Parasitology (1975), 71, 311-326
With 6 figures in the text

Utilization of amino acids by *Trypanosoma brucei* in culture: L-threonine as a precursor for acetate

G. A. M. CROSS, R. A. KLEIN *and* P. J. LINSTEAD

Medical Research Council Biochemical Parasitology Unit, Molteno Institute, Downing Street, Cambridge CB2 3EE

(Received 7 March 1975)

SUMMARY

The amino acid compositions of several culture media have been analysed and compared. The utilization and excretion of amino acids and other metabolites have been followed during growth of *Trypanosoma brucei* S42 in a defined medium. All of the added L-threonine was metabolized by the cells, even when it was present at elevated concentrations. Glucose was consumed throughout the growth cycle: glutamine was consumed more rapidly than glutamic acid, which was itself used at about the same rate as proline. Threonine was cleaved to form glycine and acetate, both of which accumulated in the medium. Alanine and succinate were excreted together with a small amount of pyruvate, but these three products accounted for less than half of the glucose used. CO_2 production from glucose was not measured, but insignificant amounts of CO_2 were produced from threonine. Tetraethylthiuram disulphide blocked the cleavage of threonine and was a potent inhibitor of trypanosome growth.

INTRODUCTION

There have been few studies of the utilization of amino acids by the Salivarian trypanosomes. Owing to our inability to cultivate them in vitro, most studies with the haematozoic forms have been confined to investigations of uptake and short-term metabolic interconversions using freshly isolated trypanosomes suspended in buffered-salts maintenance media (Chappell, Southworth & Read, 1972; Southworth & Read, 1972; Ruff & Read, 1974; Voorheis, 1973). Even the procyclic trypomastigotes (Newton, Cross & Baker, 1973), which may be maintained in culture at 25-28 °C have been little studied in this respect, and attention has been mainly focused on the metabolism of proline (Evans & Brown, 1972).

We were drawn to the study of amino acid utilization by culture forms of *Trypanosoma brucei* through consideration of the adequacy of the amino acid concentrations provided in the defined medium, HX25 recently developed for the cultivation of these organisms (Cross & Manning, 1973). The amino acid concentrations in medium HX25 were found to be considerably lower than in other media used for cultivation of pathogenic salivarian trypanosomes and insect flagellates, especially when allowances were also made for the nutritional potential of the large amounts of blood proteins often included in these media (see Table 2). However, a preliminary examination of changes in medium amino acid levels during growth of *T. brucei* sspp. trypanosomes suggested that most amino acids were present in

abundant amounts, with the exception of L-threonine which was completely metabolized. This observation led to the more detailed studies of metabolite transformations which are the subject of this paper, and in particular to the demonstration of the role of L-threonine as a major precursor of 2-carbon units. Additionally we have shown that a compound which appeared to block the utilization of threonine was a very potent inhibitor of trypanosome growth *in vitro.*

METHODS

Organism

T. brucei strain S42 was isolated by Dr J. R. Baker in 1966 from an adult female warthog in Tanzania. It was found not to be infective when tested in one human volunteer shortly after isolation. We acquired the strain in 1969 from the Nuffield Institute of Comparative Medicine, London. The organism is pleomorphic and has remained infective to *Glossina.* The culture line used in the present work was derived in 1970 by transfer of trypanosomes from a heavily infected rat into monophasic culture medium as previously described (Cross & Manning, 1973). Since its transfer into culture the strain has been maintained by twice-weekly subculture in undefined monophasic blood-containing medium (MCM) or, since mid-1972, in defined media HX25 or HX25M (see below).

Cultivation media

With the exception of specific modifications mentioned in the text, organisms were grown in either medium HX25 (Cross & Manning, 1973) or (since November 1973) in HX25M which contained different amounts (c.f. Table 5 in Cross & Manning, 1973) of certain constituents viz : glucose, 3620 mg/l; L-proline, 193 mg/l; L-glutamate, 223 mg/l; L-threonine, 360 mg/l; L-arginine. HCl 400 mg/l; D(+)-glucosamine.HCl 73 mg/l. In addition, medium 199 10x concentrate was replaced by dried powdered medium 199 (Weilcome Reagents Ltd, Beckenham, Kent), 9.3 g/l. In this medium, stock cultures grew to between 1.5 and 2.0 x 107 cells/ml in 3-4 days. Additionally it should be noted that owing to difficulty in obtaining supplies, coenzyme Q6 was sometimes omitted from the medium with no apparent effect on cell growth.

The addition *en bloc* of the constituents of tissue culture medium 199 to the defined media HX25 and HX25M was inconvenient when it was necessary to make certain deletions from the media for the purpose of assessing their growth-promoting properties, or for the purpose of adding radioactive precursors for metabolic studies. For these reasons a further medium HX28 was devised which followed closely the composition of HX25, but in which all the components were added separately. Medium HX28 (Table 1) is similar to HX25M except for the omission of citric, succinic and linoleic acids, glycine, alanine, asparagine and the D-amino acids present in medium 199 (Morgan, Morton & Parker, 1950). Growth of stock cultures in medium HX28 was, surprisingly, less satisfactory than in HX25. It should be noted that none of these media represents a minimal medium for trypanosome growth.

Table 1. *Composition of Medium HX28**

	mg/l		mg/l
HEPES†	19000	NaCl	7000
Glucose	4500	KH2P04	1000
CH3COONa. 3H2O	680	NaHCO3	1000
EDTA, disodium salt	80	KCl	350
Defatted bovine serum albumin fraction V	400	CaCl2	180
		MgSO4.7H20	180
D(+)-glucosamine.HCl	220	FeCl3	1
L-aspartic acid	266	FeSO4.7H2O	1
L-arginine.HCl	420	ZnSO4.7H2O	1
L-cysteine.HCl	80	MnSO4.4H2O	1
L-cystine	60	CoSO4.7H20	05
L-glutamic acid	295	CuSO4.5H2O	05
L-glutamine	290	H3BO4	0.5
L-histidine.HCl	190	CrCl3 .6H2O	0.1
L-isoleucine	260	NiCl2 .6H2O	0.1
L-leucine	260	KI	0.05
L-lysine.HCl	360		
L-methionine	75ATP		10
L-phenylalanine	165	Adenine	5
L-proline	230	Adenosine	20
L-serine	210	Guanine	5
L-threonine	357	Guanosine	20
L-tryptophan	102	Cytosine	5
L-tyrosine	180	Cytidine	20
L-valine	235	Uracil	5
		Uridine	20
DL-a-tocopherol	4	Thymine	5
DL-a-lipoic acid (oxidised form)	04	Hypoxanthine	5
		Xanthine	5
Menadione	0.4	D-ribose	5
Trans-retinoic acid	0.4	2-deoxy-D-ribose	5
Coenzyme Q6	0.4	Phenol red	5
Coenzyme Q10	0.4	Haemin	10
D-biotin	1	Cholesterol	0.5
D-calcium pantothenate	1	Tween 40	5
Choline chloride	1	Tween 80	5
Folic acid	11		
L-inositol	2	p-aminobenzoic acid	0.2
Nicotinamide	1	Ascorbic acid	0.2
Nicotinic acid	0.2	Vitamin B12	1
Pyridoxal.HCl	1	Formyl tetrahydrofolic acid	1
Pyridoxine.HCl	0.2		
Riboflavin	0.1	Calciferol	0.2
Thiamine.HCl	1	Glutathione	0.2

* Adjusted to pH 7•40 with NaOH.

† N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid.

Measurement of cell multiplication

Growth was followed using an electronic cell counter (Coulter Electronics Ltd, Coulter Counter Model B, 70 µm aperture). Under normal conditions at 25 °C the doubling time was about 24 h.