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EFFECT OF T.B. BRUCEI INFECTION ON TOTAL LIPID PROFILE OF RAT ERYTHROCYTE GHOST MEMBRANE.

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ABSTRACT

White albino rats were infected with 10^6 *T. b. brucei* parasites (*Federe/CT/28/NIIR)* per rat and levels of parasitaemia and packed cell volume (PCV) were monitored daily. Erythrocytes were harvested and ghost membranes prepared daily for total phospholipid and neutral lipid analysis. The result showed a significant decrease in PCV values with increase in parasitaemia ($P < 0.01$). There was also a significant variations in the total lipid components of erythrocyte ghost membranes of infected animals and values such as 79.6% and 104.2% of control values for total phospholipid and neutral lipid respectively were obtained ($P<0.05$). This implies an abnormal lipid composition of erythrocyte membrane which can cause it's damage resulting into anaemia.

INTRODUCTION

Anaemia is recognised as being one of the most important features of clinical trypanosomiasis in both man and the domestic animals (Kobayashi et al, 1976). It is possible that death occurs as a direct result of a severe haemolytic crisis (Losos and Ikede, 1972).

Haemolytic activity has been demonstrated in lysates of *T. brucel* as well as in *T. congolense* and *T. vivax* (Murray et al., 1979). However, the nature of the haemolytic factors particularly those responsible for the haemolytic activity in the plasma are yet to be extensively investigated. A range of enzymes that play a role in red cell damage has now been identified in African trypanosomes. These include proteases (Lonsdale-Eccles and Grab. 1986). Phospholipases (Mellors, 1985) and neuraminidases (Esievo, 1983). There could be more factors yet unknown. Only the proteolytic and perhaps membrane fraction phospholipase activities have been demonstrated as originating from trypanosomes by using extracellular fractions derived from the organism (Londsdale-Eccles and Grab. 1983).

Membranes of cells and intracellular organelles are highly organised systems composed mainly of proteins and lipids. Red cell destruction must necessarily be preceded by changes on the membrane component thereby making it susceptible to lytic agents.

Lipid play a very essential role in maintaining the integrity of membranes.

This work is aimed at determining the total phospholipid and neutral lipid present on erythocyte ghost membranes at various levels of T. b. brucel infection with a view to assessing the effect of trypanosome infection on the profile of these lipids and hence its effect on haemolysis.

MATERIALS AND METHODS

Trypanosoma brucel brucel (Federe/CT/28/NITR) originally isolated from cattle and since then kept alternatively in liquid nitrogen was used It was sub-passaged thrice in rats before use for this work.

White albino rats *(Rattus norvergicus)* weighing between 200-250g were obtained from the NITR small animal house.

Infecting rats

Rats were kept in 8 groups of 5 animals each in cages and fed with rat pellets and water *ad libitum*. Except the control group, the rats were injected with 10^6 *T. b. brucel* per rat intraperitoneally (Baker, 1970).

Blood sample collection

Rats from each group were sacrificed daily and blood pooled together except in the last group where only one rat survived. Rats were anaesthetized and blood collected by cardiac puncture using anticoagulant. Packed cell volume (PCV) and parasitaemia were estimated as described by Herbert and Lumsden, (1976)

Lipid profile analysis

Erythrocyte ghost membrane were obtained essentially using the method described by Ralstron, (1976) as modified by Sodipo (1989). The phospholipid and neutral lipid of ghost membrane were extracted and quantified using the methods of Folch et al (1957) and Amenta (1964) methods respectively.

RESULTS AND DISCUSSION

Fig. 1 shows a plot of changes in values of PCV and parasitaemia with days of infection. The result obtained with control (uninfected) rats are presented as day 0 while those on days 1-7 are infected with *T. b. brucel* and infection allowed to progress from day 1 to 7.

As parasite used in this study is a virulent one. As the parasitaemia increases 1,2,3 and 4 rats died on days 4,5,6 and 7 respectively. The PCV values also decreases. Anaemia therefore developed rapidly in infected rats with the PVC values dropping significantly ($P<0.01$) from an average value of 0.27 + 0.36 of control rats to values as low as 0.16 + 0.05 of control rat in the last group.

It was also observed from Figure 2, that there was fluctuations in amount of total phospholipids and neutral lipids. The highest and lowest value of total phospholipid were recorded on days 3 and 7 respectively while that of neutral lipid has days 6 and 2 respectively. Thus by day 3, the total phospholipid has increased significantly to 132.8% which dropped to 79.6% of control value on day 7 ($P<0.05$). Similarly the total neutral lipid has significantly increased by day 6 to 104.2% of control value ($P<0.05$) for only 2 survived rats on day 6.

T. brucel and *T. congolense* have been shown earlier to generate an active phospholipase A on autolysis (Mellors, 1985). While phospholipases themselves may have a direct effect on red cell membrane, their main activity would appear to be through their action on endogenous phosphatidylcholine with the release of large quantities of free fatty acids (Mellors, 1985). The result obtained in this study agreed with this finding since on day 6, many trypanosomes were autolysing, thereby generating phospholipase which act on erythrocyte ghost membrane by hydrolysing

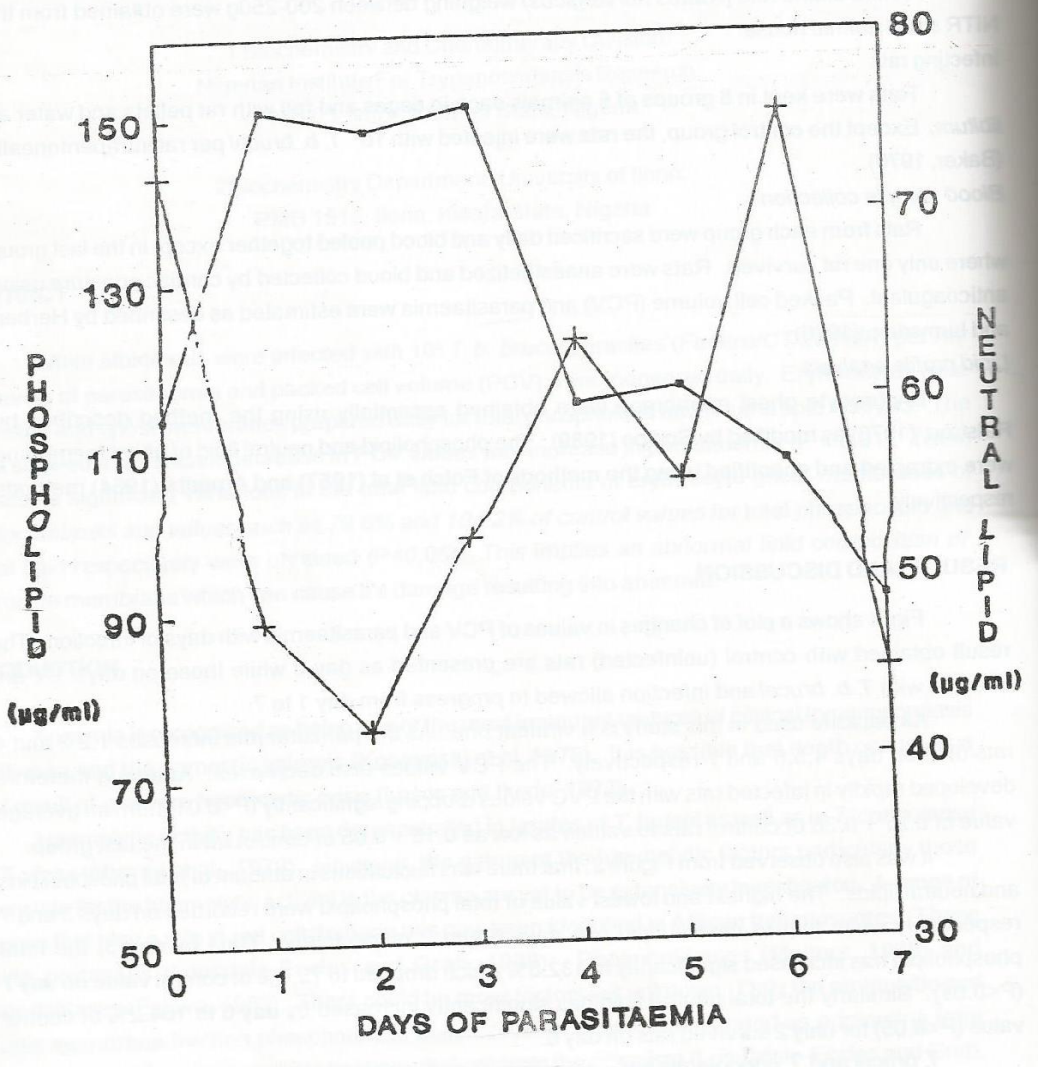

FIG.2 TOTAL PHOSPHOLIPID AND NEUTRAL LIPID IN ERYTHROCYTE GHOST MEMBRANE

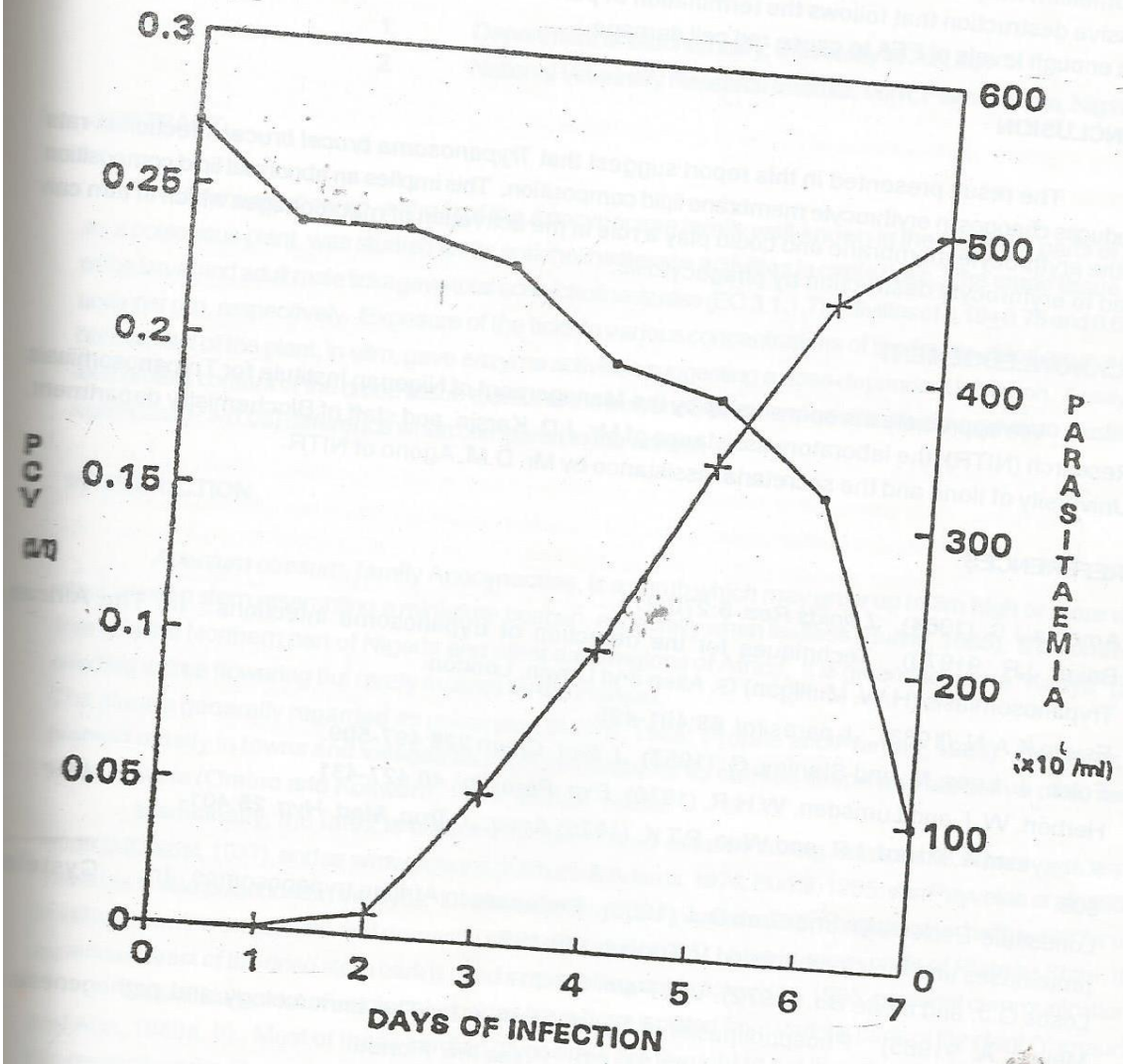

FIG.1 pcv and parasitaemia of rats infected with *T. BRUCEI*

phospholipid releasing large quantities of free fatty acid. This may therefore give an account of the observe decrease in the value of total phospholipid and increase is total neutral lipid of day 6. Furthermore, it was also observed by Murray and Dexter (1988) that, trypanosomes particularly *T. congolense* tend to congregate in dense clusters in capillary beds attaching to red cells or vascular endothelium may reach sufficiently high concentrations to provoke damage. In the same way, the massive destruction that follows the termination of parasitaemic wave could result in a transient but high enough levels of FFA to cause red cell damage.

CONCLUSION

The result presented in this report suggest that *Trypanosoma brucel brucel* infection in rats produces changes in erythrocyte membrane lipid composition. This implies an abnormal lipid composition of the erythrocyte membrane and could play a role in the activation of macrophages which in turn can lead to erythrocyte destruction by phagocytosis.

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REFERENCES

Amenta J.S. (1964). *J. lipids Res.* **5**:270-275.

Baker J.R. 91970). Techniques for the detection of trypanosome infections. In: The African Trypanosomiasis (H.W. Mulligan) G. Allen and Unwin, London.

Esiero K.A.N. (1983). *J. parasitol.* **69**:491-495.

Folch, J., Lees, M. and Stanley, G. (1957). *J. Biol. Chem* **226**:497-509.

Herbert, W.J. and Lumsden, W.H.R. (1976). *Exp. Parasitol*, **40**:427-431.

Kobayashi A; Tizard, I.R. and Woo, P.T.K. (1976) *Amer. J. Trop, Med. Hyg.* **25**:401-606.

Londsdale-Eccles J.D. and Grab D.J. (1986). Preteases in African trypanosomes In: Cysteine proteinases and their inhibitors ed V. Turk pp 189-195.

Losos G.J. and Ikede Bo. 91972). *Vet. Path.* 9:1-71.

Mellors A. 91985). Phospholipases in trypanosomes In: The immunology and pathogenesis of trypanosomiasis ed. I.R. Tizard pp. 67-74. CRC Press Inc. Florida.

Murray, M. and Dexter T.M. (1988). *Acta Tropica* 45:389-432.

Murrary, M., Clifford D.J. and Bray R.S. (1979). Cattle disease and trypanosomiasis in Gambian Clinical studies. OAU/STRC. Pub. No. 110 pp. 83-91.

Ralstron G.B. (1976). *Biochem. Biophys. Acta.* **455**: 1563-1072.

Sodipo O.A. (1989). A biochemical study of endogenous saponins of Garciniakola, Heckel Ph.D. Thesis, University of Ilorin.