Threonine as a Preferred Source of 2-Carbon Units for Lipid Synthesis in *Trypanosoma brucei*

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Culture forms of *Trypanosoma brucei* S42 were maintained in defined media by serial subculturing (Cross & Manning, 1973; Klein et al., 1975). Under these conditions considerable quantities of L-threonine were metabolized and equimolar concentrations of glycine and acetate were excreted. Glycine was identified by using an amino acid analyser and acetate was characterized as the p-bromophenacyl derivative (Cross et al., 1975).

The pathway by which acetate is produced from L-threonine by culture form *T. brucei* was shown to involve the NAD⁺-dependent L-threonine dehydrogenase (EC 1.1.1.103) and 'aminoacetone synthase' (McGilvray & Morris, 1969). In homogenates the enzymic sequence is interrupted and aminoacetone is produced; this was identified as the semicarbazide (R. A. Klein & D. J. Linstead, unpublished results). The breakdown of L-threonine is shown diagrammatically in Fig. 1.

Acetate produced from radioactively labelled L-threonine (1.23 mM) was found to be a preferred source of lipid carbon even in the presence of both exogenous acetate (0.66 mM) and a tenfold molar excess of glucose (13.2 mM).

The incorporation of radioactivity from labelled L-threonine into chloroform/methanol-soluble lipid material was decreased by adding unlabelled sodium acetate (6.76 mM) to the culture medium. However, the dilution effect (0.623 ± 0.015) was very much less than expected on the basis of the relative concentrations used. This is shown in Fig. 2, where (a) represents the radioactivity incorporated from L-[U-¹⁴C]threonine alone, (b) from L-[U-¹⁴C]threonine in the presence of unlabelled sodium acetate.

![Diagram of L-threonine breakdown](image)

Fig. 1. *Pathway for L-threonine breakdown by culture form T. brucei*

The steps to the right of the broken line occur non-enzymically in homogenates.
Fig. 2. *L*-Threonine incorporation into lipid by *T. brucei*, in the presence and absence of added acetate

Further information is given in the text.

(6.76 mm) and (c) the expected dilution by added acetate. Incorporation of radioactive carbon from *L*-threonine is compared with that for *32P* orthophosphate to correct for differing rates of cell growth.

T.l.c. of the lipids from organisms grown in the presence of radioactively labelled [1-*14C]*acetate, or *L*-[U-*14C]*threonine in the presence or absence of unlabelled acetate, gave patterns of incorporation which were indistinguishable throughout the growth cycle.

Inhibition of the *L*-threonine dehydrogenase (EC 1.1.1.103) with the thiol reagent tetraethylthiuram disulphide decreased the production of acetate and glycine from *L*-threonine and was trypanocidal. At the same time incorporation into lipid of radioactive carbon from *L*-threonine was markedly inhibited; acetate incorporation into lipid was, however, also affected.

The assimilation of carbon units for lipid synthesis during growth from both *L*-threonine and acetate was decreased by a similar amount in the presence of an exogenous source of fatty acids in the form of Tween 40. In a series of experiments, specific-radioactivity measurements on isolated fatty acid methyl esters by two different techniques showed that acetate contributed between 1 and 1.5 carbon atoms to the palmitate chain (C16:0) and 2.5 and 7 carbon atoms to the stearate chain (C18:0). On the other hand, *L*-threonine contributed 3–7 carbon atoms to the palmitate chain and 6–11 carbon atoms to the stearate chain.

Alanine was incorporated into the fatty acid chains to an extent comparable with that for acetate. Significant amounts of radioactive carbon from D-[U-*14C]*glucose,
d-[2-14C]glucose and d-[6-14C]glucose also appeared in the cell lipid fatty acyl chains.

We conclude that L-threonine is a preferred source of lipid carbon for culture form T. brucei under the conditions used. This appears to be unusual since the organism is neither energy- nor carbon- and nitrogen-limited in our growth medium (compare McGilvray & Morris, 1969). We also believe that this is the first report of the 'aminoacetone' pathway being utilized by a eukaryotic cell for the generation of acetate units to be used in lipid biosynthesis.


Biosynthesis of Phosphatidylcholine and Phosphatidylethanolamine by Germinating Soya Bean

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Phosphatidylcholine is the major phospholipid in almost every plant tissue that has been examined (Galliard, 1973). Not only is it an important component of cellular membranes but it may also play a role as acceptor (Vijay & Stumpf, 1971) or as substrate during fatty acid desaturation (Gurr et al., 1969; Roughan, 1970; Willemot & Verret, 1973).

In spite of these important functions, study of the synthesis of phosphatidylcholine has been rather neglected in higher plants. The molecule can be synthesized, via methylation, from phosphatidylethanolamine or by a CDP-base pathway. The latter can also form phosphatidylethanolamine, though it is not known whether the same enzymes are involved throughout (Galliard, 1973). We have studied these reactions by using the germinating soya bean, a plant which has a high content of the nitrogen-containing phospholipids (Harwood, 1975; Wagner & Wolff, 1964).

Phosphatidylcholine and phosphatidylethanolamine were both rapidly labelled, after an initial lag phase, from [32P]orthophosphate or [1-14C]acetate. Their specific radioactivities and the inhibition, by protein-synthesis inhibitors, of [1-14C]acetate incorporation into them were similar (Harwood, 1975). However, there were small differences in both radioactive and total fatty acid patterns between the two lipids.

The time-courses of labelling of phosphatidylcholine from [Me-14C]choline and of phosphatidylethanolamine from [2-14C]ethanolamine are shown in Fig. 1. In both cases there was a rapid increase in the labelling at about 16h germination, at a time when esterification of free fatty acids into phospholipids also increases (Harwood, 1975). Labelling then increased at a more steady rate, corresponding to emergence of the radicle. The rapid rates of labelling are surprising in view of the fact that the soya bean contains a large amount of stored phospholipid. The limiting factor for incorporation of the isotopes does not appear to be the entry of the radioactive precursors into the seed, since these are present in large amounts after very short times. Initially, the rate of entry of isotope corresponds to the amount of water imbibed. There was no labelling of phosphatidylcholine from [2-14C]ethanolamine, indicating the absence of the methylation pathway at this stage of germination.

The lag phase in labelling of phosphatidylethanolamine and phosphatidylcholine from radioactive precursors could be due to a need for enzyme synthesis to take place. The effect of the protein-synthesis inhibitors chloramphenicol and cycloheximide, neither of which caused much lowering of [14C]acetate incorporation (Harwood, 1975), was therefore tested. Cycloheximide (10μg/ml) and chloramphenicol (50μg/ml) inhibited labelling of the phospholipids to different extents, but these