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Opinion

The activity of crude bromoacetyl-L-carnitine preparations against *Trypanosoma brucei* and the roles of threonine/pyruvate in non-hexose/glycerol ATP production

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The *Trypanosoma brucei* group trypanosomes (*Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*) cause an invariably fatal disease in humans, and *Trypanosoma brucei brucei* a fatal disease in cattle, if left untreated [1]. *Trypanosoma brucei* group trypanosomes are transmitted by the bite of a Tsetse fly, and this disease is annually responsible for approximately 50,000 deaths in sub-Saharan Africa, although during pandemics the total deaths can be very much greater [1]. When in the bloodstream of a mammalian host, the Long Slender (LS) trypomastigote forms rely almost entirely upon the aerobic glycolysis of bloodstream glucose for their energy [2]. Their glycolytic pathway can be summarized by the net equation shown below.

$$C_6H_{12}O_6 + O_2 \rightarrow 2C_3H_4O_3 + 2H_2O$$

In this pathway, a glucose molecule is converted into two pyruvic acid molecules, and a mere two ATP molecules are produced during this process. The host's liver, via gluconeogenesis, converts the pyruvate which is excreted into the bloodstream by the trypanosomes, back into glucose. Thus the host's liver essentially acts as a remote ATP source for the parasite, enabling them to function with only a nine-enzyme energy pathway [3].

In the bloodstream, LS trypomastigote forms glycolysis occurs in specialized organelles called glycosomes. Glycosomes appear to be essential to facilitate the extreme glycolytic rates observed in trypanosomes and for their regulation [2,3]. *Trypanosoma brucei* LS trypomastigotes can also survive anaerobically, but this decreases their ATP yields by half [2,3]. In mammalian cells, the NADH generated earlier in the glycolytic pathway by glyceraldehyde-3-phosphate dehydrogenase is used to reduce the pyruvic acid to lactic acid, but in trypanosomes, this NADH is indirectly oxidized using molecular oxygen to generate water, and the pyruvic acid is excreted. However, the net ATP yield (2 ATPs/glucose) is thought to be identical in the parasite's aerobic glycolysis and the host's anaerobic glycolytic pathways [2]. Studies concerning changes in the composition of the culture media after incubation with *Trypanosoma brucei* trypomastigotes revealed a highly disproportionate consumption of the amino acid threonine. Threonine, through the action of threonine dehydrogenase, appears to be the major source of acetyl CoA required for fatty acid chain elongation in *Trypanosoma brucei* bloodstream trypomastigotes. Fatty acid chain elongation occurs within the mitochondrion [4,5]. The transport of acetyl CoA produced by threonine dehydrogenase into the

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mitochondrion utilizes the enzyme carnitine acetyltransferase [4,5]. Bromoacetyl carnitine is a covalent irreversible inhibitor of carnitine acetyltransferase in mammals and *Trypanosoma brucei* [6]. It was synthesized in the Klein laboratory, in a simple one-flask reaction, by the action of bromoacetic anhydride on carnitine. This gave the desired product bromoacetyl carnitine, plus an equimolar quantity of bromoacetate [6]. In the early 1980s, it was wrongly assumed by the Klein laboratory that the contaminating bromoacetate was a totally innocuous product. Therefore, no attempt was made to separate the bromoacetyl carnitine from the bromoacetate, and the product was utilized without further purification [6]. However, bromoacetate, like iodoacetate, is known to be a potent, although less famous, inhibitor of the glycolytic pathway enzyme glyceraldehyde-3-phosphate dehydrogenase, in mammals and trypanosomes. Furthermore, like iodoacetate, bromoacetate is rapidly trypanocidal *in vitro* and possesses *in vivo* trypanocidal activity in mice [7]. This calls into question the interpretation of all results performed using impure bromoacetyl carnitine preparations. In his Ph.D. thesis [8], Jackson confirmed the *in vitro* activity of bromoacetate on trypanosome motility, and the *in vivo* trypanocidal activity of bromoacetate in mice. However, he found that while the actions of bromoacetate on the motility and infection time course were very similar to Klein's bromoacetyl carnitine preparations, they were not totally identical, and thus bromoacetyl carnitine could have some activity of interest.

Despite the methodological error discussed above, the disproportionately high metabolism of threonine and other components could contribute to LS trypomastigotes' energy production. However, the inhibition of such an alternative energy-producing pathway(s), would not be expected to be rapidly lethal. Similarly, the inhibition of acetyl-CoA production and lipid chain elongation would also not be expected to exhibit rapid short-term toxicity. This contention is supported by the observation that LS trypomastigotes are perfectly viable for many hours at 37 ºC in PBS containing just glucose and 0.15% BSA as potential carbon/energy sources. However, the initial media composition studies by Klein from the early 1980s were correct, and bloodstream LS trypomastigotes (at least some of them), used threonine and a few other media components at rates inconsistent with their use for synthetic purposes only, but more consistent with their use as an energy source. Thus, in the late 1980s at Yale, we experimented with the survival of bloodstream LS trypomastigotes in RPMI-based media containing no glucose, to see if any trypomastigotes remained motile, and a small proportion were found to remain highly motile. We then progressively deleted more components to produce a simple non-glucose-containing media that could support this sub-population of trypanosomes. The recipe we finally settled on was composed of the following: PBS, BSA 0.15%, threonine, α-ketoglutarate, proline, glutamine, and pyruvate; these latter components were present at 1 mM. Some of these components could be eliminated at the cost of reduced motility. These trypanosomes could be re-purified and separated from the non-motile trypanosomes, and the cellular debris on a DEAE 52 cellulose column, in this glucose-free buffer when adjusted to pH 8.0. These unpublished results were intriguing. Furthermore, these trypanosomes were as infective to mice and rats as regular bloodstream LS trypomastigote forms, and would readily transform to procyclic forms when transferred to Cunningham's medium. It is conceivable that the pathways that permit this behavior are present but to a lesser degree in the bloodstream LS trypomastigotes form which could not survive without a hexose or glycerol energy source.

Others have produced evidence that bloodstream trypomastigote forms can derive energy from non-hexose/glycerol sources such as threonine [9]. Furthermore, a detailed metabolic study of acetate, succinate, and acetyl-CoA biosynthesis from glucose and threonine, in LS bloodstream forms confirmed the earlier findings [4-6] and supported an important role for threonine in fatty acid biosynthesis [10]. Acyl thioesters are high-energy compounds. Therefore, the reversal of the acetyl CoA synthetase, and/or the succinyl CoA synthetase reactions could provide a thermodynamically sound mechanism for ATP generation by LS trypomastigotes [10]. This would also account for the role of pyruvate in supporting LS bloodstream form ATP generation in the absence of hexose/glycerol, as pyruvate gives rise to high-energy acetyl CoA in the mitochondrion [10]. The concentration of threonine in the bloodstream is, however, considerably lower than that of glucose [11]. Thus, glucose is the preferred energy source for LS trypomastigotes, despite the lower net investment in synthetic energy costs per ATP synthesized when utilizing threonine. It should be noted that therapeutic strategies based on the inhibition of bloodstream LS trypomastigotes' ATP-generating pathways would also need to target non-hexose/glycerol ATP-yielding pathways as well. In addition, since threonine dehydrogenase activity is required for LS trypomastigotes to produce the acetyl CoA, which is required for fatty acid chain elongation [10], inhibition of this pathway alone is likely to be deleterious to the parasite's survival. However, this action would not be as immediately lethal as disrupting energy metabolism.

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References

1. World Health Organization. The world health report 2004 - changing history (WHO, Geneva, 2004).

2. Opperdose FR, Nwagwu M. Suborganellar localization of glycolytic enzymes in the glycosome of Trypanosoma brucei. The host-ivander interplay. Janssen Research Foundation (Van den Bossche H. ed.) Elsevier/North Holland Biomedical Press, Amsterdam.

3. Opperdoes FR, Borst P. Localization of nine glycolytic enzymes in a microbody-like organelle in Trypanosoma brucei: the glycosome. FEBS Lett. 1977 Aug 15;80(2):360-4. doi: 10.1016/0014-5793(77)80476-6. PMID: 142663.

4. Linstead DJ, Klein RA, Cross GA. Threonine catabolism in Trypanosoma brucei. J Gen Microbiol. 1977 Aug;101(2):243-51. doi: 10.1099/00221287-101-2-243. PMID: 925677.

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5. Gilbert RJ, Klein RA. Pyruvate kinase: a carnitine-regulated site of ATP production in Trypanosoma brucei brucei. Comp Biochem Physiol B. 1984;78(3):595-9. doi: 10.1016/0305-0491(84)90104-4. PMID: 6478790.

6. Gilbert RJ, Klein RA, Johnson P. Bromoacetyl-L-carnitine: biochemical and antitrypanosomal actions against Trypanosoma brucei brucei. Biochem Pharmacol. 1983 Nov 15;32(22):3447-51. doi: 10.1016/0006-2952(83)90375-1. PMID: 6651867.

7. Webb JL. Enzyme and Metabolic Inhibitors. Academic Press. London & New York. 1966; 3.

8. Jackson AJS. Nitroheterocycle Metabolism in Trypanosoma brucei, PhD Thesis, 1987 Cambridge University.

9. Pineda E, Thonnus M, Mazet M, Mourier A, Cahoreau E, Kulyk H, Dupuy JW, Biran M, Masante C, Allmann S, Rivière L, Rotureau B, Portais JC, Bringaud F. Glycerol supports growth of the Trypanosoma brucei bloodstream forms in the absence of glucose: Analysis of metabolic adaptations on glycerol-rich conditions. PLoS Pathog. 2018 Nov 1;14(11):e1007412. doi: 10.1371/journal.ppat.1007412. PMID: 30383867; PMCID: PMC6245841.

10. Mazet M, Morand P, Biran M, Bouyssou G, Courtois P, Daulouède S, Millerioux Y, Franconi JM, Vincendeau P, Moreau P, Bringaud F. Revisiting the central metabolism of the bloodstream forms of Trypanosoma brucei: production of acetate in the mitochondrion is essential for parasite viability. PLoS Negl Trop Dis. 2013 Dec 19;7(12):e2587. doi: 10.1371/journal.pntd.0002587. PMID: 24367711; PMCID: PMC3868518.

11. Baños G, Daniel PM, Moorhouse SR, Pratt OE. The movement of amino acids between blood and skeletal muscle in the rat. J Physiol. 1973 Dec;235(2):459-75. doi: 10.1113/jphysiol.1973.sp010397. PMID: 4763998; PMCID: PMC1350756.

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