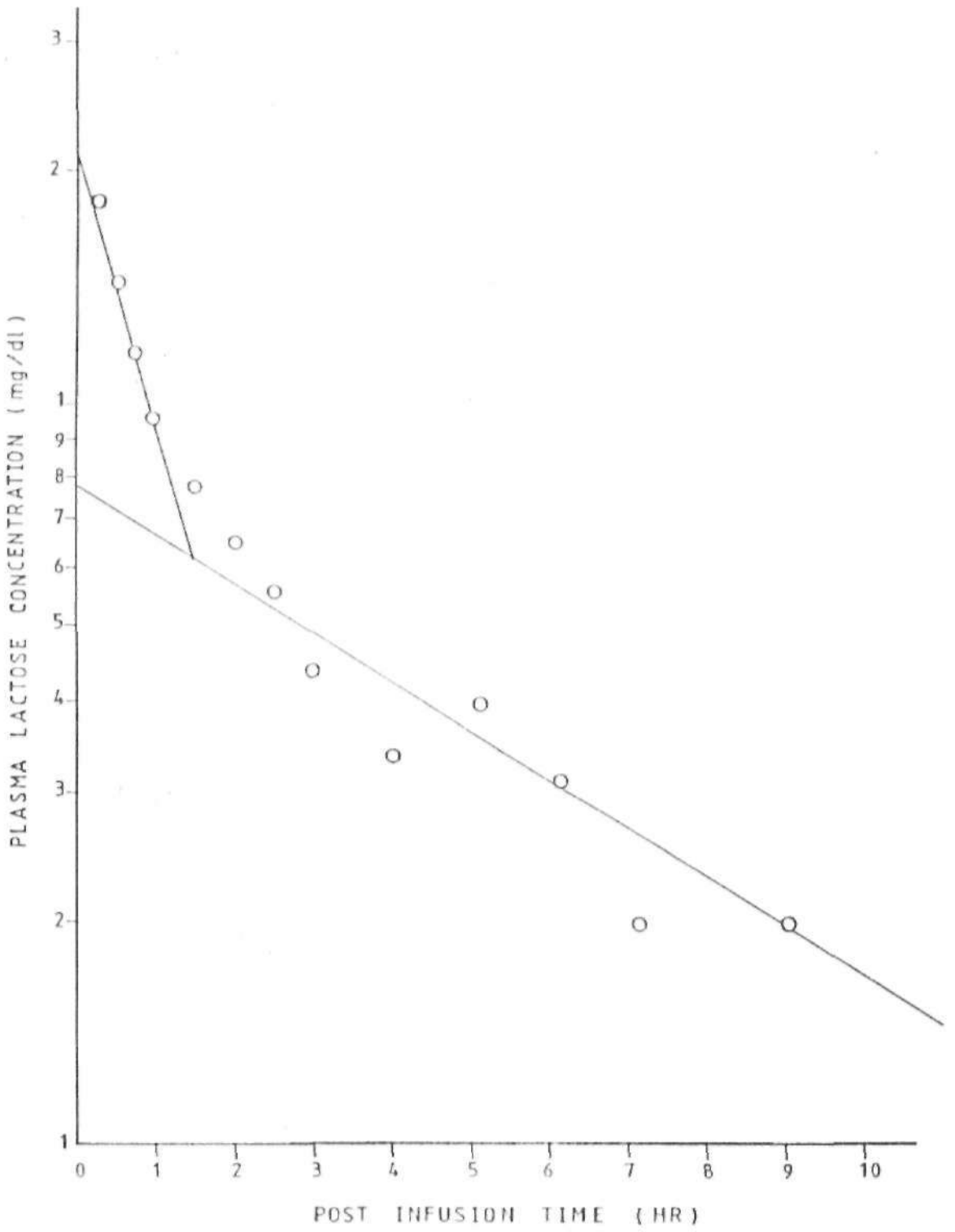


Fig. 4.2.5. Semi-log plot of plasma lactose concentrations of a healthy zebu calf.

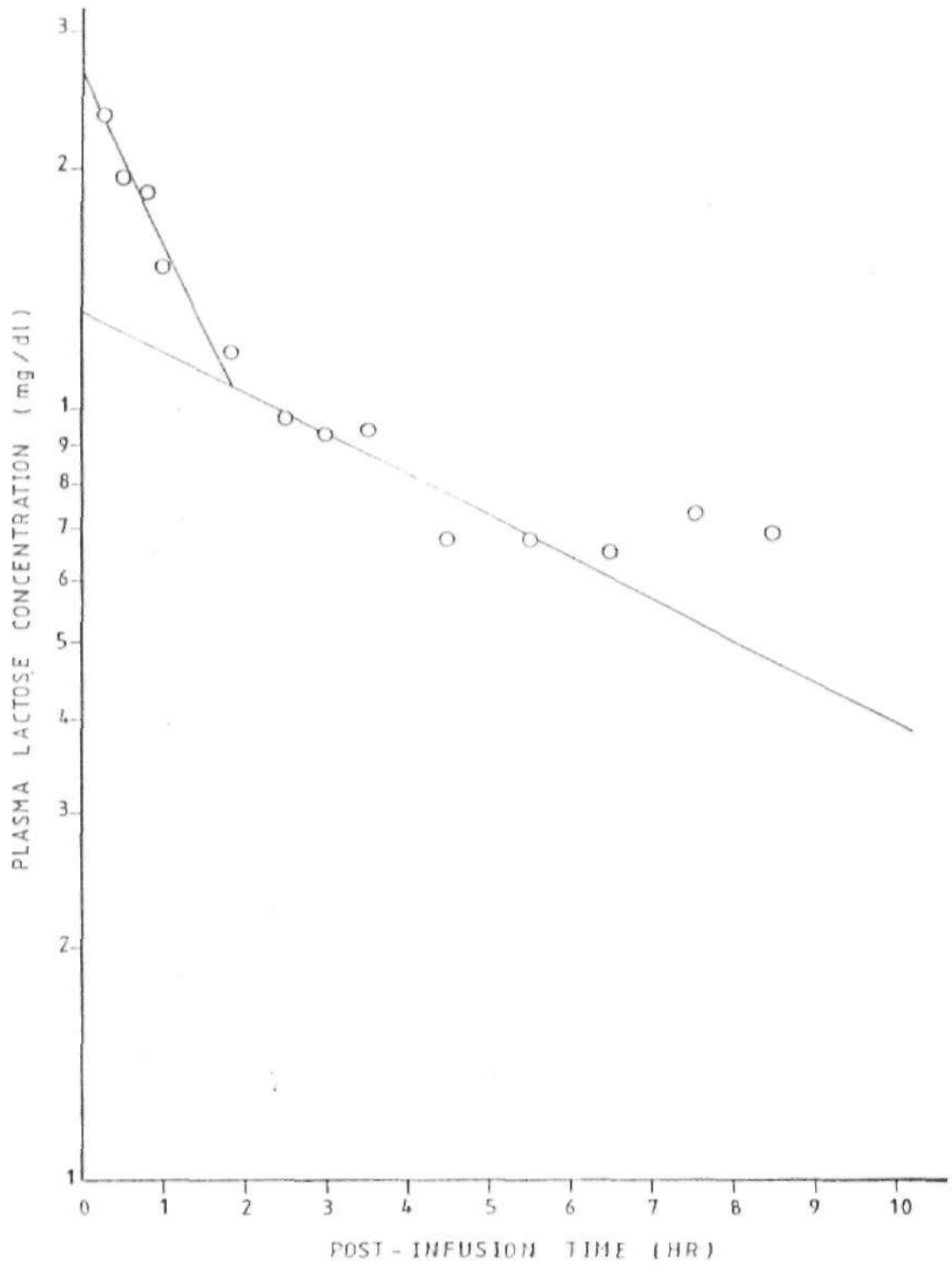


Fig. 4.2.6. Semi-log plot of plasma lactose concentrations of a healthy zebu calf.

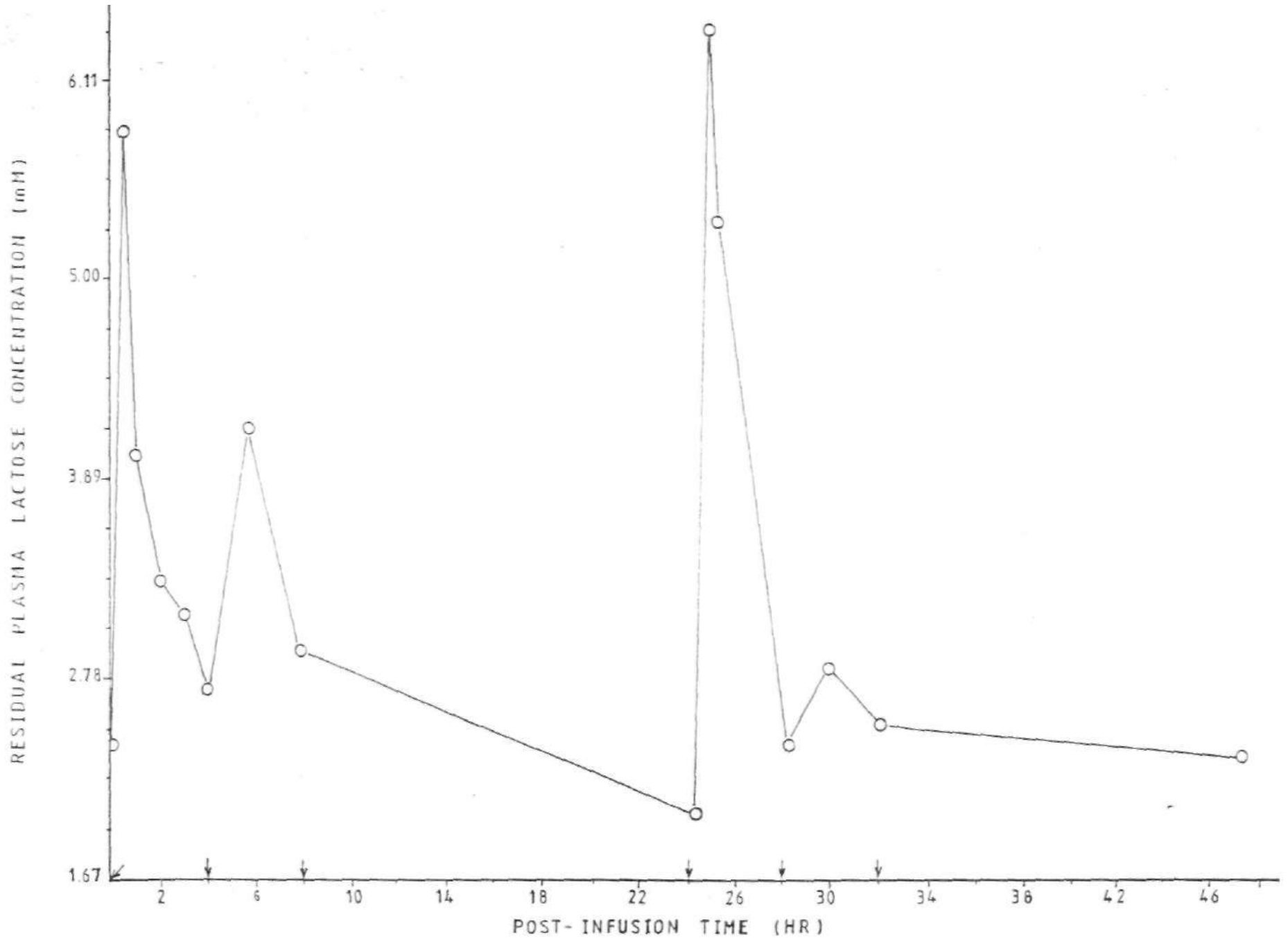


Fig. 4.3.1. Profile of plasma lactose in uninfected control calf 3 after three four hourly doses of lactose daily .

↓ indicate point of infusion

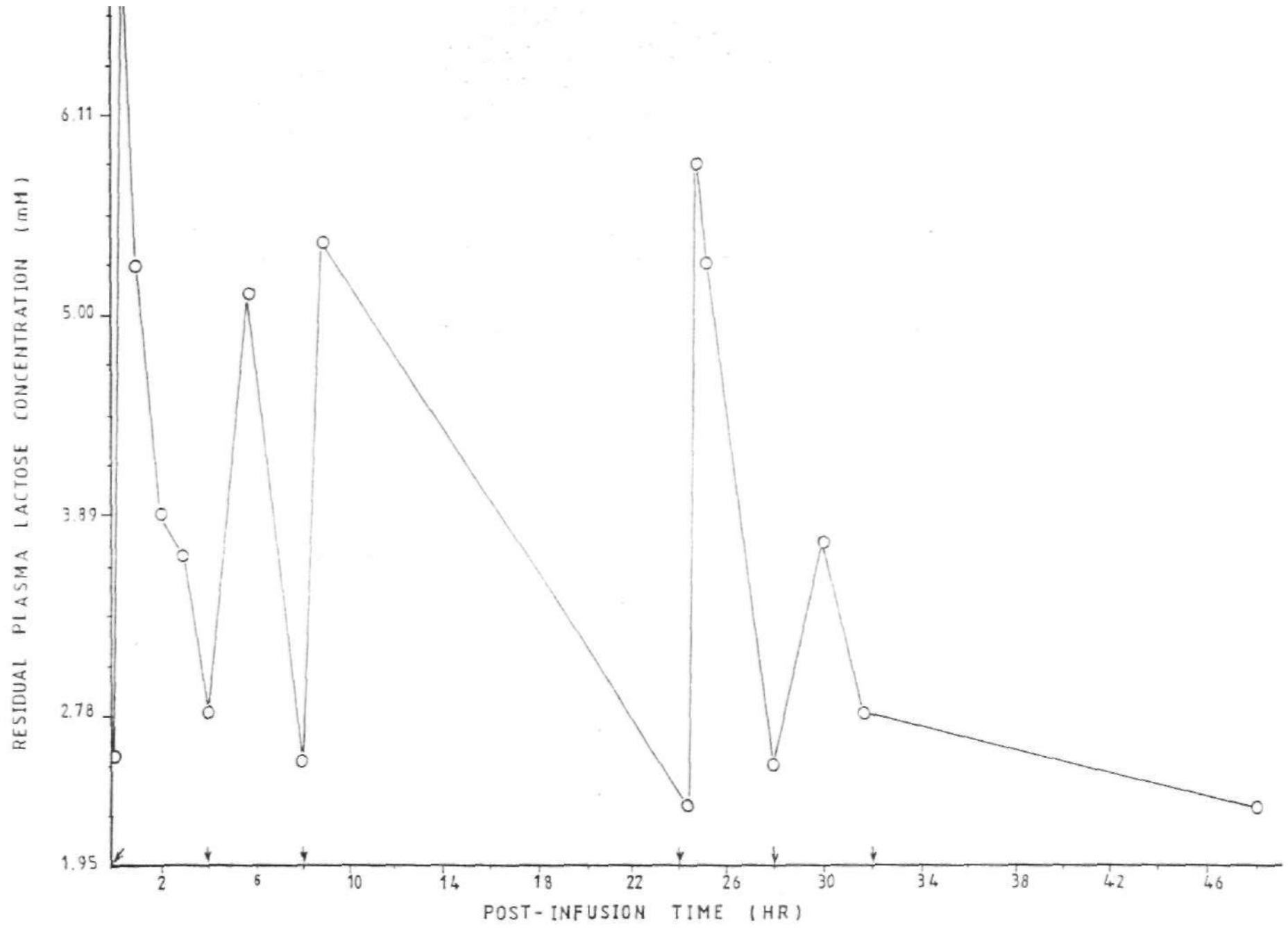


Fig. 4.3.2. Profile of plasma lactose in uninfected control calf 4 after three four hourly doses of lactose daily.

↓ indicate point of infusion

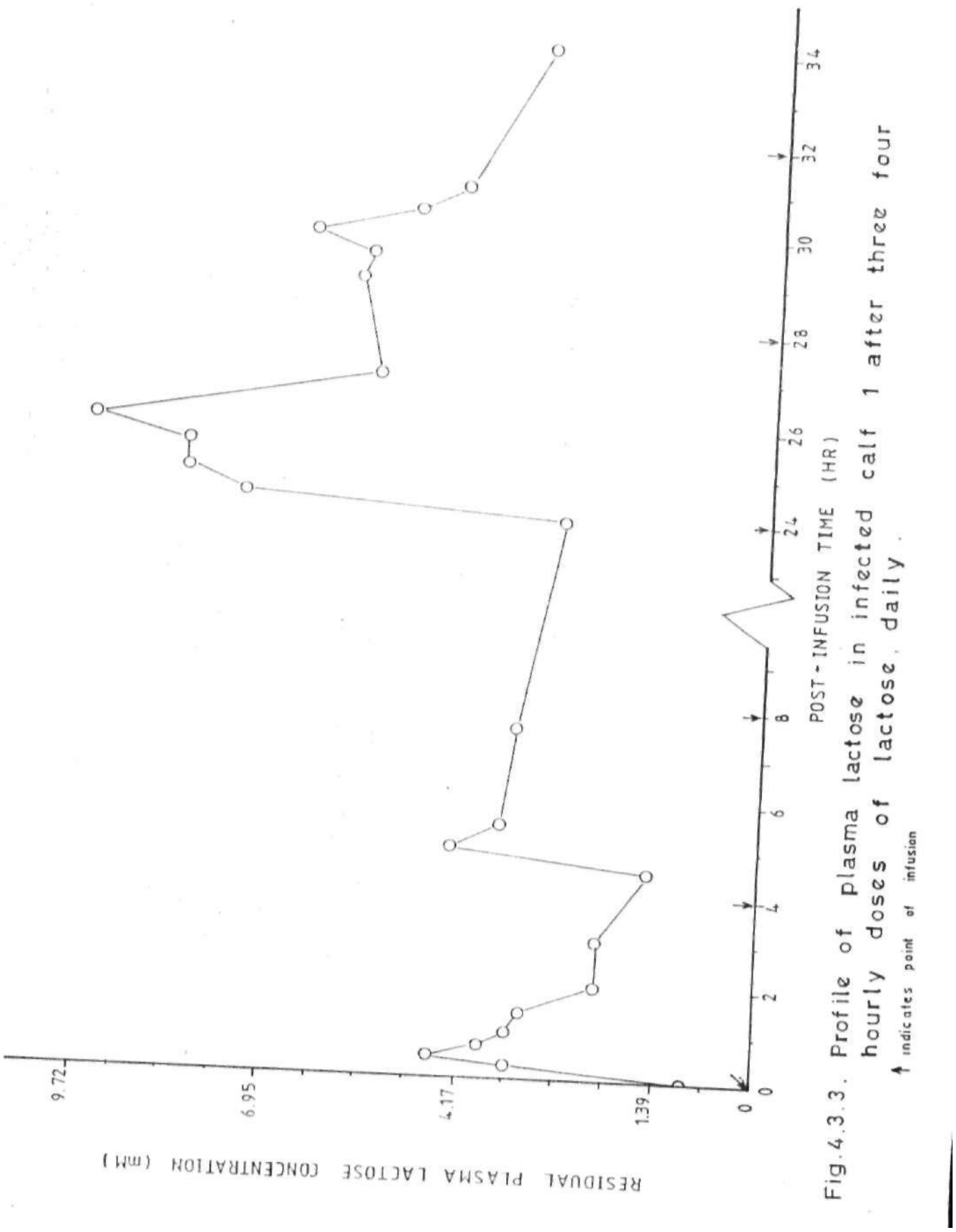


Fig.4.3.3. Profile of plasma lactose in infected calf 1 after three four hourly doses of lactose, daily.

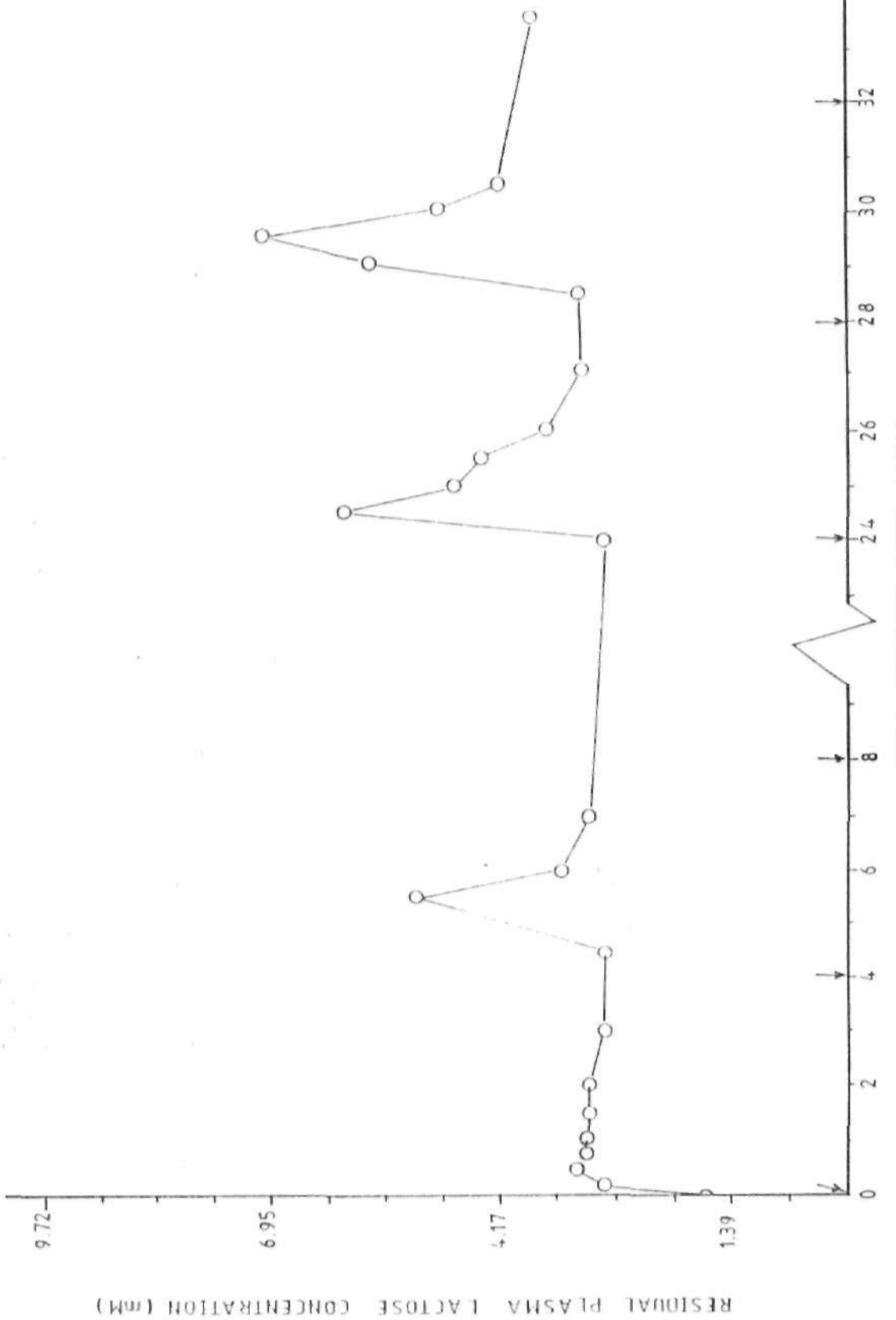


Fig. 4.3.5. Profile of plasma lactose in infected calf 3 after three four hourly doses of lactose daily.

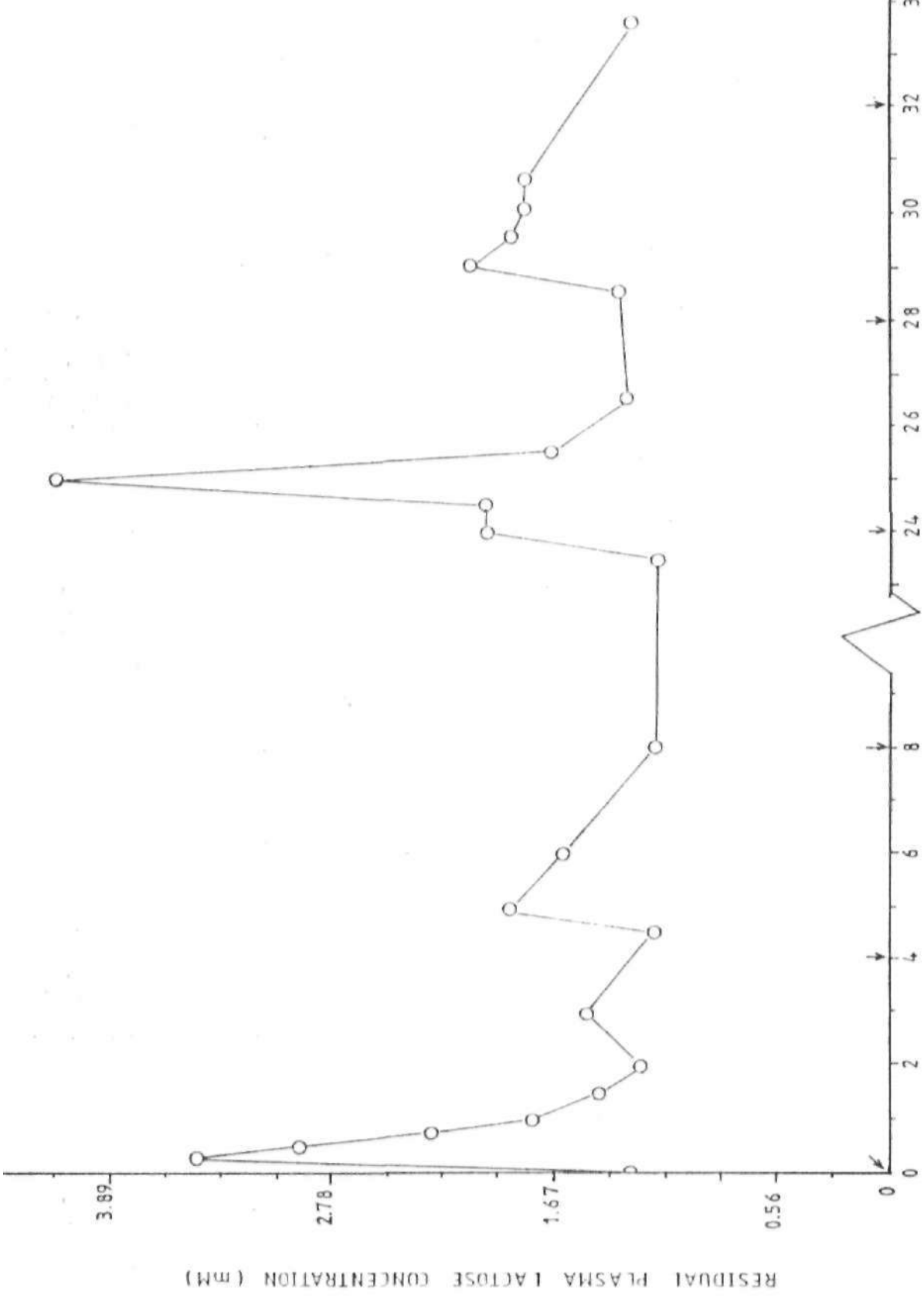


Fig. 4.3.4. Profile of plasma lactose in infected calf 2 after three four hourly doses of lactose daily.

↓ indicate point of infusion

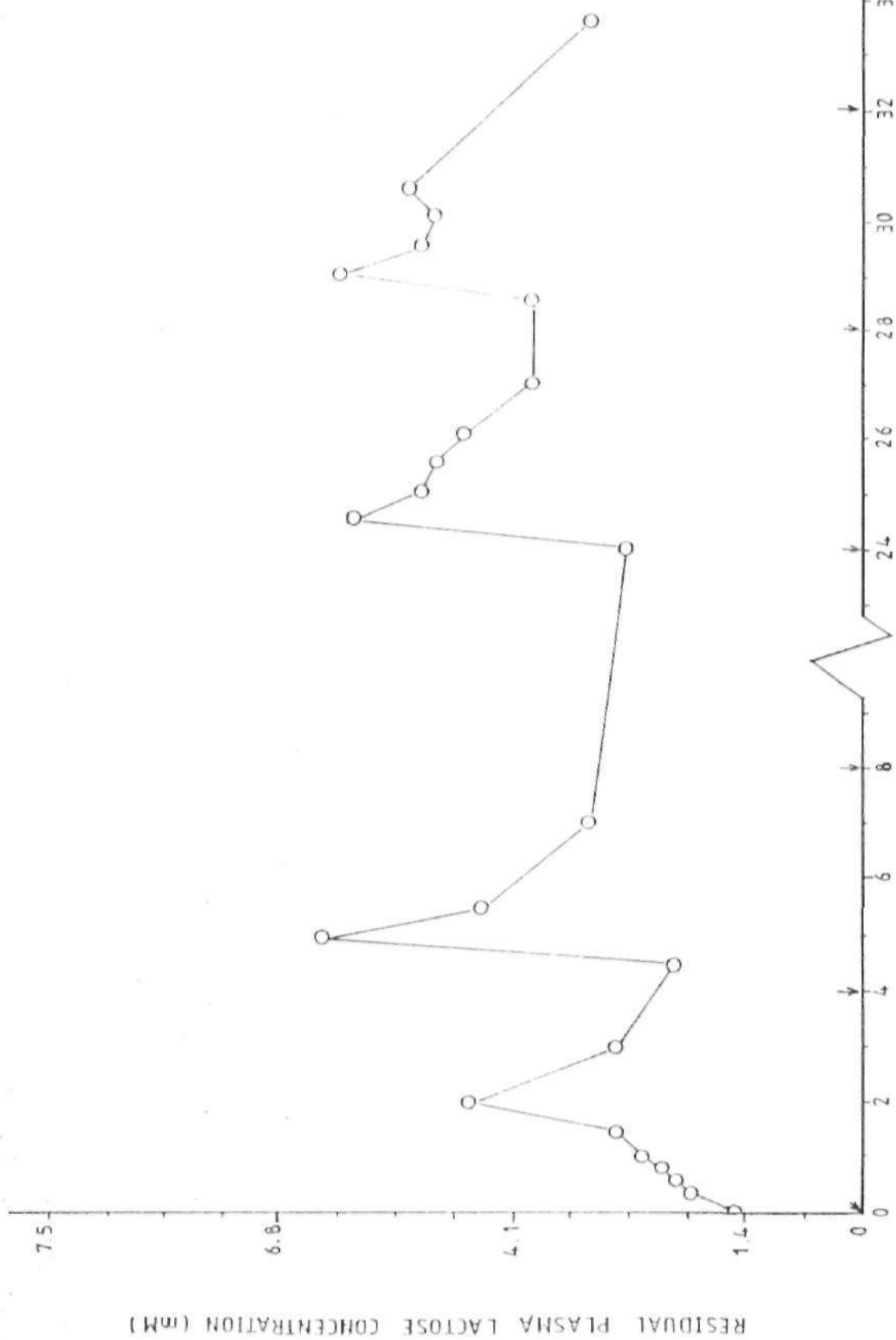


Fig.4.3.6. Profile of plasma lactose in infected calf 4 after three four hourly doses of lactose daily.

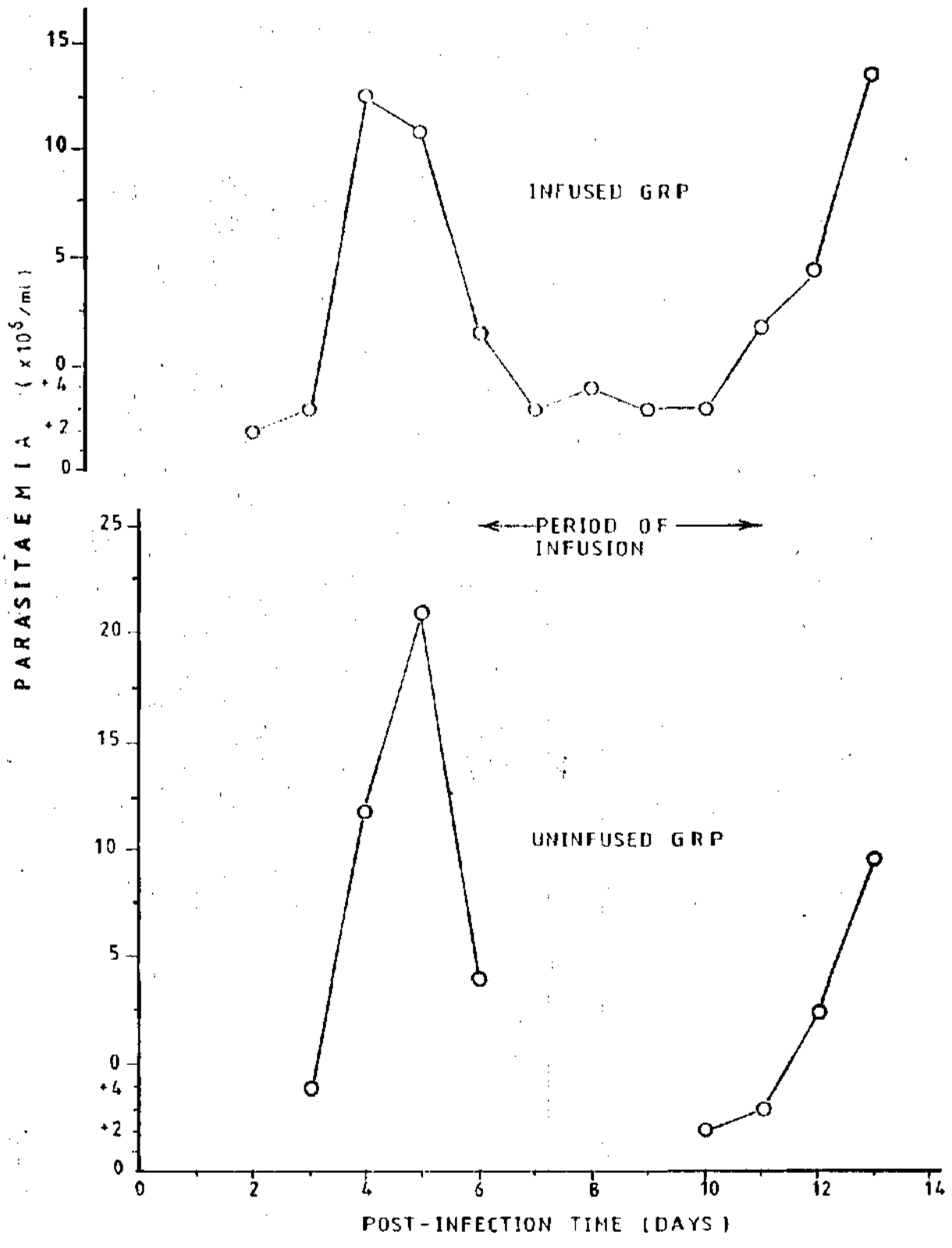


Fig. 4.3.7. Profiles of mean parasitaemia in *T. vivax*-infected zebu calves infused and-uninfused with lactose.

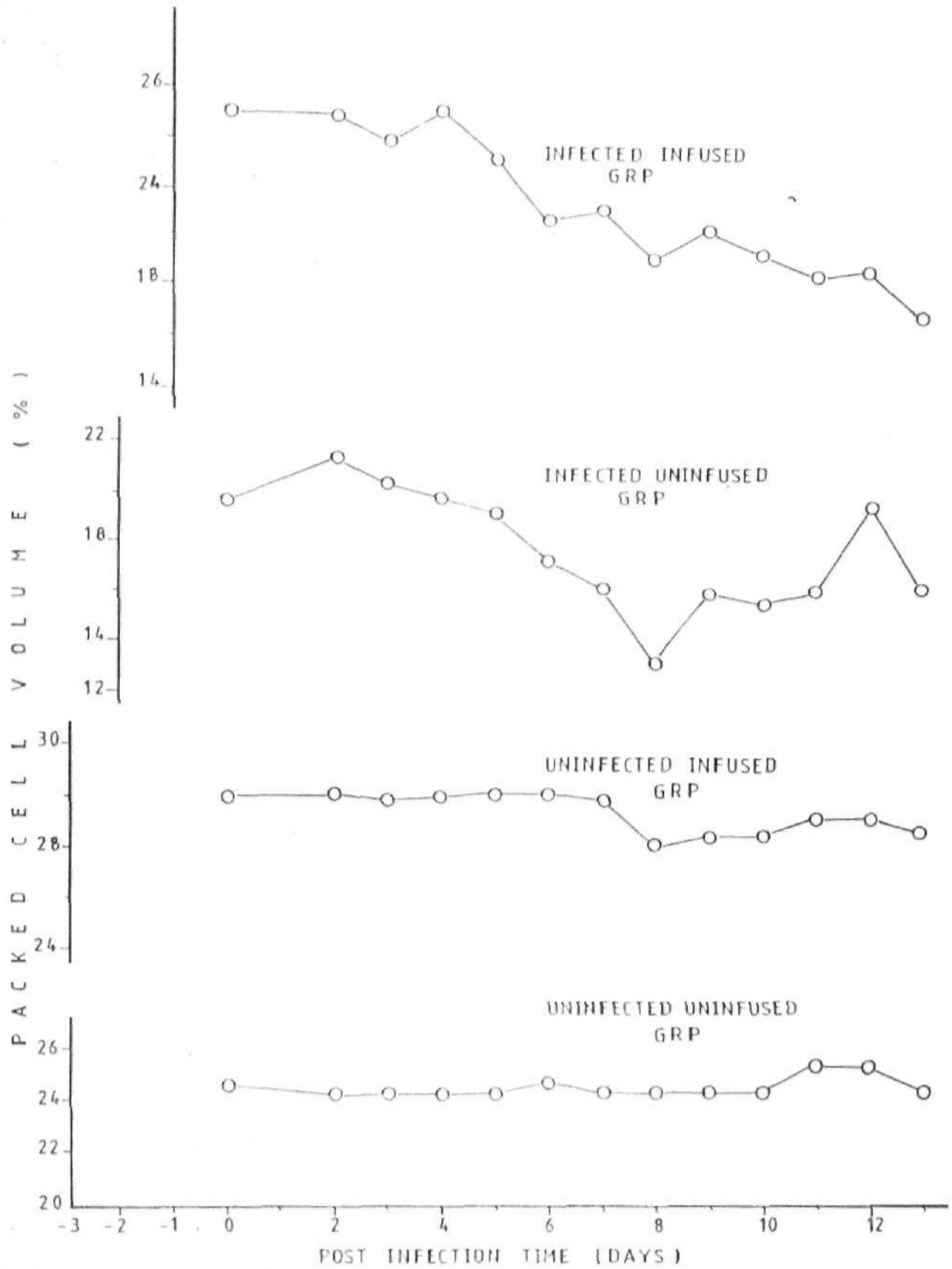


Fig. 4.3.8. Profiles of mean PCV for all groups.

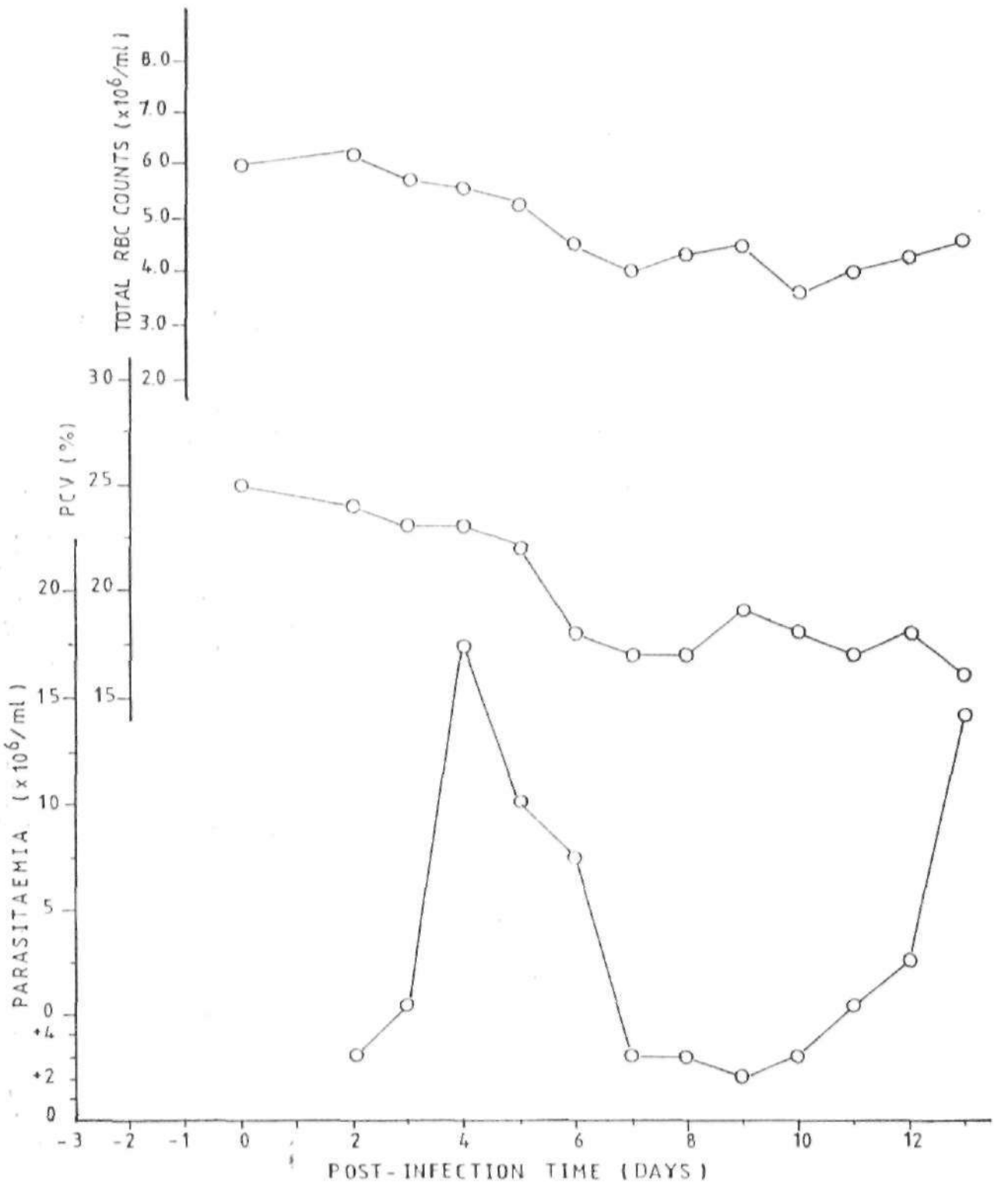


Fig. 4.3.9. Profiles of parasitaemia, PCV and total RBC counts for lactose-infused T. vivax-infected calf 1.

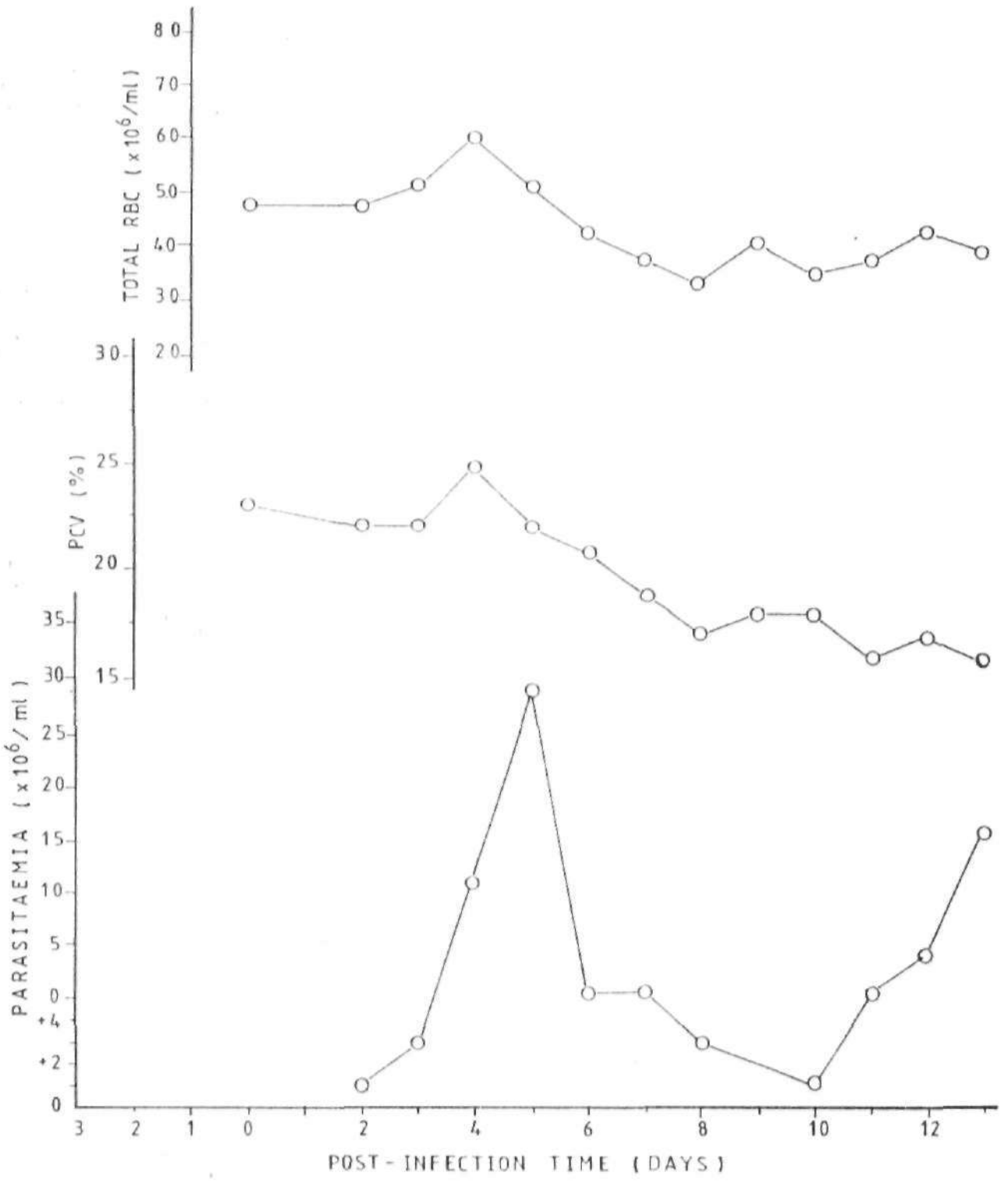


Fig. 4.3.10. Profiles of parasitaemia, PCV and total RBC counts for lactose-infused *I. vivax*-infected calf 2.

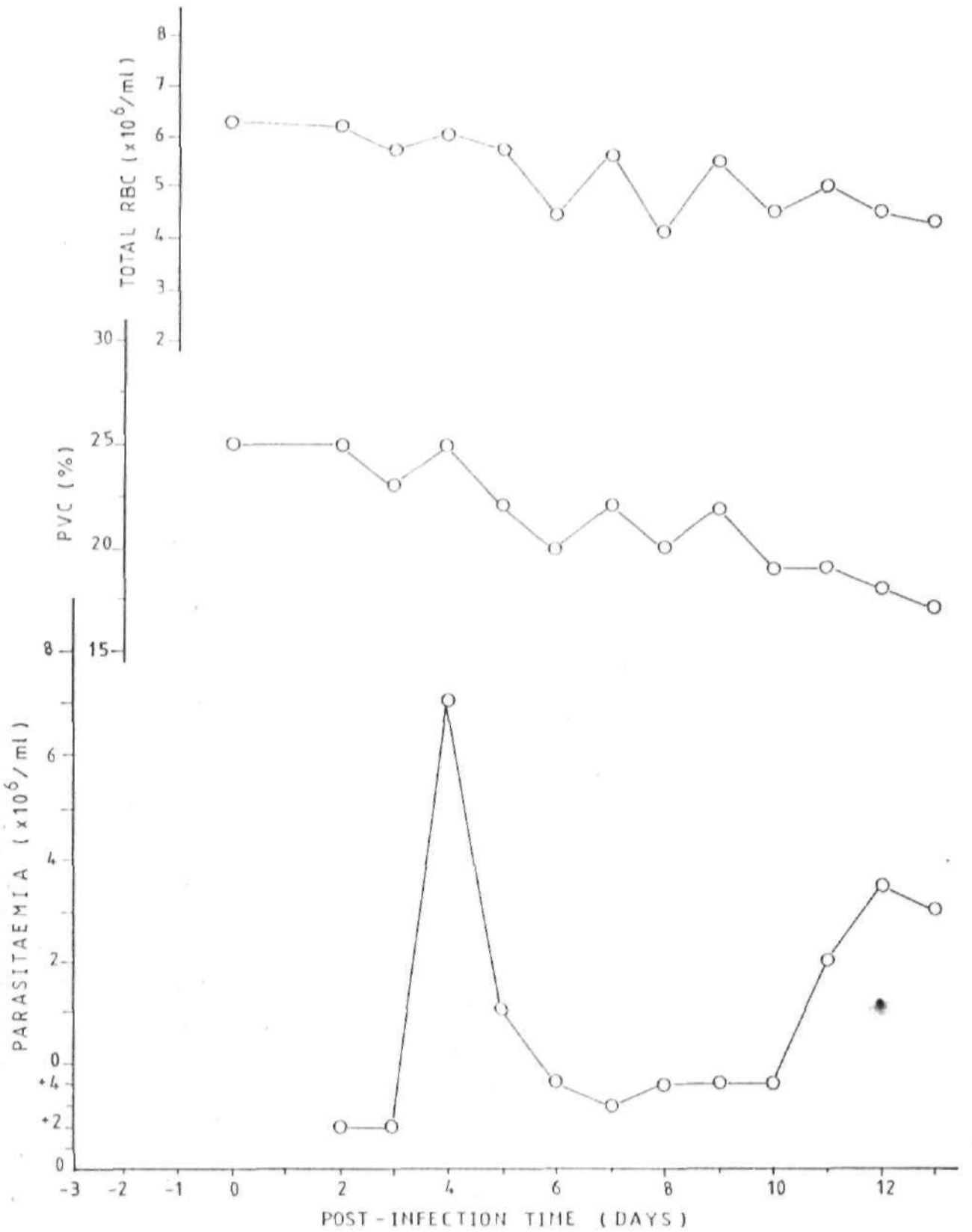


Fig. 4.3.11. Profiles of parasitaemia, PCV and total RBC counts for lactose-infused *I. vivax*-infected calf 3

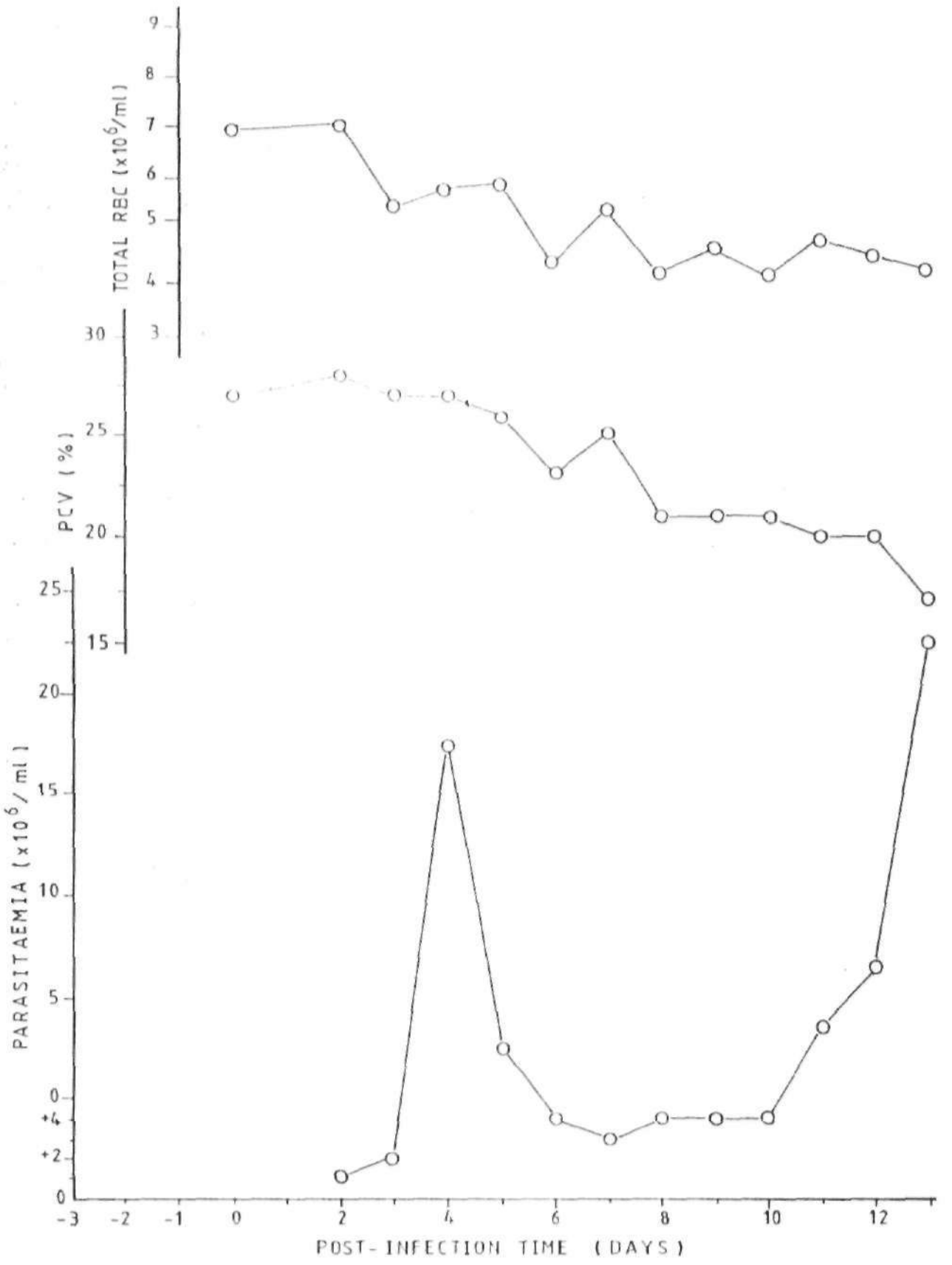


Fig. 4.3.12. Profiles of parasitaemia, PCV and total RBC counts for lactose-infused *T. vivax*-infected calf 4.

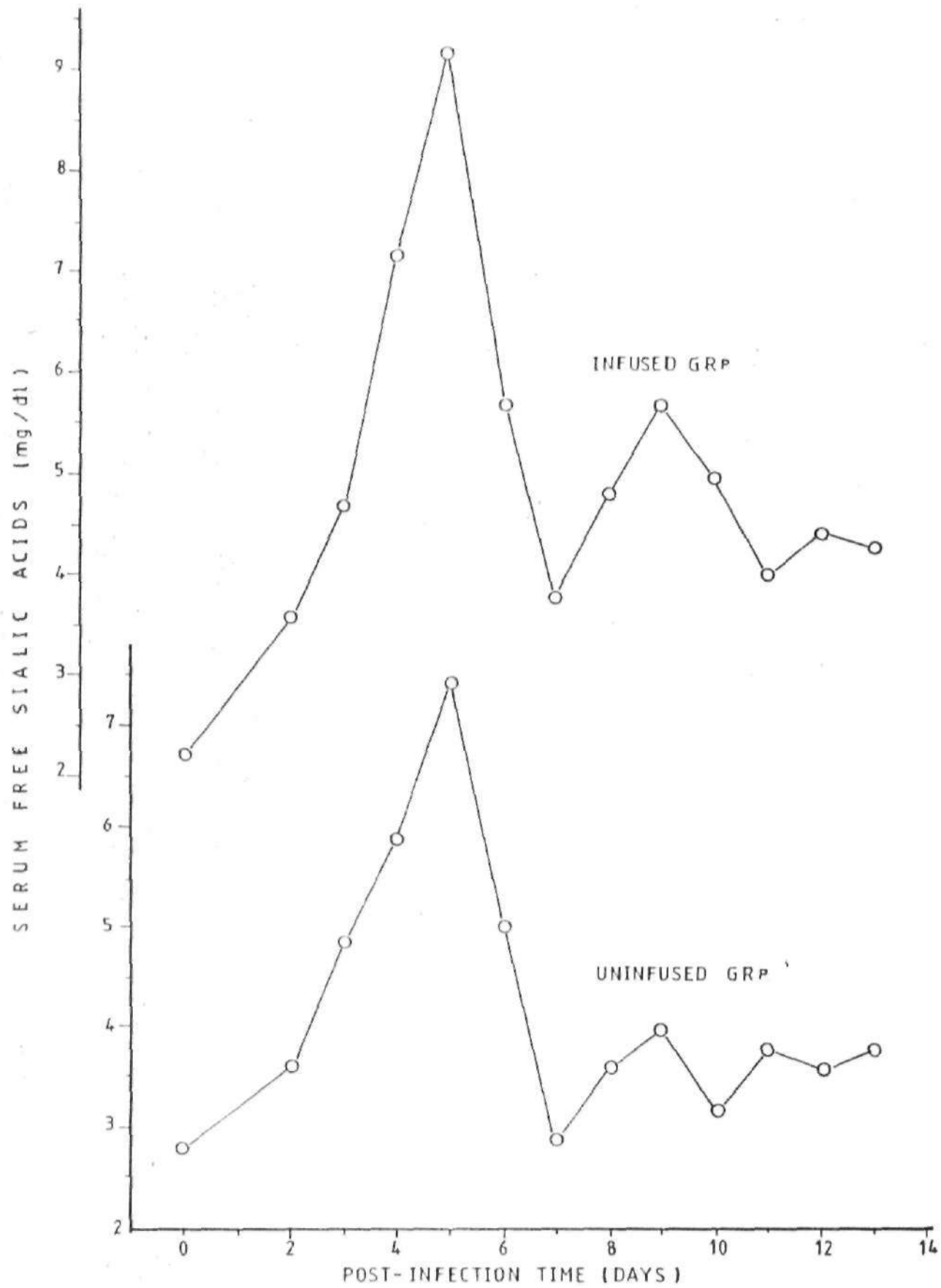


Fig. 4.3.13. Profiles of mean serum free sialic acids for *T. vivax* - infected calves.

CHAPTER FIVE

DISCUSSION

The plasma lactose concentrations in the adult healthy animals exhibited a wide variation (Tables 4:1:1 and 4:1:2). The low values recorded in the adult Zebu indicated that lactose is not a major constituent of the plasma. This was not surprising as lactogenesis occurs principally in the mammary glands and hence free lactose is found in nature in appreciable amounts only in the milk (Smith et al., 1985). The higher values seen in the calves might have been due to the fact that these were young animals that had only recently been weaned from a predominantly milk diet (Smith et al., 1985). In infant humans urinary out-put of lactose is higher during the nursing period or soon after weaning (Pilleggi and Szustkiewicz, 1974). In adult females urinary lactose is higher during pregnancy and lactation or following a long-term exclusive milk diet. These reports presuppose that plasma levels may be concomitantly higher under the conditions mentioned. The normal adult human urine contain approximately 12-40 mg lactose per day (Date, 1958); considering that human adult voids 600 to 2500 ml urine per day (Smith et al., 1985), the average lactose out-put comes to about 1.67 mg lactose/100 ml urine. The normal human infant excretes up to 1.5 mg lactose/100 ml of urine. Though no corresponding values for ruminants have come to the author's notice, it is possible that these observations are also applicable.

Administered lactose is excreted in the urine mainly unchanged, indicating the absence of the lactase enzyme at sufficiently high levels in the plasma to cause hydrolysis. The rapid rise in plasma lactose observed upon administration was indicative of rapid

distribution in the vascular system from the site of administration. The initial rapid fall seen after the concentration peaked (Figs. 4:2:1, 4:2:2, 4:2:3 and 4:2:4) represented the period of distribution of lactose into the extracellular spaces, as indicated in the semi-logarithmic plots in figs. 4:2:5 and 4:2:6. This phase was followed by a more gradual elimination phase. The non-accumulation of lactose upon repeated dosing was due to the rapid elimination of lactose (perhaps in the urine) which indicated that elimination of lactose, possibly by the kidneys, is an efficient process.

The mean $t_{1/2}$ of 5.14 $\pm$ 0.29 h and Ke of 0.138 $\pm$ 0.008 h^{-1} indicated that for lactose to be effectively used as a therapeutic agent as it was administered in the experiment, repeated doses must be given at fairly frequent intervals. Perhaps this was why Muller et al., (1981) found it necessary to administer lactose in another species of ruminant (rabbit) at four - hourly intervals to maintain a plasma concentration that reduced the rate of sequestration of injected radio-labelled homologous desialylated erythrocytes from the blood stream.

The apparent volume of distribution for lactose fell within the range of V_d for substances that are fairly well distributed (Grahame-Smith and Aronson, 1992) indicating that lactose was fairly well distributed in the body fluids.

Trypanosoma vivax infection in Zebu calves led to accumulation of lactose in the blood stream upon repeated dosing. It also significantly increased the biological half-life ($t_{1/2}$) and reduced elimination constant (Ke) of lactose in the calves. The slower rate of elimination of lactose, and hence its accumulation, may be attributed to a number of factors all of which are a direct consequence of the infection. Trypanosome infection

causes generalised degenerative changes in organs and tissue, including the kidneys (Valli et al., 1979b). However, since the duration of the experiment may not have been enough to cause such significant degenerative changes, the more likely factor contributing to the increased $t_{1/2}$ of lactose in infected animals may be the proliferation of the reticuloendothelial system which occurs during animal trypanosomiasis (Valli and Forsberg, 1979; Jennings et al., 1974; Murray, 1974; Jennings, 1976; Saror, 1980; Esievo et al., 1982; Anosa and Kaneko, 1983a; b). In a bid to fight off the infection the splenic macrophage and hepatic kupffer's cells proliferate and more monocytes released into circulation. This leads to an overall increase in the number of the cells of the mononuclear phagocytic system (MPS) in the body. These cells have been shown to have beta-D-galactoside specific lectins on their surfaces (Kuster and Schauer, 1981; Muller et al., 1983; Kelm et al., 1986a; Kelm and Schauer, 1986; Ibrahim et al., 1995) through which they bind desialylated erythrocytes (Kuster and Schauer, 1981; Omage et al., 1995b) as well as beta-galactoside compounds such as lactose (Kolb and Kolb-Bachofen, 1978; Kolb et al; 1980; Kelm and Schauer, 1986). It is probable that in infected calves lactose got bound to the macrophage surfaces and the amount of bound lactose was bound to be higher in the infected than the uninfected animals; thus making lactose, albeit temporarily, unavailable for excretion. Thus the rate of elimination of lactose from the infected animals became lower and lactose accumulated. The inability of lactose to accumulate in calf 2 of the infected group could only be attributed to individual variation.

The overall results indicated that the infected animals developed a mild infection. This was consistent with results of earlier works done with another T. vivax strain (Y-58)

in Zebu calves (Esievo et al., 1982) and bulls (Sekoni et al., 1990). This may be attributed to various factors which include host resistance, virulence of the trypanosome strain (Anosa, 1988).

The pre-patent period in the infected, uninfused group was three days post-infection, while it was two days in the infused group. The parasitemia peaked on day 4, after infection, for the infected, infused group; and day 5 for the uninfused group. This rapid appearance and rise in the number of parasites may be due to the heavy parasite dose (11×10^6 parasites per animal) given to the animals at the time of infection. The persistent presence of parasites in the blood of the lactose - infused group between day 7 and day 10 after infection; contrary to the situation in the uninfused group; was attributed to the lactose in circulation. The energy demands of trypanosomes in the blood stream depends upon adequate supplies of glucose (Voorheis, 1969) and other carbohydrates. Though the ability of trypanosomes to utilise lactose for energy and survival was not investigated in this work it is conceivable that the presence of lactose in circulation provided additional source of nourishment enough to sustain the parasite even at the height of the body's ability to control the parasites. Alternatively, perhaps lactose in some yet obscure way interfered with the destruction of trypanosomes by the body's defence mechanism. The decline in PCV and total RBC count in the infected groups coincided with the decrease in parasitemia, reaching the lowest values 8 days after infection in the uninfused group, after which an attempt at normalising the PCV and total RBC count was made. These results are in agreement with those obtained in earlier experiments (Esievo et al., 1981; Sekoni et al., 1990). The cumulative mean percentage

drop in PCV and total RBC count in the period before lactose therapy was commenced (Day 0 - Day 6, pi) for both infected groups were statistically the same.

The difference between the mean cumulative percentage changes in PCV of the infused and uninfused groups during the period of lactose infusion (Day 6 to Day 10, pi) in the infused group was significant ($P<0.025$); with the uninfused group recording a further mean drop of $7.5 \pm 2.9\%$ while the infused group recorded a marginal recovery of $0.01 \pm 3.2\%$. The difference in mean cumulative percentage change in the PCV after lactose infusion was statistically significant ($P<0.01$). In the uninfused group a recovery of $8.4 \pm 12.0\%$ was recorded while in the infused group a further drop of $13.2 \pm 2.2\%$ in the PCV was recorded in the same period.

The percentage changes in total RBC count in the two infected groups followed a similar pattern as described for the PCV.

Trypanosomes produce sialidase (Esievo, 1979; 1980; Pereira, 1983; Pereira and Hoff, 1986; Engstler et al., 1991 Engstler and Shauer, 1993) which is known to remove the masking sialic acid coat which exists on the surfaces of animal erythrocytes (Durocher et al., 1975; Seaman et al., 1977, Esievo et al., 1982). Recently it was observed in cattle infected with T. vivax that the desialylated erythrocytes have on their surfaces newly exposed galactoside residues (Omage et al., 1995). Earlier reports showed that exposed galactosides bind to beta-galactoside - specific lectins on the surface of macrophages (Kuster and Schauer, 1981; Muller et al., 1983) and are eventually phagocytised. Such desialylated erythrocytes had been found to be rapidly sequestrated from the blood stream (Durocher et al., 1975; Aminoff et al., 1978; Muller et al., 1981).

Thus during the course of T.vivax infection the sialidase that the parasite produced (Esievo, 1979; 1980) increased with the number of parasites (Esievo, 1983) and hence hydrolysed more sialic acids from the surface of erythrocytes (Esievo et al., 1982) as parasitemia increased. Further evidence in support is the steady increase in the serum free sialic acids with increasing parasitemia and more marked anaemia observed by Esievo et al., (1982) and confirmed in this work.

Erythrophagocytosis is a two - step process (Kuster and Schauer, 1981; Muller et al., 1983; Kelm et al., 1986a; Kelm and Schauer, 1986), involving an initial rate-limiting step of binding of desialylated erythrocytes to macrophage surface, and a subsequent phagocytic step which requires the presence of certain serum complement substances. The rate-limiting binding step has been shown to be inhibited in vitro by beta-glycosidically bound beta-D-galactoside residues (Kolb and Kolb-Bachofen, 1978; Nagamura and Kolb, 1979; Kolb et al., 1980; Schlepper-Schafer et al., 1980). Lactose has been shown to perform this inhibitory role in vitro (Kelm and Schauer, 1986) and in vivo in rabbits (Muller et al., 1981). Thus the prevention of further fall in PCV and total RBC count of the infected lactose-treated group of cattle recorded in this study was attributed to the inhibitory role of circulating lactose in preventing the binding of the desialylated erythrocyte in circulation to macrophage for sequestration, thereby apparently prolonging the life span of these cells as previously reported by Muller et al., (1981). This finding is even more significant in view of the existing parasitaemia (Fig. 4.3.7) and thus circulating active trypanosomal sialidase, as evidenced by high levels of serum free sialic acids (Fig. 4:3:14) during this period of infusion. In the absence of lactose in the

uninfused group PCV and total RBC counts, however, continued to fall even when the parasites were no longer detectable in the blood and with greatly reduced activity of trypanosomal sialidase as indicated by low levels of serum free sialic acids during this same period.

The significant drop in PCV and total RBC count recorded in the lactose-treated infected group in the period soon after the discontinuation of lactose infusion further supports the role attributed to lactose in preventing sequestration of desialylated erythrocytes during the period of infusion. This rapid drop, without an attempt to normalize, may have been due to the fact that during the period of infusion, parasites, and hence sialidase, were still present; expectedly these would continually remove the erythrocyte-surface sialic acids, thus accumulate, in circulation, desialylated erythrocytes which may not be sequestrated by the macrophages due to the inhibitory role of lactose. In support of this is the further decline of PCV and total RBC count as soon as the inhibition was removed. Also in the uninfused group, within the same period the attempt at recovery in the PCV and total RBC count was partially successful, probably due to the short period of absence of parasites from the blood, hence low sialidase activity.

The cause of anaemia in the early stages of animal trypanosomal infection has been attributed, principally, to erythrophagocytosis and reduced erythrocyte life-span (Mackenzie and Cruikshank, 1973; Mamo and Holmes, 1975; Dargie et al., 1979b; Esievo et al., 1982; Esievo and Saror, 1991). Immunological involvement at this early stage may not be significant (Jennings et al., 1974) since the host may not have mounted enough immunological response (Esievo and Saror, 1991). Since the severity and duration of

anaemia early in the infection is a major contributory factor to the general deterioration of condition in diseased animals (Hornby, 1921; Fiennes, 1954; Murray, 1974; Esievo et al., 1982; Sekoni et al., 1990a; c) an early prevention of anaemia may prevent other deleterious effects of the disease. It is pertinent to note that the relative resistance to trypanosomal infection by the N'dama breed of cattle, as compared with the Zebu, has been associated with the higher concentration of sialic acids (Esievo et al., 1986) and more hydrolytically resistant sialic acids (Shugaba et al., 1994) on the surfaces of their erythrocytes. Perhaps, an agent such as lactose when used as an adjunct in trypanocidal drugs may improve effectiveness and efficiency of therapy, since lactose can provide additional protection to the animal by retarding the development of anaemia.

The leucopenia recorded early in infection in this work was in agreement with earlier reports (Valli et al., 1979; Esievo and Saror, 1983; Igbokwe and Anosa, 1989). Earlier researchers (Anosa and Isoun, 1983; Esievo and Saror, 1983) reported that the leucopenia was due to lymphopenia, neutropenia and eosinopenia, though monocytosis also occurred. Similar results were observed in this study. The eosinopenia was presumably due to depression of the granulocyte precursors in the bone marrow by trypanosome toxins (Anosa, 1983). The persistent neutropenia at low parasitemia was attributed (Esievo and Saror, 1983) to the slow mechanism of neutrophil response in cattle (Valli et al., 1979). Neutropenia in T. vivax infection was postulated to be caused by the depression of granulocyte precursors in the bone morrow coupled with trapping of neutrophils in the spleen due to hypersplenism (Anosa, 1983). Monocytosis was observed in the lactose- infused infected animals abut not in the uninfused animals. Though

monocytosis has been consistently reported in animal trypanosomiasis (Isoun, 1975; Anosa and Isoun, 1980; Esievo and Saror, 1983; Anosa, 1988) there are few reports in which this was not recorded (Igbokwe and Anosa, 1989). Monocytosis was recorded in the infused group but not in the uninfused group in this experiment. It is not clear whether this difference was due to lactose infusion in the infused group.

It has been suggested that the early leucopenia observed in the course of bovine trypanosomiasis may be influenced by massive amounts of trypanosome antigens (Mackenzie et al., 1978) and plasma trypanosomal sialidase (Esievo, 1979). The possible role of sialidase in the process was, perhaps, further supported by the report that viral sialidase, which is antigenically similar to trypanosomal sialidase (Esievo, 1983); cleaves sialic acids from leucocytes and thus divert such desialylated leucocytes form the lymph nodes and spleen to the liver for destruction (Woodruff and Woodruff, 1976). The amelioration of leucopenia by lactose in the lactose-infused group observed in this experiment suggests that the sequestration of desialylated leucocytes by liver kupffer cells may be quite similar to the process of erythrophagocytosis which was also inhibited by lactose-infusion.

T. vivax infection caused a significant drop ($p<0.005$) in plasma total proteins in the infected uninfused group. Lactose therapy of infected animals however prevented this fall and went further to cause a significant increase in the plasma total proteins. There have been conflicting reports of changes in plasma total proteins that occur during ruminant trypanosomiasis; with some reports of increases (Anosa and Isoun, 1976; Igbokwe and Mohammed, 1992), and decreases (Kalu et al., 1989, Sekoni et al., 1990).

In view of the results obtained in the present study it is pertinent to note that during trypanosomiasis the proteinuria (Bruijn, 1987) and hepatocellular damage (Ingh et al., 1976; Saror, 1980; Anosa and Isoun, 1983) that occur could lead to reduced albumin levels (Anosa, 1988) also Esievo et al., (1984) have reported depletion of serum haptoglobin in acute bovine trypanosomiasis. The reason for lactose's seeming ability to prevent the trypanosome-induced fall in plasma total proteins can only be speculated upon; might it be that lactose in circulation provided an additional source of metabolic energy for the parasites thus making it less necessary for the parasite to utilise the proteins and/or amino acids in the blood of the host; or more likely, that lactose having ameliorated anaemia also slowed down degenerative changes in tissues and organs, especially the liver, thus causing minimal changes in the metabolic activities of such tissues and organs?

The levels of serum free sialic acids during the course of T. vivax infection increased concomitantly with increasing parasitemia and declined as the parasitemia decreased. In the lactose infused group the sialic acids level in the serum was persistently higher than the pre-infection level throughout the period of the experiment. This situation was not observed in the uninfused group. This difference was attributed to the persistent presence of parasites in the blood of the infused animals even on days when parasites could not be detected in the blood of the uninfused animals. An additional factor which may be significant, is the recent report (Engstler and Schauer, 1993) that trypanosomal sialidase is activated by di- and tricarboxylic acids and some saccharides, especially by lactose.

T. vivax produces sialidase (Esievo, 1979; 1980) which depletes the erythrocyte surface sialic acids in infected animals (Esievo et al., 1982). Although Esievo et al. (1982) recorded depleted erythrocyte - surface sialic acids of T. vivax (stock Y-58)-infected Zebu calves they observed an increase, but not the expected concomitant significant increase in serum free sialic acids. Kraemer (1966) had suggested that sialyltransferases present in calf thyroid gland (Spiro and Spiro, 1968) regenerated sialic acids on the surfaces of cells; thus the turn-over of serum free sialic acids may be quite high, and as such free sialic acids are being metabolically utilized as soon as they are being produced.

CONCLUSIONS AND RECOMMENDATIONS

The plasma lactose concentration in adult Zebu cattle was very low while that in Zebu calves was significantly higher. It is, however, pertinent to note that in both groups of Zebu the concentrations of lactose recorded indicated lactose is not a major constituent of plasma such as glucose. The plasma kinetics of lactose in healthy Zebu calves indicated that lactose was fairly well and rapidly distributed in the body as well as being rapidly eliminated, to the extent that it did not accumulate in the blood even upon repeated dosing at four - hourly intervals.

T. vivax infection in Zebu calves prolonged the biological half-life of lactose, infused intravenously, by reducing the rate of its elimination from the infected animals, thereby leading to accumulation of lactose upon repeated dosing in these animals. Infusion of lactose, on the other hand, ameliorated the T. vivax - induced anaemia, leucopenia and hypoproteinemia during the period of infusion. Amelioration of the trypanosomal anaemia lends further credence to the assertion that trypanosomal sialidase, reduced erythrocyte life-span and erythrophagocytosis play prominent roles in the aetiology of trypanosomal anaemia in the early stages of the disease. Lactose, however, had the unfortunate effect of sustaining the trypanosomes in the blood, even at periods when the parasites should have disappeared from the blood, and so might lead to more RBC destruction.

In this work lactose therapy was began only after anaemia had set in, to establish the existence of the disease; it would be of interest to see whether the anaemia could altogether be prevented by commencing lactose treatment as soon as the animals were

infected. It would also be interesting to investigate how or why lactose sustained the parasites in the blood; was it by acting as an additional source of nutrient for the parasite, or by interfering, somehow, with the body's ability to destroy the trypanosomes?

The amelioration of trypanosomal anaemia and leucopenia by lactose is very significant from the view-point of chemotherapy of African bovine trypanosomiasis. With further research, lactose has potential of becoming part of the common chemotherapy of African bovine trypanosomiasis - perhaps as an adjunct.

Lactose, however, proved to have some major short-comings: on the one hand enormous quantities of lactose were required to elicit the observed effects, while on the other it was eliminated rather rapidly from the body. However, the most serious problem is its ability to sustain the parasite in the blood of the infected animal. It is suggested that another beta-D-galactoside,preferably with several galactoside residues per molecule, which would not be eliminated as rapidly, nor required in such large quantities nor indeed influence the body's ability to deal with the parasite, may prove to be more effective and efficient. Alternatively lactose could be chemically modified by attachment of a ligand which would increase its biological half-life in the body and reduce the amount required for therapeutic effect. Such a complex could be administered orally (without the fear of enzymatic degradation), intramuscularly or subcutaneously to prolong its bioavailability.

Since evidence is mounting that trypanosomal sialidase plays a major role in trypanosomal anaemia, will treatment with sialic acids containing compounds (e.g. glycosides), acting as competitive inhibitors, prove to be effective? Will anti-serum prepared against trypanosomal sialidase, as suggested by Esievo and Saror (1991), be the

long-awaited effective vaccine against bovine trypanosomiasis? These and many other questions come to mind as a result of the findings in this experiment. It remains to be seen what the results of attempts at answering these questions would be; and what effects they would have on the treatment and/or prevention of African bovine trypanosomiasis.

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APPENDIX I

CHEMICALS

1. Ethylene Diamine Tetra Acetic acid (BDH)
2. Sodium Chloride (Fisher Scientific Co).
3. Phenyl hydrazine hydrochloride (BDH)
4. Sodium Metabisulphite (BDH)
5. Glacial acetic acid (Hopkins an Williams)
6. Zinc acetate (BDH)
7. Benzoic acid (BDH)
8. Glucose (BDH)
9. Lactose (BDH)
10. Sialic acid standards (BDH)
11. Periodate (Sigma)
12. Sodium arsenite (Analar)
13. 2- Thiobarbiturate (BDH)
14. Butanol (Hopkins and Williams)
15. Sulphuric acid (BDH)
16. Hydrochloric acid (Hopkins and willims)
17. Iodine crystals (BDH)
18. Potassium iodide (East Anglia chem.)
19. Nutrient broth. (Sigma)

EQUIPMENT

1. Microhaematocrit centrifuge - Gelman Hawksley Ltd. England
2. Haemocytometer - Gelman Hawksley Ltd. England
3. Microscope - Erma optical works Ltd., Tokyo, Japan

4. Centrifuge

5. Water bath - Gesellschaft fur Laborte-chmik M.B.H. and Co. W/Germany.

6. Spectronic 20

7. Hot plate - S & J Juniper and Co., Harlow, Essex, England.

ESTIMATION OF PLASMA LACTOSE BY COMBINED METHODS OF HOROWITZ *ET AL* (1951) AND WAHBA (1965)

PRINCIPLES: Saccharomyces carlsbergensis was first used to removed interfering monosaccharides and the lactose left in the plasma was reacted with phenylhydrazine to give the yellow-coloured lactosazone which absorbs maximally at 370nm.

REAGENTS:

1. **Stock Phenylhydrazine Solution**: One gram of phenylhydrazine hydrochloride was dissolved in 80ml of distilled water and 5g of Sodium metabisulphite added. The contents were mixed and diluted to 100ml with distilled water.

2. **Working Phenylhydrazine Solution**: This was always prepared fresh by diluting 10ml of stock solution to 100ml with glacial acetic acid.

3. **Deproteinising Solution**: A 2% w/v acqueous solution of Zinc acetate was prepared.

4. **Standard Lactose Solution**: A 0.1g sample of lactose hydrate was dissolved in 0.2% w/v acqeous solution of benzoic acid.

5. **Yeast Extract Solution**: A 0.5% aceous solution of yeast extract was prapared .

6. **Fermenting Organism**: Actively growing broth culture of Saccharomyces carlsbergensis.

PROCEDURE: The cells in an actively growing broth culture of S. Carlsbergenis were harvested and washed thrice in sterilised distilled water. The cells were resuspended in sterilised distilled water to obtain an absorbance reading of 0.6 at 680nm.

To 2.5ml of plasma (test) and 2.5ml of distilled water (test blank) in seperate test-tube was add 1.0ml distilled water and 1.5ml of 0.5% acqueous solution of yeast extract. The tubes were plugged and autoclaved at 15lbs pressure for 15 minutes. The cooled tubes were then asceptically inoculated with 2.0ml of the above yeast suspension, and incubated at 37°C for 24 hours. The contents of the tubes were then diluted to 20.0ml with distilled water and centrifuged at 3000g for 20 minutes to remove the cells. The supernatant was filtered until clear. To 1.0ml aliquots of the filtrate were added 1.0ml 2% (w/v) acqueous zinc acetate and 1.0ml distilled water and allowed to stand for 10minutes. The precipitate was filtered off. The reagent blank was prepared by using 1.0ml of distilled water instead of the supernatant above. For the standard 1.0ml of the working standard solution was used. All tubes were allowed to stand for five minutes and theprecipitate removed by filtration.

From each filtrate 0.5ml was placed in a fresh test tube and 5.0ml of the working phenyl hydrazine solution added. The tubes were plugged and placed in a boiling water bath for 1 hour. The tubes were cooled and absorbance read against the reagent blank at 370nm.

APPENDIX II

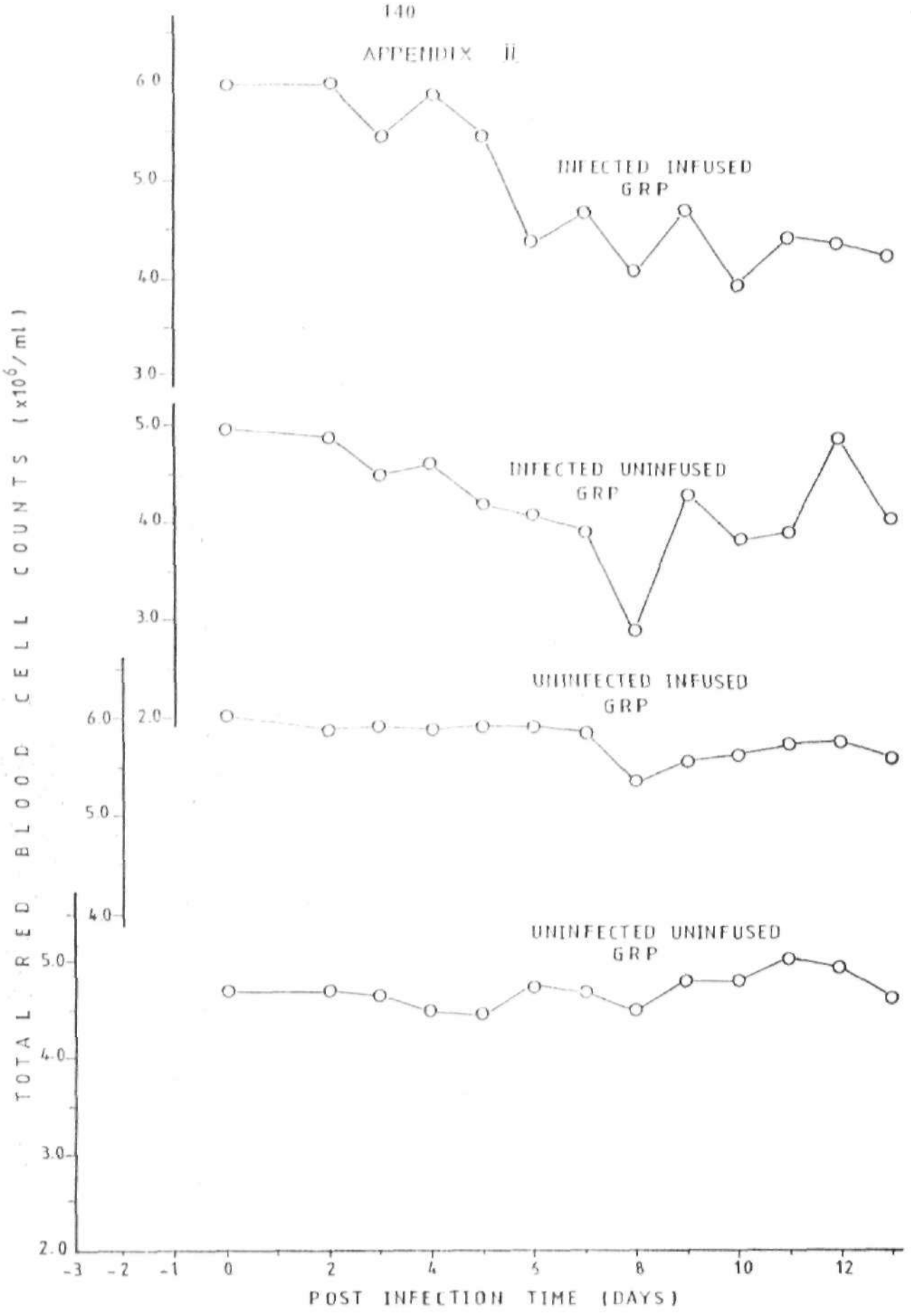


Fig. A2-1. Profiles of mean total RBC counts in all groups.

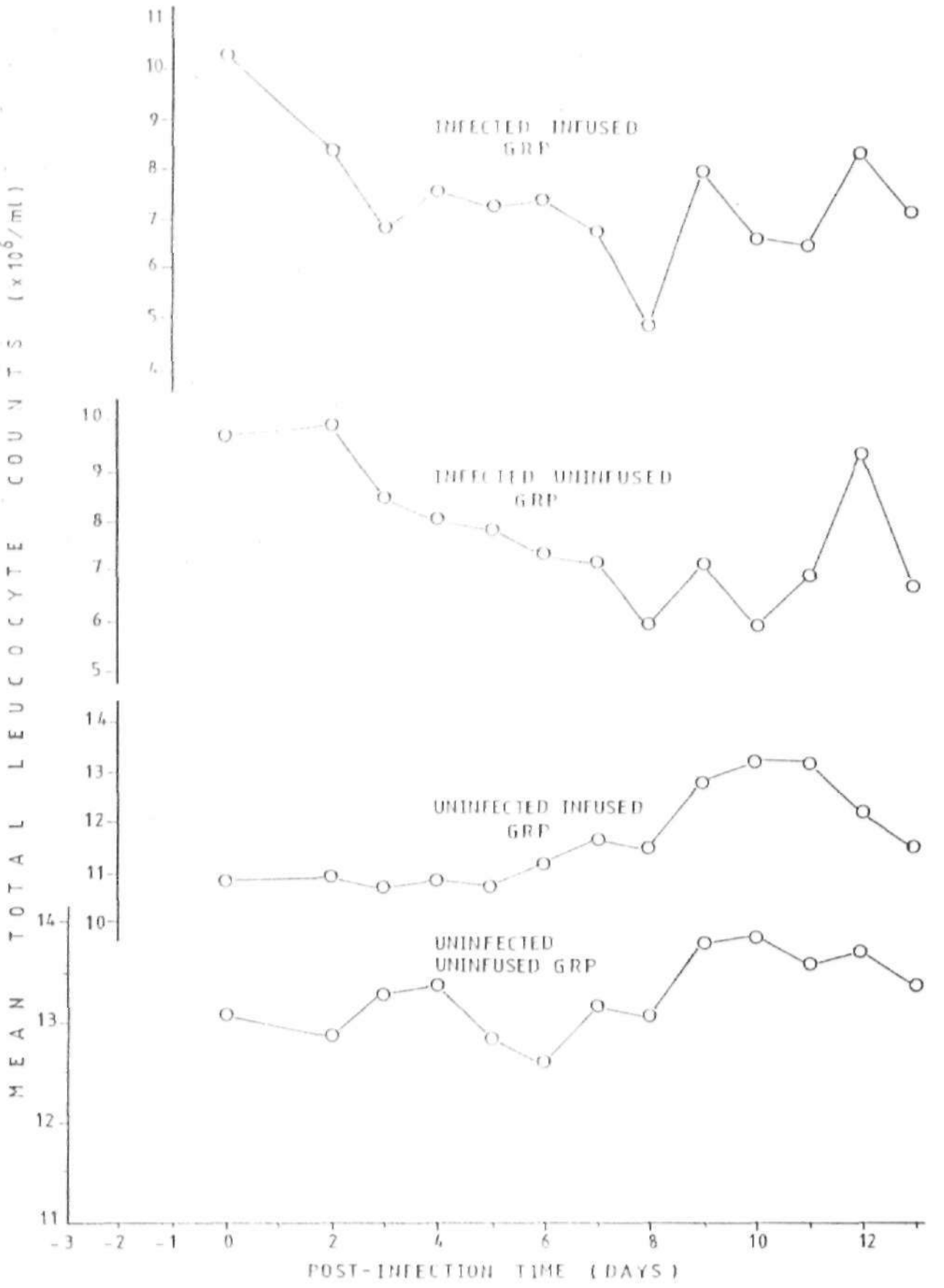


Fig. A2-2. Profiles of mean total leucocyte counts for all groups.

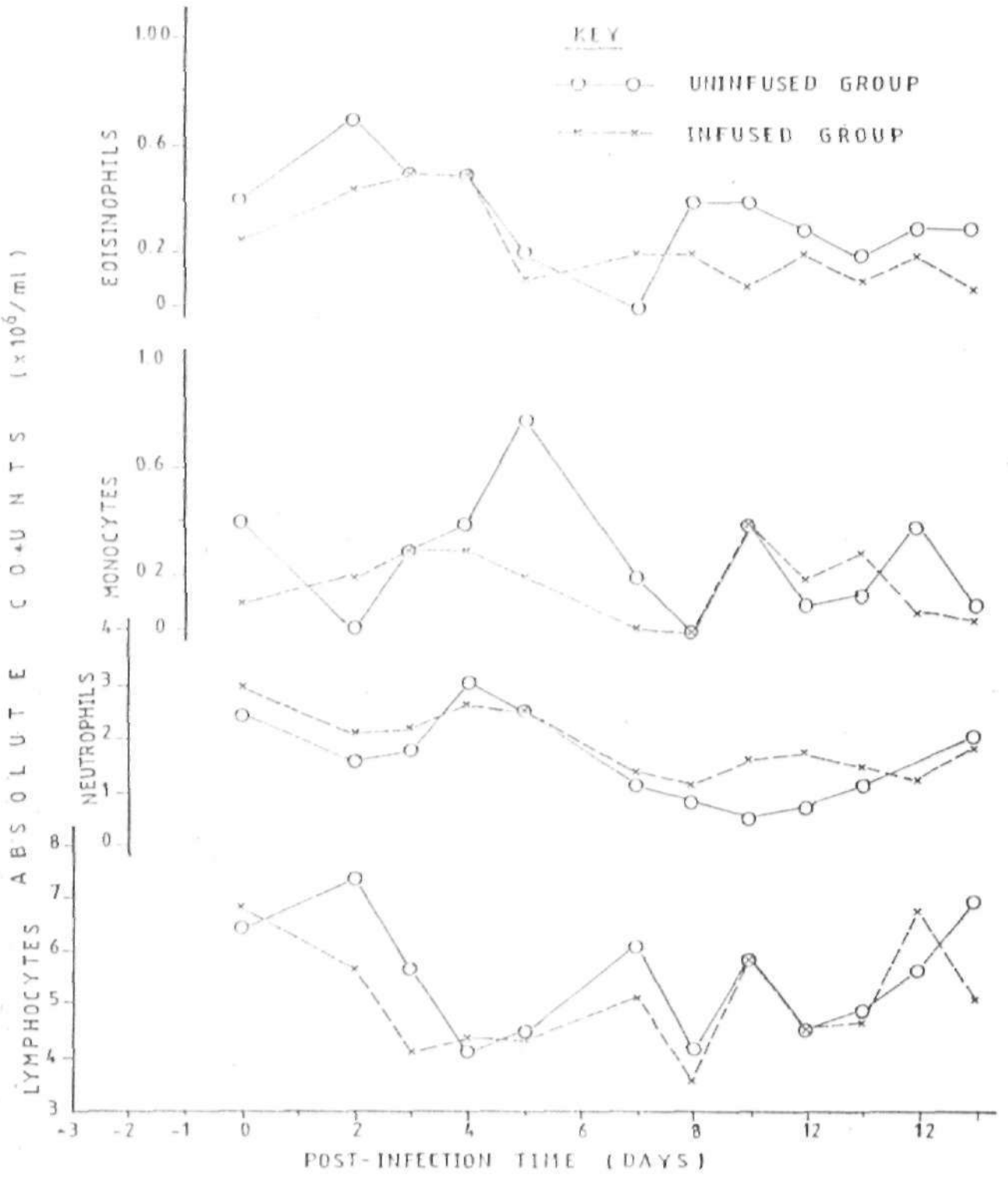


Fig. A2-3. Profiles for differential leucocyte counts for the two T. vivax-infected groups.

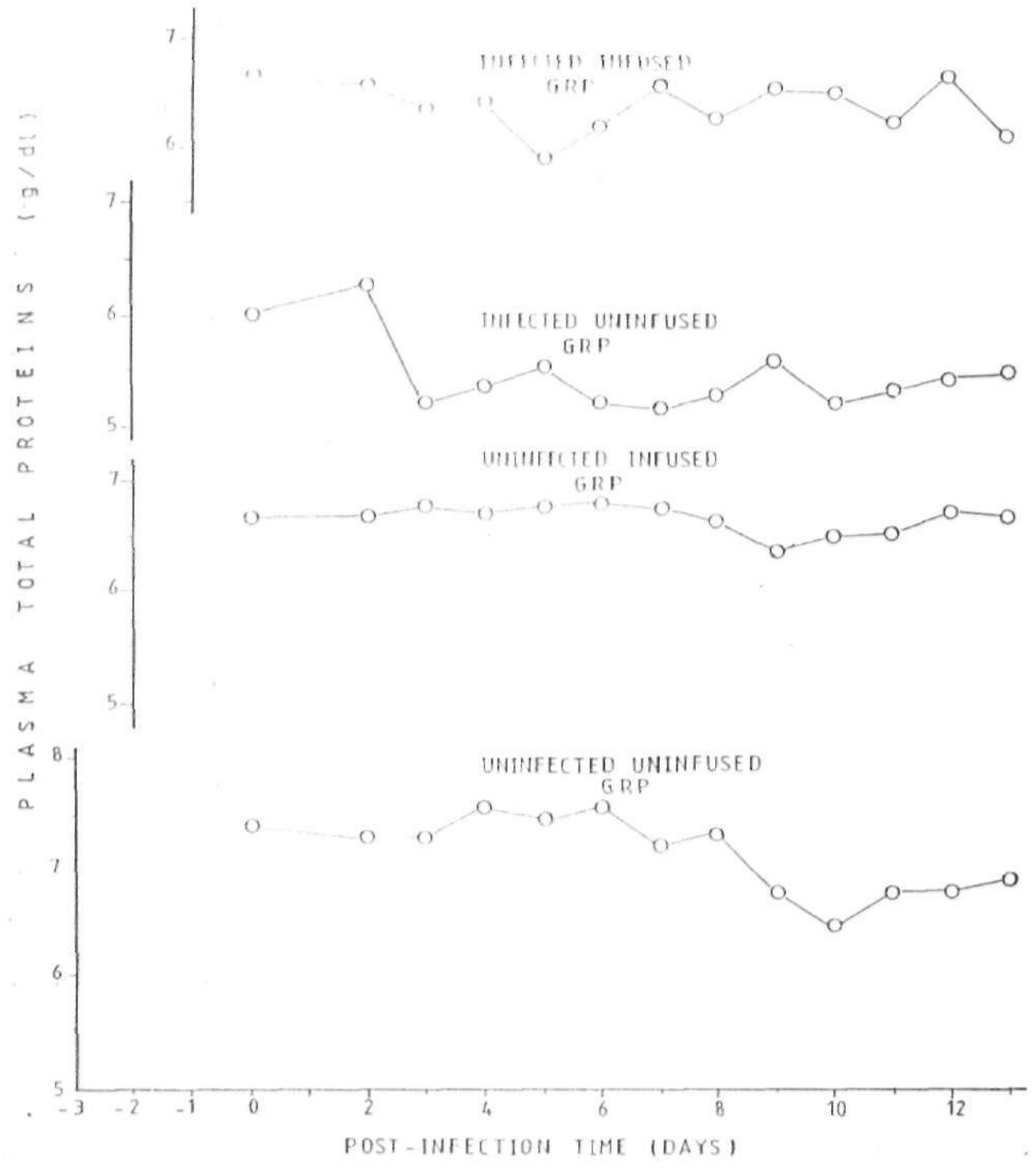


Fig A2-4. Profiles of mean plasma total proteins in all groups.

Table A2-1: Residual Lactose Concentrations at Specified Time Intervals in Plasma after a single dose of Lactose (0.5g Lactose per Kg Body Weight)

CALF 1		CALF 2		CALF 3	
POST-INF TIME (HR)	PLASMA LACTOSE CONC (mM)	POST-INF TIME (h)	PLASMA LACTOSE CONC(mM)	POST-INF TIME (h)	PLASMA LACTOSE CONC. (mM)
0.00	1.25	0.00	2.35	0.00	0.93
0.30	5.36	0.30	6.52	0.30	5.09
0.60	3.33	0.50	5.53	0.50	3.97
0.83	3.19	0.75	5.24	0.75	3.32
1.10	3.05	1.00	4.23	1.00	2.68
1.60	2.64	1.80	3.31	1.50	2.16
2.10	2.36	2.50	2.67	2.00	1.82
2.60	2.08	3.00	2.56	2.50	1.57
3.10	2.08	3.50	2.61	3.00	1.23
4.10	1.94	4.50	1.88	4.00	0.93
5.10	1.81	5.50	1.88	5.10	1.12
6.40	1.53	6.50	1.82	6.10	0.85
22.40	0.97	7.50	2.03	7.10	0.56
48.30	1.11	8.50	1.93	9.00	0.56
72.00	0.69	23.40	2.33	24.00	0.73
124.00	1.31	71.20	1.97	29.10	0.74
		78.40	1.90	48.00	0.74
		96.90	1.69	71.70	0.95
		120.30	2.20	98.80	0.65
		126.40	1.92		

Table A2-2: Residual Plasma Lactose Concentrations at Specified Post-Infusion Time Intervals after Three Four Hourly Infusions

CALF 1		CALF 2		CALF 3		CALF 4	
POST-INF. TIME (h)	PLASMA LACT. CONC (mM)	POST-INF. TIME (h)	PLASMA LACT. CONC (mM)	POST-INF. TIME (h)	PLASMA LACT. CONC (mM)	POST-INF. TIME (h)	PLASMA LACT. CONC (mM)
0.00	1.57	0.00	1.89	0.00	2.10	0.00	0.78
1.00	4.08	0.50	4.70	0.50	7.66	0.70	10.48
1.60	3.92	1.30	3.03	1.00	5.31	2.30	2.88
2.60	3.40	2.00	2.35	2.20	4.75	3.40	1.57
3.80	1.94	3.20	1.67	3.00	4.17	4.00	1.61
4.80	2.61	4.80	3.06	4.20	3.69	4.80	5.16
5.60	2.09	5.80	2.09	4.70	6.33	5.30	4.46
6.70	2.09	7.40	1.89	5.20	5.04	6.00	2.93
7.80	1.85	8.40	3.76	6.20	4.31	6.80	2.04
8.40	4.18	9.40	2.76	7.20	3.12	7.80	1.15
9.40	2.78	24.50	2.04	8.40	7.23	8.40	7.12
25.10	1.57	48.40	1.31	9.40	4.84	9.40	3.79
48.10	1.05			24.00	2.03	24.00	1.22
				48.00	1.68	48.40	0.72

Table A2-3: Residual Lactose Concentration in Plasma at Specified Post-Infusion Time Intervals of Un-Infected Control Calves

CALF 1		CALF 2		CALF 3		CALF 4	
POST-INF. TIME (h)	PLASMA LACT. CONC (mM)	POST-INF. TIME (h)	PLASMA LACT. CONC (mM)	POST-INF. TIME (h)	PLASMA LACT. CONC (mM)	POST-INF. TIME (h)	PLASMA LACT. CONC (mM)
0.00	1.32	0.00	2.68	0.00	2.40	0.00	2.56
0.60	4.13	0.60	8.89	0.50	5.83	0.50	6.94
1.10	3.58	1.10	6.27	1.00	4.04	1.00	5.28
2.10	2.95	2.10	5.14	2.00	3.33	2.00	3.89
3.10	2.59	3.10	4.60	3.00	3.14	3.00	3.67
4.10	2.24	4.10	3.63	4.10	2.73	4.10	2.81
4.70	3.68	4.80	7.64	5.80	4.17	5.90	5.10
5.80	2.74	5.80	3.47	7.80	2.93	8.00	2.55
6.80	2.42	6.80	2.74	24.30	2.04	9.00	5.39
7.80	2.01	7.80	2.44	24.80	6.40	24.20	2.28
24.20	1.49	24.30	2.79	25.30	5.31	24.80	5.84
24.70	5.57	24.80	8.26	28.30	2.43	25.10	5.34
25.30	4.68	25.30	5.56	30.10	2.84	28.10	2.51
26.40	3.38	28.00	2.25	31.91	2.54	30.13	3.75
28.10	2.68	28.50	5.85	47.30	2.33	31.80	2.84
30.60	4.45	29.50	4.56			48.10	2.27
31.70	4.10	30.50	2.84				
47.30	1.22	31.80	2.40				
		47.50	2.27				

Table A2-4: Residual Lactose Concentration in Plasma at Specified Post-Infusion Time Intervals of T.Vivax -Infected Calves

CALF 1		CALF 2		CALF 3		CALF 4	
POST-INF. TIME (h)	PLASMA LACT. CONC (mM)	POST-INF. TIME (h)	PLASMA LACT. CONC (mM)	POST-INF. TIME (h)	PLASMA LACT. CONC (mM)	POST- INF. TIME (h)	PLASMA LACT. CONC (mM)
0.00	2.43	0.00	1.25	0.00	1.74	0.00	1.56
0.25	3.47	0.25	3.39	0.20	2.94	0.25	2.08
0.50	4.51	0.50	2.88	0.50	3.30	0.50	2.22
0.75	3.82	0.75	2.24	0.75	3.13	0.75	2.40
1.00	3.47	1.00	1.75	1.00	3.13	1.00	2.64
1.50	3.30	1.50	1.41	1.50	3.13	1.50	2.95
2.00	2.26	2.00	1.22	2.00	3.13	2.00	4.66
3.00	2.26	3.00	1.37	3.00	2.95	3.00	2.94
4.50	1.56	4.50	1.15	4.50	2.95	4.50	2.26
5.00	4.27	5.00	1.85	5.50	5.21	5.00	6.42
5.50	3.65	6.00	1.60	6.00	3.47	5.50	4.51
7.50	3.47	8.00	1.15	7.00	3.13	7.00	3.27
24.00	2.84	23.50	1.15	24.00	2.94	24.00	3.10
24.50	6.81	24.00	1.98	24.50	6.05	24.50	6.05
25.00	5.19	24.50	1.98	25.00	4.74	25.00	5.23
25.50	4.46	25.00	4.09	25.50	4.41	25.50	5.07
26.00	4.09	25.50	1.66	26.00	3.60	26.00	4.74
27.00	3.89	26.50	1.28	27.00	3.24	27.00	3.92
29.00	3.10	28.50	1.34	28.50	3.27	28.50	3.92
29.50	5.72	29.00	2.05	29.00	5.72	29.00	6.21
30.00	6.54	29.50	1.85	29.50	7.03	29.50	5.23
30.50	5.07	30.00	1.79	30.00	4.90	30.00	5.07
31.00	4.41	30.50	1.79	30.50	4.25	30.50	5.39
34.00	3.27	33.50	1.28	33.00	3.78	33.50	3.27
48.00	3.35	48.00	1.14	48.00	3.89	48.00	2.98
72.00	3.54	72.00	1.39	72.00	4.26	72.00	3.52
96.00	3.76	96.00	1.28	96.00	4.08	96.00	3.30
120.00	3.99	120.00	1.17	120.00	3.98	120.00	3.12
144.00	4.00	144.00	1.48	144.00	4.16	144.00	3.28
168.00	2.43	168.00	1.12	168.00	3.21	168.00	2.79

TABLE A2 - 5: Parasitaemias in Lactose Infused and Uninfused T.Vivax - Infected Zebu Calves

POST-INFECT. TIME (DAYS)	UNINFUSED GROUP [X10⁶/ml BLOOD]				INFUSED GROUP [x10⁶/ml BLOOD]				
	CALF 1	CALF 2	CALF 3	MEAN ± SEM	CALF 1	CALF 2	CALF 3	CALF 4	CALF 4
0	0	0	0	0	0	0	0	0	0
2*	0	0	0	0	3+	1+	1+	1+	2+
3*	4+	4+	4+	4+	4+	3+	3+	2+	3+
4	9.50	11.50	15.50	12.±1.61	15.50	11.00	11.00	15.50	12.50±1.84
5	28.00	16.00	19.40	21.13±3.58	10.00	29.00	29.00	2.50	10.88±6.32
6	3.74	5.40	2.30	3.81±0.90	5.50	0.33	0.33	0.30	1.61±1.30
7*	0	0	0	0	3+	4+	3+	3+	3+
8*	0	0	0	0	3+	4+	4+	4+	4+
9*	0	0	0	0	2+	2+	4+	4+	3+
10*	3+	1+	2+	2+	2+	2+	4+	4+	3+
11*	4+	2+	2+	3+	0.50	0.50	3.00	3.50	1.88±0.80
12	6.50	0.10	0.10	2.37±2.07	4.00	4.00	4.50	6.50	4.38±0.83
13	6.0	10.50	10.50	9.33±1.69	16.00	16.00	4.00	20.50	13.63±3.49

* Parasitaemias on these days were too low to be counted by the haemocytometer. They were estimated by microhaematocrit method were 1+ = an occassional parasite perfield; 2+ = 1 to 5 parasites per buffy coat area; 3+ = 5 to 10 parasites per buffy coat area; 4+ = 10 - 20 per buffy coat area.

TABLE A2-6: The Percentage Packed Cell Volumes of Uninfected Zebu Calves Infused and not Infused with Lactose.

SAMPLING TIME (DAYS)	UNINFUSED GROUP (%)				INFUSED GROUP (%)				
	CALF 1	CALF 2	CALF 3	MEAN±SEM	CALF 1	CALF 2	CALF 3	CALF 4	MEAN ± SEM
0	23.00	23.00	28.00	24.67 ± 1.67	32.50	27.00	27.00	34.00	30.10±1.84
2	23.00	22.00	28.00	24.33 ± 1.86	33.00	27.50	27.50	32.50	30.0 ±1.60
3	23.00	23.00	27.00	24.33 ± 1.33	32.00	27.50	27.50	33.00	29.75±1.62
4	23.00	23.00	27.00	24.33 ± 1.33	33.00	27.00	27.00	32.00	29.88±1.54
5	23.00	23.00	27.00	24.33 ± 1.33	32.50	27.50	27.50	33.00	30.13±1.52
6	23.00	23.00	28.00	24.67 ± 1.67	33.00	27.00	27.00	33.00	30.00±1.73
7	23.00	22.00	28.00	24.33 ± 1.86	33.00	27.00	27.00	32.00	29.75±1.60
8	23.00	23.00	27.00	24.33 ± 1.33	32.00	26.00	26.00	27.50	28.00±1.37
9	23.00	22.00	28.00	24.33 ± 1.86	32.00	26.50	26.50	28.50	28.38±1.30
10	23.00	22.00	28.00	24.33 ± 1.86	33.00	26.00	26.00	28.50	28.38±1.65
11	25.00	23.00	28.00	25.33 ± 1.46	33.00	27.00	27.00	28.50	28.88±1.42
12	25.00	23.00	28.00	25.33 ± 1.46	32.00	27.50	27.50	29.00	29.00±1.06
13	25.00	23.00	27.00	24.33 ± 1.33	31.50	27.50	27.50	28.50	28.50±1.06

TABLE A2-7: The Percentage Packed Cell Volumes of T. vivax - Infected Zebu Calves Infused and Uninfused with Lactose.

POST-INFECT TIME (DAYS)	UNINFUSED GROUP (%)				INFUSED GROUP (%)				
	CALF 1	CALF 2	CALF 3	MEAN±SEM	CALF 1	CALF 2	CALF 3	CALF 4	MEAN±SEM
0	20.00	19.00	20.00	19.67 ± 0.34	25.00	23.00	25.00	27.00	25.00 ± 0.82
2	22.00	20.00	22.00	21.33 ± 0.67	24.00	22.00	25.00	28.00	24.75 ± 1.25
3	20.00	20.00	21.00	20.33 ± 0.34	23.00	22.00	23.00	27.00	23.75 ± 1.11
4	20.00	19.00	20.00	19.67 ± 0.34	23.00	25.00	25.00	27.00	25.00 ± 0.82
5	21.00	18.00	18.00	19.00 ± 1.00	22.00	22.00	22.00	26.00	23.00 ± 1.00
6	19.00	16.00	16.00	17.00 ± 1.00	18.00	21.00	20.00	23.00	20.50 ± 1.04
7	19.00	14.00	15.00	16.00 ± 1.53	17.00	19.00	22.00	25.00	20.75 ± 1.75
8	17.00	12.00	11.00	13.33 ± 1.86	17.00	17.00	20.00	21.00	18.75 ± 1.03
9	19.00	14.00	14.00	15.67 ± 1.67	19.00	18.00	22.00	21.00	20.00 ± 0.92
10	18.00	15.00	13.00	15.33 ± 1.46	18.00	18.00	19.00	11.00	19.00 ± 0.57
11	18.00	15.00	14.00	15.67 ± 1.20	17.00	16.00	19.00	20.00	18.00 ± 0.92
12	19.00	20.00	19.00	19.33 ± 0.34	18.00	17.00	18.00	20.00	18.25 ± 0.63
13	15.00	17.00	15.00	15.67 ± 0.67	16.00	16.00	17.00	17.00	16.50 ± 0.29

Table A2-8: The total red blood cell counts of uninfected zebu calves infused and uninfused with lactose.

SAMPLING TIME (DAYS)	UNINFUSED GROUP (x10^9/ml BLOOD)				INFUSED GROUP (X10^9/ml BLOOD)				
	CALF 1	CALF 2	CALF 3	MEAN±SEM	CALF 1	CALF 2	CALF 3	CALF 4	MEAN±SEM
0	4.95	5.15	6.01	4.70±0.66	6.94	4.98	4.80	7.35	6.02±0.66
2	4.10	4.10	5.90	4.70±0.60	7.00	4.86	4.95	6.82	5.91±0.58
3	4.30	4.35	5.35	4.67±0.34	7.05	4.80	4.95	6.96	5.94±0.62
4	4.00	4.27	5.21	4.49±0.37	7.15	4.99	4.87	6.58	5.90±0.57
5	4.10	4.30	5.01	4.47±0.28	6.96	5.16	4.80	6.89	5.95±0.57
6	4.10	4.30	5.96	4.77±0.57	7.13	4.95	4.73	6.98	5.95±0.64
7	4.21	4.00	5.96	4.72±0.62	7.13	4.95	4.80	6.64	5.88±0.90
8	4.10	4.45	5.01	4.52±0.27	6.81	4.90	4.15	5.76	5.41±0.58
9	4.35	4.10	6.01	4.82±0.60	6.81	4.75	4.80	6.01	5.59±0.50
10	4.42	4.00	5.97	4.80±0.60	7.01	4.51	4.80	6.27	5.65±0.60
11	4.95	4.31	5.90	5.05±0.46	7.16	4.85	4.87	6.18	5.77± .56
12	4.61	4.30	5.90	4.95±0.51	6.98	4.95	4.95	6.37	5.81±0.52
13	4.35	4.33	5.26	4.65±0.31	6.61	4.90	4.85	6.25	5.65±0.46

Table A2-9: The total red blood cell counts of T. vivax - infected calves infused and uninfused with lactose.

POST-INFECTION TIME (DAYS)	UNINFUSED GROUP (X10⁹/ml BLOOD)				INFUSED GROUP (X10⁹/ml BLOOD)				
	CALF 1	CALF 2	CALF 3	MEAN±SEM	CALF 1	CALF 2	CALF 3	CALF 4	MEAN±SEM
0	5.67	4.48	4.75	4.97±0.36	6.03	4.72	6.33	6.89	5.99±0.46
2	5.06	4.63	5.01	4.93±0.15	6.20	4.72	6.21	7.03	6.04±0.48
3	4.68	4.09	4.80	4.52±0.22	5.70	5.09	5.71	5.40	5.48±0.15
4	4.75	4.13	4.97	4.62±0.25	5.63	6.04	6.11	5.88	5.90±0.11
5	4.05	4.45	4.15	4.22±0.12	5.25	5.15	5.75	5.85	5.50±0.18
6	3.95	4.20	4.15	4.10±0.08	4..50	4.25	4.45	4.35	4.39±0.06
7	4.30	3.70	3.89	3.96±0.18	4.05	3.75	5.65	5.40	4.71±0.48
8	3.89	2.74	2.06	2.90±0.54	3.89	4.35	4.03	4.18	4.11±0.10
9	4.46	4.33	4.10	4.30±0.10	4.50	4.15	5.52	4.69	4.72±0.29
10	4.05	3.15	4.25	3.82±0.34	3.60	3.54	4.46	4.14	3.94±0.22
11	4.05	3.25	4.50	3.93±0.36	4.09	3.75	5.00	4.96	4.45±0.32
12	5.40	4.70	4.63	4.91±0.25	4.28	4.35	4.50	4.60	4.43±0.08
13	4.19	4.31	3.70	4.07±0.19	4.62	3.88	4.28	3.39	4.29±0.16

Table A2-10: Total leukocyte counts of uninfected Zebu calves infused and uninfused with lactose.

POST-INFECTION TIME(DAYS)	UNINFUSED GROUP (X10³/ml)				INFUSED GROUP (X10³/ml)				
	CALF 1	CALF 2	CALF 3	MEAN±SEM	CALF 1	CALF 2	CALF 3	CALF 4	MEAN ± SEM
0	8.76	14.91	15.62	13.10±2.18	10.70	10.85	7.63	14.24	10.86±1.35
2	8.61	14.62	15.38	12.87±2.14	10.70	10.85	7.85	14.00	10.89±1.26
3	8.21	15.75	15.95	13.30±2.55	10.33	10.80	7.55	14.35	10.76±1.40
4	8.34	15.70	16.15	13.40±2.54	10.68	10.67	7.73	14.31	10.85±1.35
5	9.52	14.00	15.00	12.84±1.69	10.68	10.75	7.90	14.01	10.84±1.25
6	9.35	15.20	13.30	12.62±1.72	11.91	10.60	8.30	14.90	11.20±1.20
7	9.65	14.85	15.10	13.20±1.78	12.20	11.10	8.50	14.90	11.68±1.33
8	9.30	15.00	15.00	13.10±1.90	12.35	10.85	9.00	13.95	11.54±10.6
9	10.60	15.90	14.95	13.82±1.63	13.45	12.10	10.90	14.90	12.85±0.88
10	11.35	14.85	15.41	13.87±1.27	14.30	12.85	11.20	14.90	13.31±0.83
11	10.75	15.10	15.00	13.62±1.43	14.40	12.65	11.61	14.67	13.26±0.70
12	11.00	15.32	14.88	13.73±1.37	13.75	11.10	10.00	14.10	12.24±1.01
13	9.75	14.91	15.51	13.39±1.83	12.52	10.60	8.95	14.00	11.52±1.11

Table A2-11: Total leukocyte counts of T. vivax-infected zebu calves infused and uninfused with lactose.

POST-INFECTION TIME (DAYS)	UNINFUSED GROUP (X10⁶/ml)				INFUSED GROUP (X10⁶/ml)				
	CALF 1	CALF 2	CALF 3	MEAN±SEM	CALF 1	CALF 2	CALF 3	CALF 4	MEAN±SEM
0	11.05	8.60	9.40	9.68±0.72	8.35	7.00	11.35	14.35	10.26±1.64
2	12.50	6.95	10.31	9.92±1.62	7.90	6.00	8.85	10.95	8.43±1.03
3	9.90	6.70	8.91	8.50±0.95	4.55	4.90	8.30	9.40	6.79±1.22
4	8.75	7.45	8.01	8.07±0.38	4.65	6.05	8.05	11.70	7.61±1.53
5	8.20	7.80	7.64	7.88±0.17	7.30	6.25	6.75	8.75	7.26±0.54
6	7.80	7.50	6.71	7.34±0.32	7.70	6.90	6.30	9.00	7.48±0.59
7	7.50	7.70	6.50	7.23±0.37	5.40	5.00	7.25	9.50	6.79±1.03
8	7.65	3.90	6.13	5.89±1.09	5.50	2.45	5.00	6.75	4.93±0.91
9	7.90	6.70	7.01	7.20±0.36	6.60	6.65	10.00	9.40	8.01±0.99
10	6.85	4.70	6.25	5.93±0.64	5.65	6.05	7.25	7.65	6.65±0.48
11	8.25	4.80	7.55	6.87±1.05	6.40	5.50	6.00	8.30	6.55±0.61
12	10.95	8.40	9.00	9.45±0.77	8.05	8.25	7.25	9.95	8.38±0.57
13	7.60	6.55	6.05	6.73±0.46	7.50	5.45	6.45	9.25	7.16±0.81

Table A2-12: The Plasma Total Proteins Concentration of T. vivax-Infected and Uninfected Zebu Calves that received and did not receive Infusions of Lactose

SAMPLING TIME (DAYS)	UNINFECTED, UNINFUSED GROUP (g/dl)				UNINFECTED, INFUSED GROUP (g/dl)					INFECTED, UNINFUSED GROUP (g/dl)				INFECTED, INFUSED GROUP (g/dl)				
	CALF 1	CALF 2	CALF 3	MEAN ± SEM	CALF 1	CALF 2	CALF 3	CALF 4	MEAN ± SEM	CALF 1	CALF 2	CALF 3	MEA ± SEM	CALF 1	CALF 2	CALF 3	CALF 4	MEAN ± SEM
0	7.10	7.00	8.20	7.43±0.39	6.40	6.50	6.80	7.00	6.68±0.14	6.20	5.80	6.10	6.03±0.12	7.40	6.00	6.60	6.60	6.65±0.29
2	7.00	7.00	8.00	7.33±0.34	6.40	6.70	6.80	6.90	6.70±0.11	6.90	5.50	6.50	6.30±0.42	7.00	6.50	6.00	6.80	6.58±0.22
3	7.00	6.90	8.10	7.33±0.39	6.60	6.70	6.80	7.10	6.80±0.11	5.70	4.70	5.30	5.23±0.29	6.70	6.10	6.20	6.40	6.35±0.14
4	7.30	7.10	8.40	7.60±0.40	6.60	6.50	6.90	7.00	6.75±0.12	5.90	4.80	5.40	5.37±0.32	6.60	6.10	6.50	6.50	6.43±0.11
5	7.10	7.00	8.40	7.50±0.45	6.60	6.50	7.00	7.00	6.78±0.13	6.10	4.80	5.60	5.50±0.38	5.50	5.30	6.40	6.50	5.93±0.31
6	7.30	7.00	8.50	7.60±0.46	6.50	6.50	7.10	7.20	6.83±0.19	5.70	4.70	5.30	5.23±0.29	6.70	5.60	6.00	6.50	6.20±0.25
7	6.50	7.00	8.20	7.23±0.50	6.50	6.50	7.10	7.00	6.78±0.16	6.10	4.30	5.10	5.17±0.52	6.80	6.00	6.70	6.80	6.58±0.20
8	7.10	7.00	8.00	7.37±0.32	6.40	6.50	6.10	6.70	6.68±0.16	6.00	4.50	5.40	5.30±0.44	7.00	5.50	6.40	6.20	6.28±0.31
9	6.90	6.70	6.70	6.77±0.07	5.80	5.90	6.80	7.10	6.40±0.33	6.40	4.80	5.70	5.63±0.46	7.30	6.00	6.70	6.20	6.55±0.29
10	6.30	6.60	6.60	6.47±0.09	6.00	6.10	6.90	7.10	6.53±0.28	5.90	4.50	5.30	5.23±0.40	7.20	5.90	6.30	6.70	6.53±0.28
11	6.60	6.90	6.90	6.80±0.10	6.10	6.10	6.90	7.10	6.55±0.27	6.20	4.30	5.50	5.33±0.55	7.20	5.60	5.90	6.30	6.25±0.35
12	6.70	6.70	6.90	6.80±0.06	6.40	6.00	7.20	7.40	6.75±0.33	6.30	4.60	5.50	5.47±0.49	7.50	6.40	6.10	6.70	6.68±0.30
13	6.60	6.60	7.20	6.90±0.17	6.40	6.40	7.10	7.00	6.73±0.19	6.00	5.00	5.50	5.50±0.29	7.20	5.70	5.90	5.80	6.15±0.36

Table A2-13: The plasma free sialic acids concentration in T. vivax-infected calves infused and uninfused with lactose.

POST-INFECTION TIME (DAYS)	UNINFUSED GROUP (mg/l)				INFUSED GROUP (mg/l)				
	CALF 1	CALF 2	CALF 3	MEAN±SEM	CALF 1	CALF 2	CALF 3	CALF 4	MEAN±SEM
0	3.21	2.14	3.01	2.78±0.33	1.43	3.04	1.25	3.21	2.23±0.52
2	3.75	3.39	3.46	3.53±0.11	3.57	3.57	3.57	3.57	3.57±0.00
3	4.46	5.36	4.62	4.81±0.28	3.54	4.82	4.46	3.93	4.69±0.34
4	5.36	6.43	5.81	5.87±0.31	8.21	6.61	6.79	7.14	7.19±0.36
5	6.25	7.86	6.64	6.92±0.49	11.07	9.29	7.50	8.93	9.20±0.74
6	5.01	5.01	5.00	5.01±0.01	7.52	6.01	3.34	6.01	5.72±0.87
7	2.67	3.01	2.93	2.87±0.10	5.34	2.84	3.17	5.60	3.78±0.68
8	3.34	4.34	3.15	3.61±0.37	5.34	4.68	4.01	5.18	4.80±0.30
9	4.34	3.67	3.91	3.97±0.20	5.34	4.51	6.35	6.68	5.72±0.50
10	3.09	2.50	3.87	3.15±0.40	6.18	3.09	3.82	6.77	4.97±0.90
11	4.06	2.81	4.40	3.76±0.49	5.94	3.13	3.13	3.75	3.97±0.90
12	4.03	2.55	4.12	3.57±0.51	4.57	4.03	4.57	4.57	4.44±0.14
13	2.15	4.84	4.35	3.78±0.83	3.36	4.57	4.57	4.57	4.27±0.31

Table A2-14: Daily percentage changes in PCV of T. vivax infected Zebu calves infused and uninfused with lactose.

POST-INFECTION TIME (DAY)	Untreated Group				Treated Group				
	1	2	3	MEAN±SEM	1	2	3	4	MEAN±SEM
	PRE-TREATMENT PERIOD								
2	+10	+5	+10	+8.3±1.7	-4	-4.4	0	-3.7	-3.0±1.0
3	-9	0	-4.5	-4.5±2.6	-4.2	0	-8	-3.6	-4.0±1.6
4	0	-5	-4.8	-3.3±1.6	0	+13.5	+8.8	0	+5.6±3.4
5	+5	-5	-10	-3.3±4.4	-4.4	-12.0	-12	-3.7	-8.0±2.3
6	-9.5	-11	-11	-10.5±0.5	-18.2	-4.5	-9	-8.4	-10.0±2.9
CUMULATIVE CHANGE	-3.5	-16	-20.3	-13.3±5.1	-30.8	-7.4	-20.2	-19.4	-19.5±4.8
	TREATMENT PERIOD								
7	0	-12.6	-6.3	-6±3.6	-4.9	-8.8	+10.8	+9.7	+1.7+4.9
8	-10.6	-14.2	-26.8	-17.2±4.9	+5.7	-4.8	-3.4	-10.3	-3.2±3.3
9	+11.8	+16.	+27.3	+18.6±4.6	-10.4	+4.5	+8.6	-1.4	+5.5±2.6
10	-5.3	6 +7.1	-7.1	-1.8±4.5	-4.6	+0.7	-12.9	+0.7	-4.0±3.2
CUMULA-TIVE CHANGE	-6.5	-3.1	-12.9	-7.5±2.9	+6.70	- 8.35	+3.1	-1.4	+0.012±3.2
	PERIOD AFTER TREATMENT								
11	0	0	+7.7	+2.6±2.6	-5.6	11.1	0	-4.8	-5.4±2.3
12	+5.6	+33.3	+35.7	+24.0±9.7	+5.9	+5.9	-5.3	0	+1.6±2.7
13	21.1	-15.0	-21.1	-19.1±2.0	-11.1	-5.6	-5.6	-15.0	-9.4±2.3
CUMULA-TIVE CHANGE	-15.5	+18.3	+22.3	+8.4±12.0	-10.8	-11.1	-10.9	-19.8	-13.2±2.2

Table A2-15: Daily percentage changes in total RBC count of T. vivax-infected Zebu calves infused and uninfused with lactose

POST-INFEC-TION TIME (DAYS)	UNTREATED GROUP				TREATED GROUP				
	1	2	3	Mean ± SEM	1	2	3	4	Mean ± SEM
	PRE-TREATMENT PERIOD								
2	-10.8	+ 3.3	+ 5.5	-0.7 ± 5.1	+2.8	0	-1.9	+2.0	+0.7± 1.1
3	- 7.5	-11.7	- 4.2	-7.8 ± 2.2	-8.1	+7.8	-8.1	-23.2	-3.9 ± 7.5
4	+ 1.5	+ 1.0	+ 3.5	+2.0 ± 0.8	-1.2	+18.7	+7.0	+8.9	-8.4 ± 4.1
5	-14.7	+ 7.8	-16.5	-7.8 ± 7.8	-6.8	-14.7	-5.9	-0.5	-7.0 ± 2.9
6	- 2.5	- 5.6	0	-2.7 ± 1.6	-14.3	-17.5	-22.6	-25.6	-20.0± 2.5
Cumm Change	-34.0	- 5.2	-11.7	-17.0 ± 8.7	-27.6	-5.7	-31.5	-38.4	-25.8± 7.1
	TREATMENT PERIOD								
7	+8.8	-11.9	-6.3	-3.1 ± 6.2	-9.1	-10.9	+27.9	+25.0	+8.2±10.6
8	-9.5	-26.0	-47.0	-27.5± 10.9	+4.4	+24.1	-20.6	-14.5	-1.7±10.1
9	+14.7	+58.0	+99.0	+57.2± 24.4	+11.5	-8.8	+32.8	+8.0	+10.9±8.6
10	-9.2	-27.3	+3.7	-10.9± 9.0	-20.5	-15.2	-19.7	-12.2	-16.9±2.0
Cumm Change	+4.8	-7.2	+49.4	+15.7± 17.2	-13.7	-10.8	+20.4	+6.3	+0.6±8.0
	PERIOD AFTER TREATMENT								
11	0	+3.2	+5.9	+3.0±1.7	+13.6	+5.9	+12.1	+19.8	+12.9±2.9
12	+33.3	+44.6	+2.9	+26.9±12.5	+4.7	+16.0	-10.0	-7.3	+0.9 ±6.0
13	-22.4	-8.3	-2.0	-10.9 ± 6.0	+7.9	-10.8	-4.9	-4.6	-3.1 ±3.9
Cumm Change	+10.9	+39.5	+6.8	+19.1 ±10.3	+26.2	+11.1	-2.8	+7.9	+10.6 ±6.0

Table A2-16: Daily Mean percentage changes and mean Cummulative Percentage Changes in total RBC count of T.Vivax-infected Zebu calves before, during and after lactose infusion in one Group of the infected animals.

	Percentage Change in RBCC before Lactose Infusion (Mean ± SEM)							Percentage change in RBCC during. Lactose Infusion (Mean ± SEM)					Percentage change in RBCC after Lactose Infusion was stopped (Mean ± SEM)			
Post-Infection Time (Days)	1	2	3	4	5	6	Cup-erc	7	8	9	10	Cup-erc	11	12	13	Cup-erc
Infected. uninfused Group (n=3)	-	-0.7 ±5.1	-7.8 ±2.2	−2.0 ±0.8	-7.8 ±7.8	-2.7 ±1.6[a]	-17.0 ±8.7	-3.1 ±6.2	-27.5 ±10.9[a]	+51.2 ±24.4[a]	-10.9 ±9.0	−15.7 ±17.2	−3.0 ±1.7[a]	+26.9 ±12.5[a]	-10.9 ±6.0[a]	+19.1 ±10.3
Infected. Lactose-infused Group (n=4)	-	+0.7 ±1.1	-3.9 ±7.5	−8.4 ±4.1	-7.0 ±2.9	-20.0 ±2.5[b] (P<0.005)	-25.8 ±7.1	−8.2 ±10.6	-1.7 ±10.1[b] (P<0.01)	+10.9 ±8.6[b] (P<0.01)	-16.9 ±2.0	+0.6 ±8.0	−12.9 ±2.9[b] (P<0.005)	−0.9 ±6.0[b] (P<0.01)	-3.1 ±3.9[a] (P<0.05)	+10.6 ±6.0

Cuperc = Mean Cummulative percentage change in RBC Count during the period indicated. Positive and negative signs indicate increases and decreases, respectively. Comparison was done down the columns and values with different superscripts are satistically different at the confidence limits indicated.

Table A2-17: Percentage changes in the mean Total Leukocyte counts in all Groups.

Sampling Time (Days)	% Changes in Mean Total Leukocyte Counts (%)			
	Uninfected Uninfused Group	Uninfected infused Group	Infected Uninfused Group	Infected. Infused Group
2	-2	0	+2	-18
3	+2	-1	-12	-34
4	+2	0	-17	-26
5	-2	0	-19	-29
6	-4	+3	-24	-27
7	+1	+8	-25	-34
8	0	+6	-39	-52
9	+6	+18	-26	-22
10	+6	+23	-38	-35
11	+4	+22	-29	-36
12	+5	+13	-2	-18
13	+2	+6	-31	-30
Mean ± SEM	+1.67 ±0.90[a]	+8.17 ±2.44[b]	-21.58 ±3.61[c]	-30.08 ±2.59[d]

Positive and negative signs indicate increases and decreases, respectively compared to pre-infection values. Values with different superscripts are significantly ($p<0.005$) different.

Table A2-18: Percentage changes in mean Absolute Lymphocyte, Neutrophil, Monocyte and Eoisinophil counts in T. vivax-infected Zebu calves Infused and not Infused with Lactose.

POST-INFECTION TIME (DAYS)		2	3	4	5	6	7	8	9	10	11	12	13	Mean ± SEM
Lymphocytes (%)	Uninfused	-14	-12	-37	-31	ND	-6	-36	-9	-30	-24	-13	-7	-16.09±5.16[a]
	Infused	-17	-41	-37	-36	ND	-25	-48	-14	-33	-31	-1	-26	-28.09±4.05[b] (P<0.005)
Neutrophils (%)	Uninfused	-35	-27	+24	+5	ND	-51	-66	-76	-68	-50	-	-17	-36.10±9.88[a]
	Infused	-30	-28	-9	-16	ND	-53	-61	-47	-44	-51	-55	-40	-39.46±5.04[a]
Monocytes (%)	Uninfused	-	-27	+11	-114	ND	-38	-	+3	-68	-60	-	-54	-13.22±16.81[a]
	Infused	+91	-127	-155	+91	ND	-78	-85	-287	+73	-136	-26	-118	+80.82±32.89[b] (P<0.0005)
Eoisinophils (%)	Uninfused	-59	+14	+7	-64	ND	-	-14	-14	-39	-52	-25	-30	-15.80±10.76[a]
	Infused	-83	+25	+4	-61	ND	-25	-31	-67	-25	-50	-17	-69	-19.55±13.65[a]

Positive and negative signs indicate increases and decreases respectively; mean values (for each parameter) with different superscripts are statistically different.

ND = Not determined (prepared slides were lost by accident)
- = Not detected in slide.

Table A2-19: The percentage changes in mean plasma total proteins in all groups

Sampling Time (DAY)	% CHANGES IN MEAN PLASMA TOTAL PROTEINS (%)			
	UNINFECTED, UNINFUSED GROUP	UNINFECTED, INFUSED GROUP	INFECTED, UNINFUSED GROUP	INFECTED, INFUSED GROUP
2	-1	0	+5	+7
3	-4	+2	-13	+3
4	+2	+1	-11	+5
5	+1	+2	-9	-4
6	+2	+2	-13	+1
7	-3	+2	-14	+7
8	-1	0	-12	+2
9	-9	-4	-7	+7
10	-13	-2	-13	+6
11	-4	-2	-12	+2
12	-1	+1	-9	+9
13	-7	+1	-9	0
Mean±SEM	-2.67±1.29[a]	+0.25±0.54[a]	-9.75±1.42[b]	+3.75±1.04[a]

Positive and negative signs indicate increases and decreses, respectively; values with different superscripts are significantly (P<0.005) different.

Table A2-20. The mean percentage changes in the mean total, absolute differential leukocyte counts and total plasma proteins.

PARAMETERS	MEAN ± SEM PERCENTAGE CHANGES IN THE PARAMETERS*			
	Healthy, uninfused group	Healthy, infused group	Infected, Uninfused group	Infected, infused group
Total leukocyte count	+1.67.0.±90[a]	+8.17±2.44[b]	-21.58±3.61[c]	-30.08±2.59[d]
Absolute Lymphocyte count	ND	ND	-16.09±5.16[a]	-28.09±4.05[b] (P<0.0005)
Absolute neutrophil count	ND	ND	-36.10±9.88	-39.46±5.04
Absolute monocyte count	ND	ND	-13.22±16.81[a]	+80.82±32.89[b] (P<0.0005)
Absolute eoisinophil count	ND	ND	-15.80±10.76	-19.55±13.65
Total Plasma proteins	-2.67±1.29[a]	0.25±0.54[a]	-9.75±1.42[b]	+3.75±1.04[c]

Positive and negative signs indicate increase and decreases, respectively; values with different superscripts are statistically different at the state confidence limit or P<0.005. ND = Not determined

* All the values are mean percentage changes from the pre-infection values throughout the duration of the experiment.