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SOME ASPECTS OF CARBOHYDRATE METABOLISM OF CULTURAL FORMS OF *TRYPANOSOMA CRUZI* (*)

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SUMMARY

Extracts from *T. cruzi* contain aconitase, fumarase and the following dehydrogenases: glucose 6 phosphate, 6-phosphogluconic, isocitric, triose phosphate and malic enzyme. The presence of 6-phosphogluconate splitting enzime is reported and a possible pathway for CO_2 uptake during anaerobic glycolisis is suggested.

INTRODUCTION

The knowledge of the metabolic pathway in *Trypanosoma cruzi* is important in the research of chemotherapy of Chagas' disease. Although several papers on this subject have been published, very little is known on the carbohydrate metabolism of this protozoa.

RYLEY [11] has found that cultural forms of *T. cruzi* are able to metabolize glucose to succinate, acetate and other undeterminated acids. Carbon dioxide is produced in aerobic conditions, and consumed in anaerobiosis. That carbon dioxide uptake found with *T. cruzi* as well as with *T. lewisi* called our attention to its carbohydrate metabolism. In the present paper I am reporting the presence of several enzymes in the homogenate of *T. cruzi*, especially those that might explain the carbon dioxide requirement for anaerobic glycolisis.

METHODS

Homogenate. — *T. cruzi* was cultivated in Noller's [7] media, with 60 γ/ml of penicillin G, at 28°C. After 15 days the protozoa was harvested by centrifugation at 3,000 g, washed twice with .9% of sodium chloride. The protozoa was suspended in M/200 sodium bicarbonate, M/200 neutralized cystein, homogenized in a Potter-Elvejn homogenizer, frozen and thawed twice. Intact cells were removed by centrifugation.

Spectrophotometric determination. — It was used a Carry recording spectrophotometer, model 14, equiped with a slidewire for 0-.1 units of optical density.

Enzymatic determinations. — Aconitase and fumarase were measured by the spectrophotometric method of RACKER [8]. Activation of aconitase was carried in the presence of 10^{-2}M ferrous chloride and 4×10^{-2}M cystein. Glucose 6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, malic dehydrogenase, lactic dehydrogenase and isocitric dehydrogenase were measured by following pyridine nucleotide oxidation or reduction [6] at 340 mμ, by adding the substrate as the last reagent. DPN-isocitric dehydrogenase was tested in the presence of 10^{-4}M AMP [5]. Triose phosphate dehydrogenase was tested with frutose diphosphate and excess crystalline aldolase.

Chemical determination. — Protein was measured in *T. cruzi* homogenates by biuret [4].

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RESULTS AND DISCUSSION

Table I shows the activity of several enzymes detected in *T. cruzi* homogenates, including enzymes of Krebs acid cycle, Embden-Meyerhof and pentose pathways. It is interesting to note the very high activity of TPN linked dehydrogenases. Isocitric dehydrogenase was reported independently and purified by AGOSIN & WEINBACH [1]. Almost no lactic and malic dehydrogenases were found. This data agrees with RYLEY'S [11] work, which showed no accumulation of lactic acid during glycolisis. It seems so that *T. cruzi* has an incomplete Krebs acid cycle.

TABLE I

Enzyme	*Cofactor*	*Activity* μ mole/mg protein/min.
Isocitric dehydrogenase	TPN	0.015
Isocitric dehydrogenase	DPN	0.000
Malic dehydrogenase	DPN	0.002
Malic enzyme	TPN	0.019
Malic enzyme	DPN	0.000
Lactic dehydrogenase	DPN	0.004
Aconitase	—	0.004
Aconitase	Fe.. + .SH	0.030
Fumarase	—	0.045
Glucose 6-P dehydrogenase	TPN	0.041
6-P gluconic dehydrogenase	TPN	0.010
Triose phosphate dehydrogenase	DPN	0.030

Aconitase of *T. cruzi* homogenates is very labil, and in a few hours no activity can be found, unless reactivation with ferrous ions and SH groups is used. This property is similar to rat liver aconitase [3].

It is also interesting to note that the enzymes described in the Table I are in the same range of activity (.010-.045).

Carbon dioxide requirement for anaerobic glycolisis was reported, but not explained by RYLEY [10, 11]. It suggests a CO_2 fixation reaction dismuting the reduced pyridine nucleotide. Malic enzyme is present in a high concentration, being probably the enzyme responsible for this dismutation.

Glucose oxidation could be carried with a high probability by the Embden-Meyerhof pathway or through the pentose shunt. The finding of BAERNSTEIN & REED [2], of a low aldolase activity seems to rule out this first pathway.

In anaerobic glycolisis through the pentose shunt, to oxidize glucose to pyruvate, 6 mole of TPNH and 3 of DPNH would be formed. It is impossible to balance this data with RYLEY'S [11] finding of oxidation of 1 mol of glucose, resulting in .61 of acetate, .67 of succinate with an uptake of .46 of CO_2.

A pathway leading to pyruvate from glucose 6-phosphate, with a smaller production of reduced nucleotides, it is the one involving WOOD & SCHWERDT [12] enzyme, which splits 6-phosphogluconate in pyruvate plus glyceraldehyde 3-phosphate.

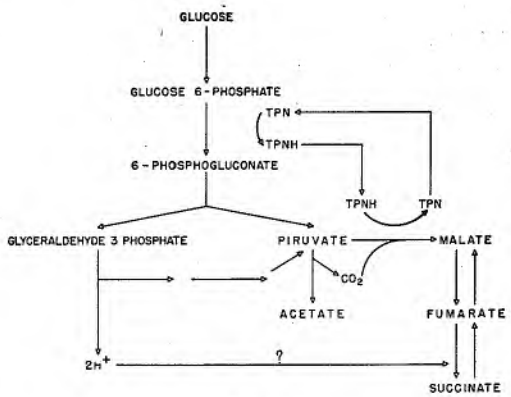

Fig. 1

Figure 1 shows a scheme which is likely to explain CO_2 uptake during anaerobic glycolisis.

TABLE II

Pyruvate from 6-phosphogluconate

System contains in 3 ml: .1M hydrazine sulfate, .003M sodium arsenite, .01M substrate, .1M tris buffer pH 7.5, 1-3 mg protein of trypanosome, 400 units of lactic dehydrogenase, .0003M DPNH and .03M magnesium chloride.

Substrate	Δ 345/min./mg protein
none	.002
ribose 5-phosphate	.002
6-phosphogluconate	.010

The presence of 6-phosphogluconate splitting enzyme was tested by lactate formation from pyruvate with purified lactic dehydrogenase. Hydrazine sulfate was added to trap the aldehyde formed, and arsenite was used to inhibit pyruvate oxidation. Table II shows a typical result. Lactate is formed from 6-phosphogluconate, but not from ribose 5-phosphate, ruling out the pentose shunt.

Further studies are being carried out to prove the present proposed pathway.

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RESUMO

Alguns aspectos do metabolismo de carboidratos em formas culturais de "Trypanosoma cruzi".

Extratos de *T. cruzi* contêm: aconitase, fumarase e as seguintes dehidrogenases: glicose 6-fosfato, 6-fosfoglucônica, isocítrica, triose, fosfato e enzima málico. A presença do enzima que cinde 6-fosfogluconato é descrita e sugere-se uma possível via metabólica para a fixação de CO_2 durante a glicólise anaeróbia.

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