LETTERS TO THE EDITORS

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Formaldehyde as an Acceptor Aldehyde for Transketolase, and the Biosynthesis of Triose

THE enzyme transketolase is biologically widely distributed, and, in presence of thiamine pyrophosphate and magnesium ions, transfers the ketol group or 'active glycolaldehyde' ($CH_2OH.CO\ldots H$) from a variety of ketose phosphates (sedoheptulose 7-phosphate, D-fructose 6-phosphate, D-xylulose 5-phosphate), from certain free ketoses (L-erythrulose, D-xylulose) and from hydroxypyruvic acid to certain 'acceptor aldehydes', thus synthesizing new ketoses. The 'acceptor aldehydes' hitherto recognized are D-glyceraldehyde 3-phosphate, D-ribose 5-phosphate, D-erythrose 4-phosphate and 2-deoxyribose 5-phosphate; also free glyceraldehyde and glycolaldehyde (for reviews see refs. 1–3). Acetaldehyde and formaldehyde are stated not to be acceptors[4], but the former appears in our preliminary tests to be weakly active.

Formaldehyde, on the other hand, we find reacts quite effectively as an acceptor when pure lithium hydroxypyruvate[5] is the donor. The product formed corresponds chromatographically, chemically and enzymically with dihydroxyacetone:

$$CH_2OH.CO.COOH + H.CHO \rightarrow CH_2OH.CO.CH_2OH + CO_2$$

Table 1 shows the manometric and other results. Table 2 shows the chromatographic identification.

Table 1. FORMALDEHYDE AS 'ACCEPTOR' FOR TRANSKETOLASE ACTION

Transketolase was prepared by the method of de la Haba, Leder and Racker (ref. 4); a sample purified by column adsorption kindly supplied by Dr. E. Racker behaved similarly. Contents of vessels: transketolase (500 μgm.), thiamine pyrophosphate (0·2 μmole), magnesium chloride (5 μmoles), *p*H 6·6 buffer to total volume of 2·5 ml. Substrates as below (formaldehyde or hydroxypyruvate, 20 μmoles of each). The values given are in μmoles after 2 hr. incubation at 37° C.

Substrate	Hydroxypyruvate	Hydroxypyruvate + formaldehyde	Hydroxypyruvate + formaldehyde (no enzyme)
Carbon dioxide produced	1·2	15·2	0
Formaldehyde utilized	0	15·7	0·8
Hydroxypyruvate utilized*	2·6	18·0	2·8
Triose formed†	none	+++	none

* Spectrophotometrically by specific dehydrogenase (cf. ref. 11).
† By glycerokinase and adenosine triphosphate (manometrically and also by formation of alkali-labile phosphate); by formation of methyl glyoxal on distillation with acid; and by chromatography (Table 2).

Table 2. CHROMATOGRAPHIC IDENTIFICATION OF DIHYDROXYACETONE FORMED

Deionized vessel-contents (Table 1) were concentrated *in vacuo* and analysed by descending chromatography on Whatman No. 1 paper; detection by aniline phosphate spray

Solvent (parts by vol.)	*R*F values: Unknown	Dihydroxyacetone	Glyceraldehyde	Erythrulose	Threose
Phenol/water (4 : 1)	0·81	0·81	0·44	0·72	0·65
Butanol/acetic acid/water (4 : 1 : 5)	0·45	0·45	0·49	0·36	0·43
Ethylmethyl ketone/acetic acid/4 per cent boric acid (9 : 1 : 1)	0·48	0·49	0·14	0·38	0·36

The interaction of transketolase with the numerous biological sources of formaldehyde opens the possibility of various new metabolic routes. The dihydroxyacetone formed in the above reaction is readily phosphorylated by 'glycerokinase' prepared from rat liver by the method of Bublitz and Kennedy[6]: the resulting phosphate could therefore enter the triose phosphate and hexose phosphate pools. Another route from L-serine to hexose, alternative to that proposed for phosphoserine by Ichihara and Greenberg[7], might proceed via serine aldolase (giving formaldehyde and glycine) in conjunction with the formation of hydroxypyruvate from L-serine by the specific transaminase of Sallach[8]. Transketolation of the hydroxypyruvate to formaldehyde could thus provide dihydroxyacetone. Whether the observed transformation in the rat of [3-^{14}C]serine[9] and of [2-^{14}C and 3-^{14}C] hydroxypyruvate[10] to liver glycogen actually follows this pathway is being investigated. The extent of formation of dihydroxyacetone from donors other than hydroxypyruvate and the question of reversibility of these reactions are also being studied by us.

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A Procedure for the Purification of Sirenin

SIRENIN (I am indebted to Dr. A. Vegis for suggesting, what seems to me, this aptly descriptive name) is the name, used here for the first time, for the chemotactic sexual hormone from the water-mould *Allomyces* which attracts the male gametes and is produced by the female gametes[1]. This hormone has now been produced and purified.

Fernbach flasks (1,800 ml.) containing 500 ml. of yeast extract–starch medium[2] were inoculated with mycelial fragments of the female strain *F*-2[1] and incubated for two days on shakers at 30° C. The plants obtained were washed with copious amounts of tap water and then placed in thin layers in dishes where they were just covered with double-distilled water. This water was replaced after 3, 8 and 24 hr., thereby inducing gamete formation and release for a total of 48 hr. The aqueous solutions, containing approximately 25–100 μgm. sirenin per litre, were freed of gametes by passage through Seitz filters and the solutes adsorbed on 'Norit *FNX* Special' charcoal used at the rate of 5 gm. per 500 ml. of solution.