

ylammonium iodide appear to be completely dissociated and their equivalent conductivities are independent of concentration to within 1%. However, the equivalent conductivity of the latter compound declines from 10.0 to 9.3 between 5×10^{-3} and $1.25 \times 10^{-2} M$ as measured in a cell with platinized platinum electrodes. This decline may be largely a viscosity effect since the viscosity increase of the solution is of this order of magnitude. Accommodation of the conductance anomalies by introduction of finite ion size parameters would require unreasonably large values, several hundred Å.

Negative deviations from the Onsager slope were exhibited by sodium thiocyanate, dimethylmorpholinium iodide and lithium nitrate. The Davies⁴ treatment gives ion pair dissociation constants of 0.021, 0.013 and 0.0011, respectively. At higher concentrations, as determined cryoscopically, lithium nitrate is largely undissociated.

(4) C. W. Davies, *Trans. Faraday Soc.*, **23**, 351 (1927).

(5) Monsanto Fellow, 1957-1958. National Science Foundation Predoctoral Fellow, 1958-1959.

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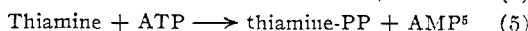
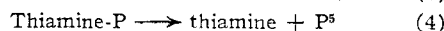
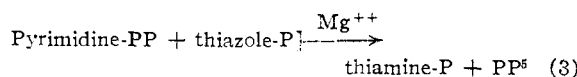
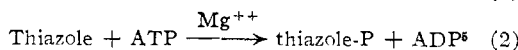
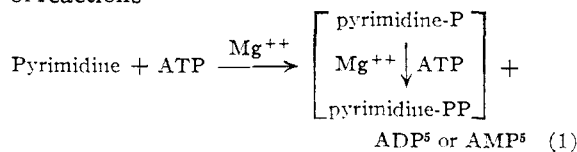
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RECEIVED APRIL 20, 1959

THE BIOSYNTHESIS OF THIAMINE AND THIAMINE PHOSPHATES BY EXTRACTS OF BAKERS' YEAST¹

Sir:

Preliminary reports from two different laboratories have established the ability of enzymes present in bakers' yeast to synthesize thiamine from the pyrimidine and thiazole moieties of the vitamin.^{2,3} Recent experiments carried out in this laboratory have shown that the biosynthesis of thiamine and thiamine-PP⁴ proceed in cell-free extracts of bakers' yeast according to the series of reactions



(1) This work was supported by a grant from the National Science Foundation.

(2) D. L. Harris and J. Yavit, *Fed. Proc.*, **16**, 192 (1957).

(3) I. G. Leder, *Fed. Proc.*, **18**, 270 (1959).

(4) Abbreviations used are: pyrimidine, pyrimidine-P, and pyrimidine-PP for 2-methyl-4-amino-5-hydroxymethylpyrimidine, the orthophosphoric acid ester and the pyrophosphoric acid ester of this compound, respectively; thiazole and thiazole-P for 4-methyl-5-β-hydroxyethylthiazole and the orthophosphoric acid ester of this compound, respectively; thiamine-P and thiamine-PP for thiamine mono- and diphosphate; AMP, ADP, and ATP for adenosine mono-, di-, and triphosphate; and P and PP for inorganic ortho- and pyrophosphate.

(5) These compounds are assumed to be the products of the reactions shown even though they have not yet been well characterized as such.

The two compounds shown as products of reaction 1 both have been isolated from enzymatic reaction mixtures. One compound was identified as pyrimidine-P by analyses which showed one mole of phosphorus per mole of pyrimidine. Pyrimidine was measured spectrophotometrically as well as by microbiological assay with a mutant of *Salmonella typhimurium* which requires this specific pyrimidine or thiamine for growth. Since the compound which is thought to be pyrimidine-PP is quite labile, it has not been possible to isolate it in large enough quantities for accurate phosphorus analyses. Indirect evidence which indicates that it is pyrimidine-PP includes (a) an elution pattern from Dowex-1 columns which is similar to that of cytidine diphosphate, (b) its relative lability to acid hydrolysis (when compared to pyrimidine-P) to yield both the free pyrimidine and pyrimidine-P, (c) the inhibition of the formation of thiamine-P (reaction 3) by PP (which presumably is a product of reaction 3), (d) its conversion to pyrimidine-P and free pyrimidine by phosphatases, and (e) its formation enzymatically from pyrimidine-P in the presence of ATP. Whether or not pyrimidine-P is an obligate intermediate in the formation of pyrimidine-PP cannot be decided from the evidence currently available.

Thiazole-P was prepared by cleaving thiamine-P with sulfite and then chromatographing on Dowex-1 (formate) to separate the compound from the other components of the reaction mixture. The isolated compound, which contained by analysis one mole of phosphorus to one of thiazole, was shown by paper chromatography to be identical with the compound formed enzymatically from thiazole and ATP (reaction 2). The isolated thiazole-P also was able to serve as substrate along with pyrimidine-PP for the synthesis of thiamine-P.

The products of reactions 3, 4 and 5 have been identified by comparing their mobilities on paper chromatograms, in a variety of systems, with the mobilities of the corresponding known compounds. Thiamine cannot be used as a substrate in place of thiazole for reaction 2. Free thiazole cannot be used as substrate for reaction 3 and thiamine-P cannot be used as substrate for reaction 5. Thus thiazole-P, thiamine-P, and thiamine are all necessary intermediates in the biosynthesis of thiamine-PP from pyrimidine-PP and thiazole.

(6) Karl T. Compton Fellow of the Nutrition Foundation (1956-1959).

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RECEIVED JUNE 1, 1959

TRIMERIC DIMETHYLAMINOBORINE

Sir:

The first of the "saturated" boron-nitrogen six membered ring compounds was reported as the trimer of N-methylaminoborine,¹ which was prepared by heating methylamine-borine. In con-

(1) T. C. Bissot and R. W. Parry, *THIS JOURNAL*, **77**, 3481 (1955).