Some plasma biochemical changes in experimental *Trypanosoma brucei* infection of Sokoto Red goats

I.O. Igbokwe [1]

A. Mohammed [2]

IGBOKWE (I.O.), MOHAMMED (A.). Variations biochimiques plasmatiques mises en évidence chez des chèvres Red Sokoto infectées expérimentalement par *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 287-290

Les variations biochimiques plasmatiques ont été étudiées durant 8 semaines consécutives chez des chèvres Red Sokoto expérimentalement infectées par $1{,}6.10^7$ *Trypanosoma brucei* injectés par voie intraveineuse. Le pouvoir infectieux de la souche 8/18 s'est révélé élevé. L'hématocrite moyen a diminué de façon significative entre la 1ère et la 8e semaine après l'infection (post-infection PI) ($P < 0{,}05$). Durant cette période, les concentrations plasmatiques moyennes en bilirubine totale ont augmenté significativement ($P < 0{,}05$). Les taux moyens de bilirubine conjuguée (directe) et de bilirubine libre (indirecte) ont augmenté significativement de la 2e à la 8e semaine PI ($P < 0{,}05$). L'albuminémie moyenne n'a pas varié de façon significative ($P > 0{,}05$), mais les concentrations moyennes en protéines plasmatiques totales et en globulines ont augmenté significativement entre la 5e et la 8e semaine PI ($P < 0{,}05$). Aucune modification significative des concentrations moyennes en bicarbonates, de la créatininémie et de la cholestérolémie n'a été notée ($P > 0{,}05$). *Mots clés* : Chèvre Red Sokoto - Trypanosomose - *Trypanosoma brucei* - Infection expérimentale - Modification hématologique - Plasma sanguin - Nigeria.

Introduction

Trypanosoma brucei infections have been reported to cause disease in ruminants, horses, dogs, cats and rodents. The level of parasitaemia in infected domestic animals is not related to the course of the disease, whereas massive parasitaemia could be related to an acute course in rodents (15). The parasite is known to invade the connective tissues causing foci of degeneration and necrosis of interstitial and parenchymatous cells as well as to induce an extensive infiltration by lymphocytes, macrophages and plasma cells (15). LOSOS and IKEDE (15) had wondered whether a tendency for the parasite to invade a particular organ depended on the infected host species. REGENDANZ and HOEPPLI (20) suggested that the pathological changes in trypanosomosis could depend not only on the duration of infection but also on the species of the infected host.

Pathological changes in the organs lead to alterations in their functions. It was the opinion of SEED and HALL (22) that greater emphasis should be given to the physiological changes which occur in the infected host in order to reach a better understanding of patient management. Therefore, they summarized reports on the changes in the biochemical parameters of infected laboratory animals which indicated liver dysfunction (22).

1. Department of Veterinary Pathology, University of Maiduguri, PMB 1069, Maiduguri, Nigeria.

2. Department of Veterinary Surgery and Reproduction, University of Maiduguri, PMB 1069, Maiduguri, Nigeria.

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Goats have been used extensively in the investigation of the infectivity and virulence of various isolates of *T. brucei* (15).

Studies on the plasma or serum biochemical changes in *T. vivax* and *T. congolense* infected goats have already been reported (2, 10, 14). The present study refers to some plasma biochemical changes in *T. brucei* intravenous experimental infection of Sokoto Red goats in Northern Nigeria.

Materials and Methods

Male Sokoto Red goats aged 8-12 months and weighing 12-14 kg were used. They were purchased from the local livestock market and housed in fly-proof concrete floored pens. They were watered *ad libitum* and fed with *Acacia albida* pods and native hay prepared form *Arachis hypogaea* (groundnut) and *Vigna unguiculata* leaves (cowpea). They were screened for infections by physical, blood and faecal examinations and were treated with thiabendazole (Thibenzole^R, MSD) orally at 50 mg/kg body weight and kept under observation for 6 months before experiment.

Four goats were infected intravenously with 1.6×10^7 of *Trypanosoma brucei* strain 8/18 obtained from the National Institute for Trypanosomiasis Research, Vom, Nigeria. Another group of 4 goats served as uninfected controls. Infection period was 8 weeks after which the animals were treated with diminazene aceturate* at 7 mg/kg body weight.

Eight millilitres of blood were collected once a week on weeks 1, 2, 5 and 8 from the jugular vein of the control and infected goats into plastic containers with heparin as anticoagulant. Packed cell volume (PCV) was determined by the microhaematocrit method with centrifugation at 12,000 g for 5 min. The plasma was harvested from the blood samples after centrifugation at 3,000 g for 10 min and stored at - 20°C in a freezer before analysis.

Total plasma proteins (TPP) and albumin (Alb) concentrations were measured by biuret and bromocresol green methods, respectively (24). Plasma globulin (Glo) concentration was calculated as the difference between TPP and Alb concentrations. Colorimetric methods were used to measure plasma total bilirubin (TB) and direct bilirubin (DB) concentrations after Van der Bergh's reaction, plasma cholesterol (Cho) concentration after Liebermann-Buchard's reaction and plasma creatinine (Cre) after Jaffe's reaction (26). The plasma indirect bilirubin (IB) concentration was calculated as the difference between TB and DB concentrations. Plasma bicarbonate (HCO^-_3) concentration was measured by a titrimetric method (24).

The data were summarized as mean ± standard deviation (SD) and significant difference was assessed after a paired Student's t-test adapted for small sample size (17).

* Berenil^R, Hoechst A.G., Germany.

Results

The *T. brucei* strain 8/18 was highly infective. The infected goats developed parasitaemia at about 4 days post-infection (PI). The changes in the PCV and plasma biochemical parameters of the control and infected goats are presented in table I. The mean PCV of the infected goats significantly decreased from 1 to 8 weeks PI ($P < 0.05$). The mean plasma total bilirubin (TB) concentration of the infected goats significantly increased from 1 to 8 weeks PI ($P < 0.05$). The mean plasma DB and IB concentrations increased at 1 week PI, but not significantly ($P > 0.05$) and from 2 to 8 weeks PI, both parameters significantly increased ($P < 0.05$). The mean TPP and Glo concentrations of the infected goats significantly increased between 5 and 8 weeks PI ($P < 0.05$) and the plasma Alb was unaffected throughout the period of infection. There were no significant changes in the mean plasma bicarbonate, creatinine and cholesterol concentrations ($P > 0.05$).

Discussion

The erythrocyte changes in this experiment have already been reported by IGBOKWE and MOHAMMED (13).

There was a concurrent hyperbilirubinaemia and as the mean plasma total bilirubin concentration increased, the mean PCV decreased in the infected goats. EDWARDS *et al* (10) reported no change in the plasma bilirubin concentration in trypanosome-infected goats.

The observed hyperbilirubinaemia suggested a severe extravascular haemolysis in the infected goats. The increase in plasma indirect bilirubin could be associated with haemolysis but the increase in plasma direct bilirubin may suggest either hepatic or extrahepatic involvements (7). The liver did not develop obvious lesions in *T. brucei*-infected goats (15) but ultrastructurally, a small number of hepatocytes were found to be degenerated in *T. brucei* infection of mice (4). Furthermore, AROWOLO *et al* (5)

TABLE I Changes in the PCV and plasma biochemical parameters (mean ± SD) of 4 control and 4 **T. brucei** *— infected goats.*

Parameters*	Weeks post-infection				
	0	1	2	5	8
PCV (%)	+ 29.9 ± 3.9 ++ 27.8 ± 0.5	33.0 ± 0.1[a] 25.3 ± 2.2[b]	31.1 ± 1.2[a] 21.0 ± 3.6[b]	30.3 ± 1.8[a] 18.0 ± 2.5[b]	33.0 ± 0.3[a] 13.3 ± 3.8[b]
TB (µmol/l)	8.1 ± 0.7 8.1 ± 0.8	8.5 ± 0.6[a] 10.5 ± 2.0[b]	8.8 ± 0.7[a] 14.1 ± 2.3[b]	8.9 ± 0.9[a] 14.2 ± 1.5[b]	8.4 ± 1.2[a] 21.6 ± 7.9[b]
DB (µmol/l)	3.7 ± 0.8 3.5 ± 0.8	2.2 ± 1.8 4.7 ± 2.5	3.4 ± 0.8[a] 5.8 ± 2.8[b]	2.0 ± 1.8[a] 6.6 ± 3.0[b]	3.9 ± 0.7[a] 11.5 ± 6.5[b]
IB (µmol/l)	4.4 ± 0.8 4.6 ± 0.6	4.9 ± 0.9 5.8 ± 1.5	4.8 ± 0.7[a] 8.4 ± 3.0[b]	4.9 ± 0.8[a] 7.7 ± 1.9[b]	5.1 ± 1.2[a] 10.1 ± 3.0[b]
TPP (g/l)	59.9 ± 12.1 56.5 ± 12.1	67.0 ± 10.9 50.5 ± 14.6	56.0 ± 12.2 57.3 ± 13.2	59.1 ± 11.8[a] 74.0 ± 8.0[b]	68.2 ± 10.7[a] 88.3 ± 6.2[b]
Alb (g/l)	39.4 ± 8.5 38.0 ± 9.1	48.0 ± 7.0 37.8 ± 7.5	42.0 ± 7.2 43.0 ± 6.5	48.0 ± 7.5 47.0 ± 3.2	46.3 ± 6.8 43.3 ± 5.0
Glo (g/l)	19.8 ± 7.0 18.5 ± 7.3	19.2 ± 7.1 12.8 ± 8.3	14.9 ± 8.8 14.3 ± 6.7	15.6 ± 4.8[a] 27.0 ± 9.1[b]	19.2 ± 6.5[a] 45.0 ± 4.1[b]
HCO^-_3 (mmol/l)	25.4 ± 5.0 25.5 ± 5.8	28.0 ± 4.0 24.0 ± 6.2	20.0 ± 5.9 20.3 ± 4.5	20.0 ± 6.1 23.5 ± 8.7	20.8 ± 6.9 19.8 ± 2.6
Cre (mmol/l)	82.0 ± 11.1 80.3 ± 12.0	68.0 ± 19.1 64.8 ± 19.8	89.1 ± 11.0 82.0 ± 18.1	77.8 ± 11.7 79.3 ± 9.5	83.4 ± 10.5 114.8 ± 37.9
Cho (mmol/l)	1.0 ± 0.4 1.0 ± 0.5	1.1 ± 0.4 0.8 ± 0.5	0.9 ± 0.5 0.7 ± 0.2	0.8 ± 0.5 0.5 ± 0.2	1.1 ± 0.4 0.8 ± 0.2

** Packed cell volume (PCV) ; total bilirubin (TB) ; direct bilirubin (DB) ; indirect bilirubin (IB) ; total plasma protein (TPP) ; albumin (Alb) ; globulin (Glo) ; bicarbonate (HCO^-_3) ; creatinine (Cre) ; cholesterol (Cho).*
[a], [b], Mean ± SD with different superscripts are significantly different ($P < 0.05$).
+ control ; ++ infected.

have shown that there may be depressed liver function in *T. brucei* infection of mice after observing an increase in serum alkaline phosphatase and a decrease in serum cholinesterase. MOON *et al* (18) reported an increase in bromosulfophtalein retention in mice infected with *T. rhodesiense* which seemed to suggest a hepatocellular damage ; but it should be noted that bromosulfophtalein clearance could be impeded in hyperbilirubinaemic mice (7).

There have been reports of decreased plasma albumin concentrations in several trypanosome infections (1) and it is thought that the decrease could be due to plasma expansion (1), proteinuria (6) or hepatocellular damage (3, 21, 25). We found that the plasma albumin concentrations in the infected goats were unaltered which seemed to suggest that plasma expansion, proteinuria or hepatocellular damage might not have occurred significantly.

It was observed that while the plasma albumin concentration was unaltered, the total plasma protein concentration increased due to an increase in the plasma globulin concentration in the infected goats (table I). The total plasma protein concentration was reported to be decreased (14), unaltered (10) or increased (2) in trypanosome infections of goats. It was shown that total plasma protein concentration did not increase in trypanosome infected animals with little resistance, but increased in those with more resistance (1). It was proposed that there could be a non-specific polyclonal stimulation of B-lymphocytes in infected animals (16, 27) which leads to the production of greater amounts of immunoglobulins (12). The increase in the plasma globulin fraction in our study was similar to the findings in *T. vivax* infection of goats (2, 14) and in trypanosome infections of other animal species (1).

The review by ANOSA (1) does not contain any report of the acid-base balance in trypanosome infected animals. It was reported that pCO_2 increased concurrently with the plasma creatinine concentration in *T. rhodesiense* infection of mice (18), which seemed to suggest that a renal impairment like glomerulonephritis reported in similar infections (19) might have occurred resulting in the accumulation of organic acid waste products (9). Our study did not show any significant changes in the plasma bicarbonate and creatinine concentrations in the infected goats (table I). These findings seem to suggest that there may not have been considerable renal impairment in the infected goats. LOSOS and IKEDE (15) did not report any renal damage in *T. brucei* infected goats. However, anti-basement membrane glomerulopathy was reported in *T. brucei* infection of mice (6) and plasma creatinine concentration increased in *T. brucei* infection of rabbits (11).

In the present study, plasma cholesterol concentration was unaltered in infected goats. Hypercholesterolaemia has been reported in rabbits infected with *T. gambiense* (8) and *T. brucei* (11). Hypercholesterolaemia was thought to indicate an energy deficit due to liver dysfunction in trypanosome infected animals (22). Hypercholesterolaemia has been associated with liver and kidney diseases (7, 9). The absence of hypercholesterolaemia in our study further suggests that the hepatic and renal dysfunctions may have been negligeable.

Conclusion

We showed that a high *T. brucei* experimental infection of Sokoto Red goats caused hyperbilirubinaemia and hyperproteinaemia due to hyperglobulinaemia. However, no alterations were found in plasma albumin, bicarbonate, creatinine and cholesterol concentrations. These biochemical findings do not seem to suggest any considerable hepatic and renal impairments.

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Plasma biochemical changes were studied for 8 consecutive weeks in Sokoto Red goats experimentally infected by intravenous route injection of 1.6 x 10^7 *Trypanosoma brucei*. The strain 8/18 was highly infective. The mean packed cell volume significantly decreased from 1 to 8 weeks post-infection (PI) at $P < 0.05$. During this period, the mean plasma total bilirubin concentrations significantly increased ($P < 0.05$). The mean plasma direct and indirect bilirubin concentrations significantly increased from 2 to 8 weeks PI ($P < 0.05$). The mean plasma albumin concentrations did not vary significantly ($P > 0.05$), but the mean total plasma proteins and mean plasma globulin concentrations significantly increased between 5 and 8 weeks post infection ($P < 0.05$). There were no significant changes in the mean plasma bicarbonate, creatinine and cholesterol concentration ($P > 0.05$). *Key words* : Sokoto Red goat - Trypanosomosis - *Trypanosoma brucei* - Experimental infection - Haematological change - Plasma - Nigeria.

References

1. ANOSA (V.O.). Haematological and biochemical changes in human and animal trypanosomiasis. Parts I. and II. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (1) : 65-78 ; **41** (2) : 151-164.
2. ANOSA (V.O.), ISOUN (T.T.). Serum proteins, blood and plasma volumes in experimental *T. vivax* infection of sheep and goats. *Trop. Anim. Hlth Prod.*, 1976, **8** : 11-19.
3. ANOSA (V.O.), ISOUN (T.T.). Pathology of experimental *T. vivax* infection of sheep and goats. *Zentbl. Vet. Med. (B)*, 1983, **30** : 685-700.
4. ANOSA (V.O.), KANEKO (J.J.). Pathogenesis of *T. brucei* infection in deer mice (*P. maniculatus*). Ultrastructural pathology of the spleen, liver, heart and kidney. *Vet. Path.*, 1984, **21** : 229-237.
5. AROWOLO (R.O.A.), ELHASSAN (E.O.), AMURE (B.O.). Assessing hepatic dysfunction in rabbits experimentally infected with *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (3) : 277-281.
6. BRUIJN (J.A.). Anti-basement membrane glomerulopathy in experimental trypanosomiasis. *J. Immunol.*, 1987, **139** : 2482-2488.
7. COLES (E.M.). Veterinary clinical pathology. 3rd ed. Philadelphia, London and Toronto, W.B. Saunders Company, 1980.
8. DIEHL (E.J.), RISBY (E.L.). Serum changes in rabbits experimentally infected with *T. gambiense*. *Am. J. Trop. Med. Hyg.*, 1974, **23** : 1019-1022.
9. DUNCAN (J.R.), PRASSE (K.W.). Veterinary Laboratory Medicine. Clinical Pathology. Ames, Iowa, The Iowa State University Press, 1977.
10. EDWARDS (E.E.), JUDD (J.M.), SQUIRE (F.A.). Observations on trypanosomiasis in domestic animals in West Africa. II. The effect on erythrocyte sedimentation rate, plasma protein, bilirubin, blood sugar, osmotic fra-

gility, body weight and temperature in goats and sheep infected with *T. vivax, T. congolense* and *T. brucei*. *Ann. Trop. Med. Parasit.*, 1956, **50** : 242-251.

11. GOODWIN (L.G.), GUY (M.W.). Tissue fluid in rabbits infected with *Trypanosoma (Trypanozoon) brucei*. *Parasitology*, 1973, **66** : 499.

12. GREENWOOD (B.M.), WHITTLE (H.C.). The pathogenesis of sleeping sickness. *Trans. R. Soc. Trop. Med. Hyg.*, 1980, **6** : 716.

13. IGBOKWE (I.O.), MOHAMMED (A.). The reticulocyte response to the anaemia in goats caused by experimental *Trypanosoma brucei* infection. *Vet. Res. Comm.*, 1991, **15** : 373-377.

14. KALU (A.U.), IKWUEGBU (O.A.), OGBONNAH (G.A.). Serum protein and electrolyte levels during trypanosome infection and following treatment in the West African Dwarf goats. *Bull. Anim. Hlth Prod. Afr.*, 1989, **37** : 41-45.

15. LOSOS (G.J.), IKEDE (B.O.). Review of pathology of disease in domestic and laboratory animals caused by *T. congolense, T. vivax, T. brucei, T. rhodesiense* and *T. gambiense*. *Vet. path.* (Suppl.), 1972, **9** : 1-71.

16. MANSFIELD (J.M.). Immunobiology of African trypanosomiasis. *Cell. Immun.*, 1978, **39** : 204-210.

17. MEAD (R.), CURNOW (R.N.). Statistical methods in agriculture and experimental biology. London, Chapman and Hall, 1983.

18. MOON (A.P.), WILLIAM (J.S.), WITHERSPOON (C.). Serum biochemical changes in mice infected with *T. rhodesiense* and *T. duttoni*. *Exp. Parasitol.*, 1968, **22 :** 112-121.

19. NAGLE (R.B.), DONG (S.), JANACEK (L.L.), GUILLOT (J.M.), LINDSLEY (H.B.). Glomerular accumulation of monocytes and macrophages in experimental glomerulonephritis associated with *T. rhodesiense*. *Lab. Invest.*, 1982, **46** : 365.

20. REGENDANZ (P.), HOEPPLI (R.). Die Einwanderung der Trypanosomen in das Gewebe und die dadurch bewirkten histopathologischen Veränderungen. *Arch. Schiffs-Tropenhyg.*, 1929, **33** . 376-387.

21. SAROR (D.I.). Observations on the course and pathology of *T. vivax* in Red Sokoto goats. *Res. Vet. Sci.*, 1980, **28** : 36-38.

22. SEED (J.R.), HALL (J.E.). Pathophysiology of African trypanosomiasis. *In* : TIZARD (I.). Immunology and pathogenesis of trypanosomiasis. Boca Raton, Florida, CRC Press Inc., 1985.

23. SMITH (H.A.), JONES (T.C.), HUNT (R.D.). Veterinary Pathology. 4th ed. Philadelphia, Lea and Febiger, 1972.

24. TIETZ (N.W.). Fundamentals of Clinical chemistry. Philadelphia, W.B. Saunders, 1976.

25. VAN DEN INGH (T.S.), ZWART (D.), SCHOTMAN (A.J.H.), VAN MIERT (A.S.), VEENENDAAL (G.H.). The pathology and pathogenesis of *T. vivax* infection in the goat. *Res. Vet. Sci.*, 1976, **21** : 264-270.

26. VARLEY (H.), GOWENLOCK (A.H.), BELL (M.). Practical clinical biochemistry. Vol. I. 5th ed. London, William Heinemann Medical Books, 1980.

27. WERY (M.), MULUMBA (P.M.), LAMBERT (P.H.), KAZYLUMBA (L.). Hematological manifestations, diagnosis and immunopathology of African trypanosomiasis. *Sem. Hemat.*, 1982, **19** : 83-92.