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 1.25×10^{-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 Davies[4] 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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THE BIOSYNTHESIS OF THIAMINE AND THIAMINE PHOSPHATES BY EXTRACTS OF BAKERS' YEAST[1]

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-PP[4] proceed in cell-free extracts of bakers' yeast according to the series of reactions

$$\text{Pyrimidine} + \text{ATP} \xrightarrow{Mg^{++}} \left[\begin{array}{l}\text{pyrimidine-P} \\ Mg^{++} \downarrow \text{ATP} \\ \text{pyrimidine-PP}\end{array}\right] + \text{ADP}^5 \text{ or } \text{AMP}^5 \quad (1)$$

$$\text{Thiazole} + \text{ATP} \xrightarrow{Mg^{++}} \text{thiazole-P} + \text{ADP}^5 \quad (2)$$

$$\left.\begin{array}{l}\text{Pyrimidine-PP} \\ \text{thiazole-P}\end{array}\right\} \xrightarrow{Mg^{++}} \text{thiamine-P} + \text{PP}^5 \quad (3)$$

$$\text{Thiamine-P} \longrightarrow \text{thiamine} + \text{P}^5 \quad (4)$$

$$\text{Thiamine} + \text{ATP} \longrightarrow \text{thiamine-PP} + \text{AMP}^5 \quad (5)$$

(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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TRIMERIC DIMETHYLAMINOBORINE

Sir:

The first of the "saturated" boron-nitrogen six membered ring compounds to be reported was 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).

trast, when dimethylamine-borine is heated, the principal product is dimethylaminoborine dimer.[2] However the preparation of trimeric dimethylaminoborine, $[(CH_3)_2NBH_2]_3$, has been accomplished by heating the dimer with higher boron hydrides. Both pentaborane(9) and the yellow boron hydride solids produced by the pyrolysis of diborane have been effective in this conversion.

A sample of $[(CH_3)_2NBH_2]_3$, prepared by heating the dimer at 100–110° with pentaborane(9), and purified on the vacuum line,[3] melted at 97.0–97.8°. The molecular weight (cryoscopic in benzene) was 165.3 (calcd. 170.7). In two experiments the acid methanolysis (16 hours at 85°) showed 3.48 and 3.50% active hydrogen, or an average of 5.91 moles of hydrogen produced per mole of $[(CH_3)_2NBH_2]_3$ (calcd. 6.00).

Anal. Calcd.: C, 42.25; H, 14.17; N, 24.62; B, 19.01. Found: C, 42.24, 42.14; H, 14.10, 14.21; N, 24.44, 24.35; B, 19.02 and 19.28.

The proton magnetic resonance spectrum of $[(CH_3)_2NBH_2]_3$ consisted of four signals of equal intensity with spacing of approximately 2 p.p.m., with a very strong superimposed single peak. An integration showed that the intensity of the large single peak was three times the sum of intensities of the four smaller peaks, corrected for 19% B^{10} concentration. The interpretation was that the large single peak was due to the C–H hydrogen, and that the $N(CH_3)_2$ protons are magnetically equivalent, whereas the smaller multiplet was due to the hydrogen atoms attached to B^{11}. The relative intensities, therefore, indicate that there are three hydrogen atoms bonded to carbon for each B–H hydrogen, which is in agreement with the formula $[(CH_3)_2NBH_2]_3$. This interpretation was substantiated by the B^{11} spectrum, run at 16.2 mc. in a field of 11,900 gauss. A simple triplet was observed, with a 1–2–1 intensity ratio, which strongly indicates that all boron atoms are magnetically equivalent and that each has two covalently bonded hydrogen atoms.

The dimethylaminoborine trimer has a camphor-like odor, and is quite unreactive in moist air and even when dissolved in wet acetone it does not hydrolyze measurably at room temperature. In this respect it is quite comparable to the methylaminoborine trimer.[1] In view of the nuclear magnetic resonance analysis, it appears that this compound has a cyclic structure, comparable to the phosphinoborine trimers.[4]

It was reported by Burg[5] that the reaction of pentaborane(9) with dimethylaminoborine (present in excess) produced the compound "$[(CH_3)_2N]_3B_3H_4$." No compound of this composition was obtained from this system. Since the physical properties of trimeric dimethylaminoborine were nearly identical to those of the reported "$[(CH_3)_2N]_3B_3H_4$," and the preparation process was identical to that described by Burg, it appears likely that trimeric dimethylaminoborine and "$[(CH_3)_2N]_3B_3H_4$" are the same compound.

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(2) A. B. Burg and C. L. Randolph, THIS JOURNAL, **73**, 953 (1951).

(3) The last traces of $[(CH_3)_2N]_2B_4H_8$ were removed from the trimeric dimethylaminoborine by slow distillation at 0 to 5°. The infrared spectrum of $[(CH_3)_2N]_2B_4H_8$ showed strong absorption at 4.08 μ, with a shoulder at 3.9 μ, whereas $[(CH_3)_2NBH_2)_3$ had low absorption at these wave lengths, but absorbed strongly at 4.14, 4.25 and 4.42 μ. Other absorption bands for $[(CH_3)_2N]_2B_4H_8$, which were useful in detecting its presence in trimeric dimethylaminoborine, were found at 8.68 μ and 10.35 μ. The complete absence of absorption at these wave lengths was prerequisite to further work with trimeric dimethylaminoborine.

(4) W. C. Hamilton, *Acta Cryst.*, **8**, 199 (1955).

(5) A. B. Burg, THIS JOURNAL, **79**, 2129 (1957).

ELECTRON TRANSFER FROM THE INDOLE NUCLEUS TO THE PYRIDINE COENZYMES

Sir:

We report the formation of charge transfer complexes among biologically active compounds and a concomitant strong support for the views of Mulliken[1] and Kosower[2] on the importance of charge transfer complexes in biochemical systems.

Addition of DPN[3] or TPN or of their model compound 1-benzyl-3-carboxamide pyridinium chloride to an aqueous solution of any of the indole derivatives available to us (these in Table I and yohimbine) developed *instantaneously* a faint yellow color. Spectroscopic examination indicates the appearance of a *new, quite diffuse* band as a long tail to the longer wave length side of the indole nucleus absorption.

TABLE I

DATA FOR CHARGE TRANSFER COMPLEXES OF 1-BENZYL-3-CARBOXAMIDE PYRIDINIUM CHLORIDE WITH INDOLE AND DERIVATIVES[a]

		Association constant l. mol.$^{-1}$	Molar extinction coefficient ϵ	mμ
Indole	Water	2.5	540	370
L-Tryptophan	Water	2.2	860	370
Glycyl-L-tryptophan	Water	2.9	500	400
Indole-3-acetic acid	1.7×10^{-2} *M* phosphate *p*H 6.7	4.1	1220	370
Serotonin[b]	*p*H 6.5	1.8	1410	380
Acetyltryptophan	*p*H 6.5	4.0	510	400

[a] Room temperature (25 ± 2°). [b] As creatinine sulfate.

Under comparable conditions, no other amino acid is able to replace tryptophan in this kind of interaction.

Chymotrypsinogen after preincubation with urea for a few hours also develops the charge transfer band by addition of DPN or its model, even at low *p*H's where reactivity of other amino acids is out of question.[4]

Application of the equation of Foster,[5] *et al.*,

(1) R. S. Mulliken, THIS JOURNAL, **74**, 811 (1952).

(2) E. M. Kosower, *ibid.*, **78**, 3497 (1956).

(3) These abbreviations are used: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; GPD, glyceraldehyde phosphate dehydrogenase; APDPN, the acetyl analogue of DPN.

(4) See J. van Eys, *J. Biol. Chem.*, **233**, 1203 (1958)

(5) R. Foster, D. Ll. Hammick and A. A. Wardley *J. Chem. Soc.*, 3817 (1953).