LETTERS TO THE EDITORS

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Structure of Coenzyme A

Chemical[1] and enzymic[2] studies from these two laboratories suggested that coenzyme A is best represented by formula (I) (cf. ref. 3). While the synthesis of various fragments of the molecule[4] has lent considerable support to this structure, the enzymic and chemical evidence did not agree on one point. This concerned the nature of a substance obtained by the action of nucleotide pyrophosphatase on the coenzyme and which stimulated the growth of *Acetobacter suboxydans*[5]. Although not isolated in a chemically pure state, it was thought to be a simple phosphate of pantothenic acid[6]. None of the synthetic pantothenic acid phosphates showed activity towards this organism; consequently, it was suggested[1] that this '*Acetobacter*-stimulatory factor' might be pantetheine-4′phosphate (II). An unambiguous synthesis of (II) is described here.

D : L - Pantetheine - $O^{2'}$: S dibenzyl ether was prepared from pantothenic acid - 2′benzyl ether by reaction with ethyl chloroformate and then 2-benzylthioethylamine. Phosphorylation with dibenzyl chlorophosphonate and removal of the four benzyl groups with sodium in liquid ammonia gave D : L-pantetheine-4′phosphate.

A more reliable method of assay for the '*Acetobacter*-stimulatory factor' consists in its resynthesis to coenzyme A by a partially purified enzyme system isolated from pigeon liver[7]. This system does not respond to pantetheine or to pantothenic acid phosphates. Synthetic D : L - pantetheine - 4′phosphate, when examined in this system, showed an activity equivalent to 265 units of coenzyme A per milligram, which represents a 47 per cent conversion into coenzyme A. This is in excellent agreement with the expected 50 per cent conversion for the optically inactive substance.

Pure D(+)-pantetheine-4′phosphate, $[\alpha]_D^{18}$ 10·8°, was synthesized by direct phosphorylation of pantetheine with dibenzyl chlorophosphonate followed by removal of benzyl groups with sodium in liquid ammonia. This method, although ambiguous, is very convenient in operation, and the product showed a coenzyme A activity in the above test of 450 units/mgm., which represents an 82 per cent conversion.

This synthesis establishes beyond doubt the structure of the pantetheine phosphate part of the coenzyme molecule, and it is hoped to extend these studies to the synthesis of pyrophosphates more closely related to coenzyme A.

Full details of this work will be published separately elsewhere.

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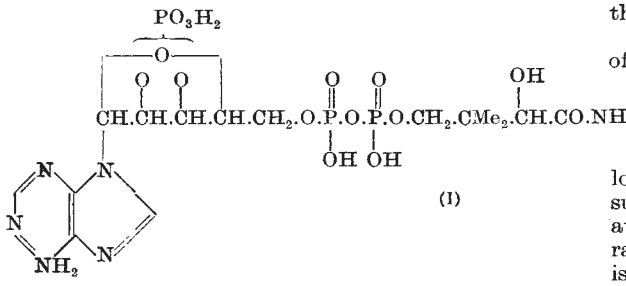
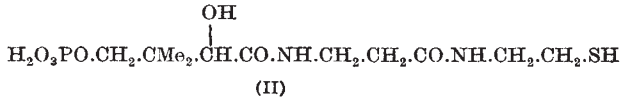


(I)



(II)

[1] Baddiley, J., and Thain, E. M., *J. Chem. Soc.*, 2253 (1951); 3783 (1952).
[2] Gregory, J. D., Novelli, G. D., and Lipmann, F., *J. Amer. Chem. Soc.*, **74**, 854 (1952).
[3] Wang, T. P., Schuster, L., and Kaplan, N. O., *J. Amer. Chem. Soc.*, **74**, 3204 (1952).
[4] Baddiley, J., and Thain, E. M., *J. Chem. Soc.*, 246, 3421 (1951).
[5] King, T. E., Locher, L. M., and Cheldelin, V. H., *Arch. Biochem.*, **17**, 483 (1948).
[6] Novelli, G. D., "Phosphorus Metabolism", **1**, 414 (Baltimore, 1951).
[7] Levintow, L., and Novelli, G. D., abstract of paper presented at the Atlantic City Meeting of the American Chemical Society, p. 33 *c* (1952).

Activation of Enzymes by Chelating Agents

Lysine and glyoxaline-4-5-dicarboxylic acid[1] and histidine[2] have been shown to activate preparations of Keilin horse-heart succinoxidase in unfavourable environments. A property common to these compounds is their ability to chelate heavy metals. We have accordingly tested the effect of other well-known chelating agents upon this system in bicarbonate buffer, which has been shown by Bonner[2] to depress its activity. In Table 1 are shown typical results.

Table 1

Enzyme in	μl. oxygen/20 min.
Phosphate buffer (0·13 *M*)	300
Bicarbonate buffer (0·025 *M*)	65
,, ,, + histidine (1·0 mgm./ml.)	270
,, ,, + pyrophosphate (1·0 mgm./ml.)	240
,, ,, + 8-OH-quinoline (0·1 mgm./ml.)	300
,, ,, + versene (0·1 mgm./ml.)	330
Phosphate buffer + versene	330

Each Warburg flask contained: 0·2 ml. enzyme (see ref. 3), 0·2 ml. cytochrome *c*, 2·4 ml. buffer (*p*H 7·3), 0·3 ml. activator to give final concentrations listed above, 0·2 ml. sodium succinate (0·4 *M*). Temp. 37° C., gassed with oxygen with phosphate buffer, and oxygen containing 5 per cent carbon dioxide with bicarbonate buffer.

The most effective reagent, versene (ethylenediaminetetracetic acid), raises the activity in bicarbonate to a level even higher than that in optimum concentrations of phosphate. The activity in phosphate can be increased to the same figure by the addition of versene.

These results suggest that the activating effect of all these reagents is due to their ability to remove heavy metals from the reacting system. This suggestion is supported by the following further evidence: (1) If commercial sodium succinate is carefully purified, the activity in bicarbonate is markedly increased, and that in phosphate is raised a little (Table 2). However, the effect of versene is still marked, which is not surprising since succinate is not the only source of heavy metals in the system. (2) Concentrations of 2×10^{-3} *M* cupric and zinc ions in phosphate buffer inhibit the succinic oxidase completely, and the activity is restored