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Artikel: Free plasma amino acid profiles of normal and "Trypanosoma brucei"-infected rats : short communication

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Free plasma amino acid profiles of normal and *Trypanosoma brucei*-infected rats

Short communication

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Attempts have been made to grow *Trypanosoma brucei* in semi-defined or defined media (Cross and Manning, 1973). The trypanosomes, under in vitro conditions transform rapidly to culture forms. Although success had been reported for the in vitro cultivation of bloodstream forms of strain *T. brucei* 427, using a complex medium with 20% foetal calf serum and bovine fibroblast-like cells (Hirumi et al., 1977), there is great need, for biochemical, immunological and pharmacological purposes, to devise semi-defined or defined media in which *T. brucei* can multiply and yet maintain the bloodstream (in vivo) properties. A knowledge of the plasma amino acid pattern of the plasma of normal and *T. brucei*-infected rats may serve as a rational clue or starting point to devise a medium for the in vitro cultivation of the blood stream forms of *T. brucei.*

There is scant or no information on the amino acid profile of rats under the stress of *T. brucei*-induced trypanosomiasis.

It is the purpose of these preliminary studies to investigate the amino acid profile of the plasma of normal and *T. brucei*-infected rats and also that of the blood stream form of the *T. brucei* organism itself.

The rats used in these experiments were inbred strains of albino rats; they have been under strict veterinary supervision. The rats showed no clinical evidence of disease. Haemoglobin and white blood cell counts of the rats were within normal range. Two rats were each inoculated intraperitoneally with 5×10^6 of a monomorphic strain of *T. brucei* (EATRO no. 1713). Blood for analysis was collected at terminal parasitaemia, 4 days post inoculation. Two other rats served as non-infected controls. Parasitaemia was checked daily.

Five ml of blood were obtained in heparinized syringes from infected and control rats. The blood was spun in a refrigerated centrifuge at 5,000 *g* for 20 min. The plasma was collected and deproteinized with sulphosalicylic acid. Norleucine was added as the internal standard. For purposes of obtaining *T. brucei* from whole blood, 10 ml of blood was obtained from a rat with high

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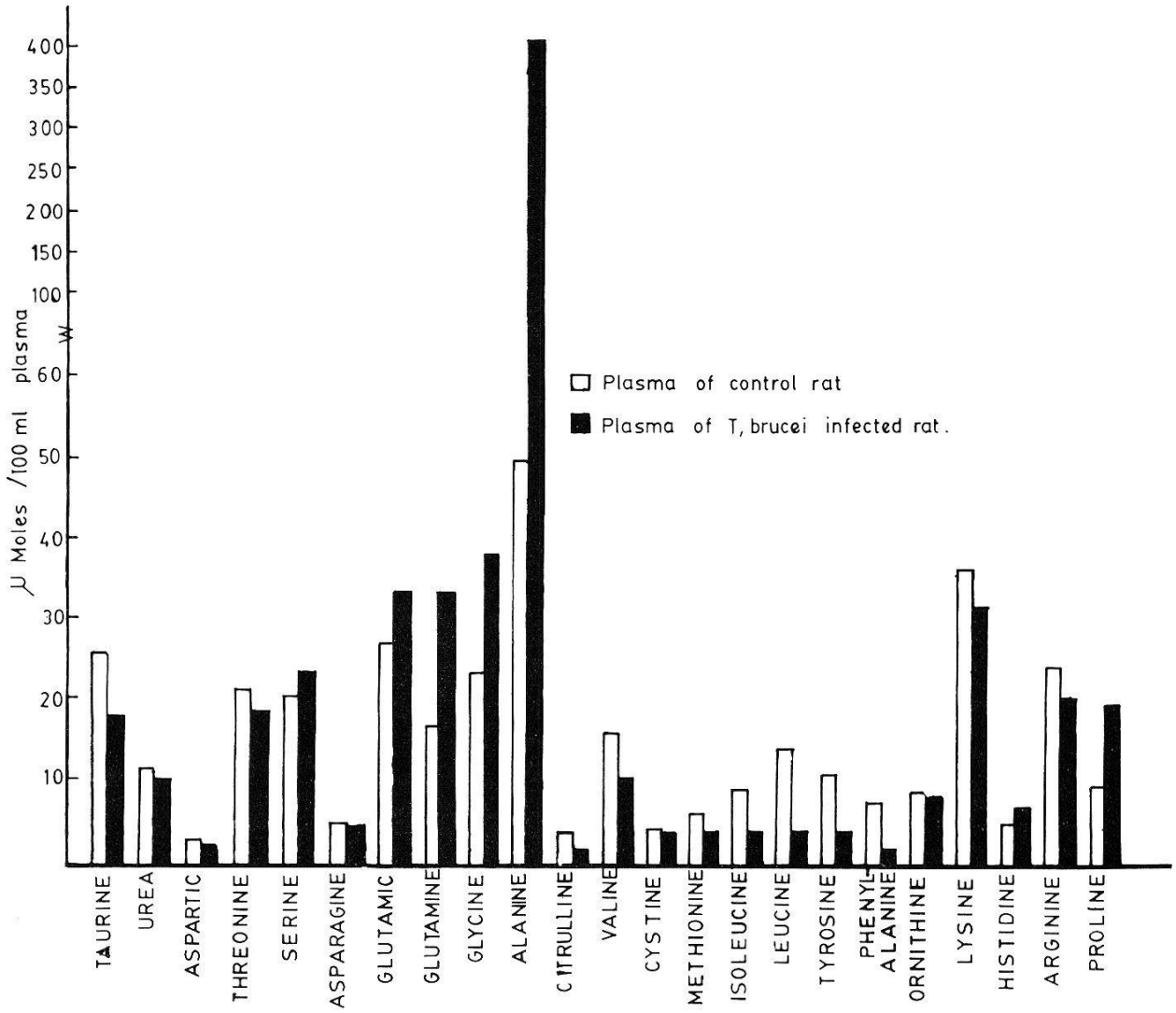

Fig. 1. Plasma amino acid profile of *T. brucei* infected rats.

parasitaemia. Trypanosomes were eluted from whole blood through DEAE cellulose column by the method of Lanham (1968) and washed in saline.

The trypanosomes (2.94×10^8) thus obtained were disrupted by repeated freezing and thawing. The lysate was centrifuged to remove nucleic acid and other cell debris. The supernatant was deproteinized with sulphosalicylic acid. Norleucine was added as the internal standard. The plasma and trypanosome amino acids were determined using an automatic amino acid analyser (Locarte Co. Ltd).

Alanine, glutamine, glycine and proline were markedly elevated; but serine, histidine and glutamic acid were also marginally increased in the plasma of rats infected with *T. brucei*. Alanine values in infected rats were raised over six fold of those in the plasma of control non-infected rats. There was about two fold decrease in the concentrations of isoleucine, leucine, tyrosine, phenylalanine in the plasma of infected rats as compared to the values in the non-infected controls. The decrease in the values of threonine, cystine, ornithine, taurine,

Table 1. Amino acid profile of *T. brucei** (μmoles/294.0$\times 10^6$ organisms)

Amino acid	0.31
Threonine	0.68
Serine	0.79
Glutamic acid	0.79
Glutamine	2.50
Glycine	3.27
Alanine	17.38
Leucine	0.27
Arginine	2.95

* 294.0$\times 10^6$ *T. brucei* were utilized for the analysis in duplicate samples. The figures represent an average of the duplicate samples. The range between the two samples was always less than 10%.

aspartic acid, asparagine, methionine, lysine and arginine in the plasma of infected rats as compared to non-infected controls, was marginal (Fig. 1).

Of the amino acids measurable, in this study, in the *T. brucei* organism, the concentration of alanine was the highest: 17.38, followed by glycine 3.27, arginine 2.95 and glutamine 2.50 μ moles/294$\times 10^6$ organisms respectively (Table 1).

The increase in the concentration of alanine in the plasma of *T. brucei*-infected rats as compared to non-infected controls is remarkable; this increase is undoubtedly much greater than the increase in the values of alanine in the plasma of *Trypanosoma vivax*-infected sheep (24 μ moles/100 ml) as compared to 20 μ moles/100 ml in control sheep (Isoun et al., in press). It would appear that the bloodstream forms of *T. brucei* secrete large quantities of alanine to the plasma of the host. A high intracellular pool of alanine in *T. brucei* (Williamson, 1964) had been previously reported. The large quantities of alanine may be brought about by the transamination of pyruvic acid – an endproduct of glucose metabolism in the blood stream forms of *T. brucei*. The presence of amino transferases in *T. brucei* has been reported (Kilgour and Godfrey, 1973).

Citrulline, a non protein amino acid, is present in only trace amounts in the plasma of normal and *T. brucei*-infected rats; it is not present in the amino acid profile of *T. brucei* organism per se. This is in contrast to the high values of citrulline in the plasma of the normal and *T. vivax*-infected sheep and also in the amino acid profile of *T. vivax* itself (Isoun et al., in press).

The distortion of plasma amino acids: elevation of serine, glutamic acid, glutamine, glycine, alanine, proline – all *non-essential* amino acids, and the depression of methionine, isoleucine, leucine, tyrosine, phenylalanine, most of them *essential* amino acids, in the plasma of *T. brucei*-infected as compared to non-infected control rats may affect, positively, the absorption of amino acids by *T. brucei* and adversely, the protein synthesis and amino acids metabolism of various organs of the infected rats.

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