activity of this compound in man and animals[6] and its possible relation to Slater's factor.[7]

In ammonium sulfate-fractionated extracts of *A. fischeri*,[8] the menadione reductase activity was present chiefly in the 30–40% saturated fraction. With this preparation it was observed that the reaction required FMN and that FAD was only slightly active (Table I). The specificity for FMN is in contrast to the *S. faecalis* menadione reductase system of Dolin.

TABLE I

EFFECT OF FLAVINS ON THE *A. fischeri* MENADIONE REDUCTASE SYSTEM

Reaction components: 1.0 ml. tris-(hydroxymethyl)-aminomethane (0.1 *M*), *p*H 8.2; 200 μg. $DPNH_2$; 25 μg. menadione; 29 μg. FMN; 50 μg. FAD; 0.05 ml. enzyme; final vol., 3.0 ml.; components incubated with enzyme for 5 min. at room temp. before $DPNH_2$ addition. The $-\Delta E_{340}$ was measured.

Reaction components	Enzyme units[a]
Enzyme	12
Enzyme + FAD	32
Enzyme + FMN	240
Enzyme + FMN (no menadione added)	26

[a] One enzyme unit = amount of enzyme which gives a change in log I_0/I of 0.001 per min. calculated from the change between 15- and 45-sec. readings.

Crude cell-free extracts of *A. fischeri* reduced cytochrome c at a relatively rapid rate but only in the presence of menadione. The purified enzyme, however, required the addition of FMN before a significant increase in the rate of cytochrome c reduction was observed (Table II).

TABLE II

EFFECT OF FMN AND MENADIONE ON CYTOCHROME C REDUCTION IN *A. fischeri*

Reaction components: 1.0 ml. tris-(hydroxymethyl)-aminomethane (0.1 *M*), *p*H 8.2; 200 μg. $DPNH_2$; 25 μg. menadione; 90 μg. FMN; 0.2 ml. cytochrome c (2×10^{-4} *M*); 0.06 ml. crude and 0.02 ml. purified enzyme [30–40% $(NH_4)_2SO_4$ fraction] used; final vol., 3.0 ml.; components incubated for 5 min. at room temp. before addition of $DPNH_2$. The $+\Delta E_{550}$ was measured.

Reaction components	Enzyme units
Crude enzyme	4
Crude enzyme + menadione	76
Purified enzyme + FMN	16
Purified enzyme + menadione	8
Purified enzyme + menadione + FMN	172

A cell-free extract of *S. faecalis*[9] from which cytochrome c reductase activity had been removed by ammonium sulfate fractionation and adsorption on calcium phosphate gel and which contained menadione reductase reduced cytochrome c at a rapid rate after the addition of menadione. Explanation of this involves two possibilities: (a) that menadione is the "prosthetic group" of cytochrome c reductase, or (b) that the product of menadione reductase reaction enzymically or spontaneously reduces cytochrome c. The latter hypothesis is favored by the observation that reduced menadione rapidly reduces cytochrome c non-enzymically. Boiled crude extracts of *S. faecalis* did not reactivate the purified extract.

A. fischeri menadione reductase is inhibited by BAL, while both BAL and $FMNH_2$ reverse menadione and cytochrome c inhibition of bacterial luminescence. It seems likely that the menadione reductase and luminescent systems are competing with each other.

BIOLOGY DIVISION
OAK RIDGE NATIONAL LABORATORY M. J. CORMIER
OAK RIDGE, TENNESSEE J. R. TOTTER

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(6) S. Ansbacher and E. Fernholz, THIS JOURNAL, **61**, 1924 (1939).
(7) E. C. Slater, *Biochem. J.*, **46**, 484 (1950).
(8) B. L. Strehler and M. J. Cormier, *Arch. Biochem. and Biophys.*, **47**, 16 (1953).
(9) M. I. Dolin, manuscript in preparation.

PYRIDOXAL PHOSPHATE AND METAL IONS AS COFACTORS FOR HISTIDINE DECARBOXYLASE

Sir:

In contrast to other amino acid decarboxylases, histidine decarboxylase has not been resolved into apoenzyme and coenzyme.[1,2] The failure to detect pyridoxal phosphate (PLP) in purified cell-free preparations of histidine decarboxylase, the comparative insensitivity of this enzyme to inhibition by ferrous ions or carbonyl-trapping reagents, and the fact that histidine decarboxylase (in contrast to ornithine decarboxylase) was not reduced in amount in cells of a *Lactobacillus* grown with reduced amounts of vitamin B_6, have led to the view that this enzyme may not require PLP for activity.[3,4,5] However, long incubation of resting cells of *Escherichia coli* with pyridoxine does increase their histidine decarboxylase activity.[6] This uncertainty concerning the role of PLP in the activity of histidine decarboxylase stimulated the following investigation.

Lactobacillus 30a of Rodwell,[5,7] proved to require vitamin B_6 for growth in a defined medium containing D-alanine[8]; however, at minimal levels of vitamin B_6, the histidine decarboxylase activity per mg. of cells obtained was less than 4% of that of cells grown with optimal levels of the vitamin (Table I). It should be noted that the concentration of vitamin B_6 required for production of an active ornithine decarboxylase is very much higher.

Cells from a complete medium were sonically disintegrated and the fraction soluble in 45% ammonium sulfate but insoluble in 55% ammonium sulfate separated. On the basis of protein content, this fraction was several hundred times more active than intact cells in decarboxylating histidine. Dialysis of this fraction at room temperature for 24 hours against 0.2 *M* acetate buffer, *p*H 3.8, resulted in almost complete loss of enzymatic activity. Addition of a boiled cell suspension restored 60 to 100% of the initial activity; PLP additions gave erratic results (A and B, Fig. 1), higher amounts frequently failing to show any reactivating

(1) O. Schales, "The Enzymes," Vol. II, Part A, J. B. Sumner and K. Myrback, Academic Press, New York, N. Y., 1951, p. 222.
(2) E. E. Snell, *Physiol. Revs.*, **33**, 509 (1953).
(3) E. S. Taylor and E. F. Gale, *Biochem. J.*, **39**, 52 (1945).
(4) H. M. R. Epps, *ibid.*, **39**, 42 (1945).
(5) A. W. Rodwell, *J. Gen. Microbiol.*, **8**, 233 (1953).
(6) E. Werle and W. Koch, *Biochem. Z.*, **319**, 305 (1949).
(7) A. W. Rodwell, *J. Gen. Microbiol.*, **8**, 224 (1953).
(8) The medium is similar to that used by Craig and Snell (*J. Bact.*, **61**, 283 (1951)). A more detailed account of the nutrition of this organism will appear later.

TABLE I

COMPARATIVE CONCENTRATION OF VITAMIN B_6 REQUIRED FOR GROWTH AND DECARBOXYLASE PRODUCTION BY *Lactobacillus* 30a

Pyridoxamine, mγ per ml.	% of maximum growth[a]	Dicarboxylase activity toward L-Histidine Q_{CO_2}[b]	Dicarboxylase activity toward L-Ornithine Q_{CO_2}
0	0	...	...
0.58	45	5.0	...
1.16	66	27	...
3.5	59	137	0
11.6	100	142	0
58.0	100	114	20
116	100	108	35
1160	100	111	206

[a] Incubated 24 hours at 37°; maximum growth was 1.38 mg. of cells (dry weight) per ml. [b] Q_{CO_2} = μl. of CO_2 evolved per mg. of cells (dry weight basis) per hour. Enzyme activity was determined manometrically at 37° in air. The reaction vessels contained 1 ml. of 0.2 *M* acetate buffer, *p*H 4.8, 0.81 mg. of cells and water to make 2.5 ml. in the main compartment, and 0.5 mg. of substrate (histidine or ornithine) in 0.5 ml. of the acetate buffer in the side arm. Equilibrated 15 minutes before mixing; Q_{CO_2} values were calculated from CO_2 evolution after 60 minutes. CO_2 evolution in absence of substrate was nil.

effect. However, addition of PLP together with ferric ion gave excellent reactivation (C, Fig. 1) similar in magnitude to that obtained with the boiled cell extract. Of several metal ions tested, Fe^{+++} and Al^{+++} were about equally effective, Co^{++} and Ni^{++} were slightly effective, and Fe^{++}, Zn^{++}, Cu^{++}, Mn^{++}, Mg^{++} and molybdate ion were either inhibitory or had no effect.

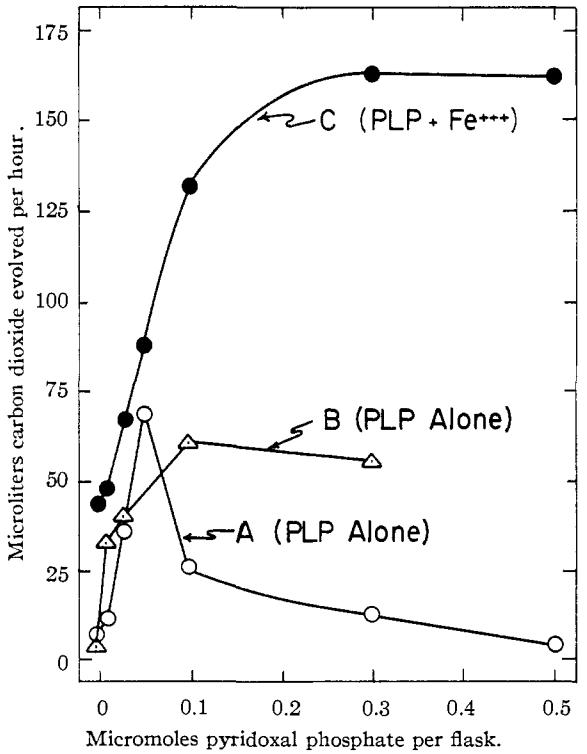

Fig. 1.—Reactivation of dialyzed histidine decarboxylase by pyridoxal phosphate and ferric ions: A and B, PLP alone (different days); C, PLP + 0.5 μatoms of Fe^{+++} per flask. A and C were run simultaneously. Assay conditions as in Table I, but with dialyzed cell-free enzyme (2γ of protein) in place of cells, 100γ of adenosine-3-phosphate in main compartment, and acetate buffer in side arm replaced by water.

Thus histidine decarboxylase, like other decarboxylases, requires pyridoxal phosphate as coenzyme, but in the cells is fully activated by vitamin B_6 concentrations insufficient to activate other decarboxylases studied. The additional requirement for ferric or aluminum ions[9] establishes a remarkable parallelism between the requirements for enzymatic and non-enzymatic catalytic actions of pyridoxal and supports the validity of the general mechanism previously proposed for such reactions.[10]

(9) Indications that magnesium ions activate kynureninase (W. B. Jakoby and D. M. Bonner, *J. Biol. Chem.*, **205**, 699 (1953)), cystathionase (S. Wijesundera and D. D. Woods, *J. Gen. Microbiol.*, (Proc.) **9**, 3 (1953)) and possibly D-serine dehydrase (C. Yanovsky, *J. Biol. Chem.*, **198**, 343 (1952)), all of which are pyridoxal phosphate enzymes, have recently appeared.

(10) D. E. Metzler, M. Ikawa and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).

THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH
AUSTIN, TEXAS
BEVERLY M. GUIRARD
ESMOND E. SNELL

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AN INITIAL REPORT ON THE STOICHIOMETRY AND KINETICS OF THE GAS PHASE REACTION OF NITROGEN DIOXIDE AND AMMONIA

Sir:

During the course of the investigation of the kinetics of the non-catalytic thermal oxidation of ammonia carried out in this laboratory,[1,2,3] it was recognized that any chain mechanism likely to afford an explanation of the experimental facts would probably involve nitric oxide and, in turn, nitrogen dioxide. Therefore, a knowledge of the reaction of ammonia with these possible intermediates was necessary in order to interpret the results obtained. Since the literature[4,5] gave little information concerning these reactions, the work reported in this communication was undertaken and is being continued.

Although the products of the gas-phase NO_2–NH_3 reaction at room temperature have been reported as N_2, H_2O, NO and NH_4NO_3 by one author[5] and as those plus N_2O, NH_4NO_2, and NH_2NH_2 by another,[4] we have been able to show that the major products are N_2, H_2O and NH_4NO_3. Under pressures of less than 50 mm. of each reactant at least 99% of the material formed is composed of these three products, giving a stoichiometry

$$2NO_2 + 2NH_3 \longrightarrow NH_4NO_3 + N_2 + H_2O$$

Traces of N_2O, NO and NH_4NO_2 have been identified—the latter two possibly being formed by the reaction of NO_2 and NH_3 with water, which is produced in the major reaction, as shown by Klevke.[6]

(1) E. R. Stephens and R. N. Pease, THIS JOURNAL, **72**, 1188 (1950).

(2) E. R. Stephens, unpublished thesis, 1951.

(3) P. S. Blatz, unpublished thesis, 1952.

(4) M. Patry, R. Garlet and S. Pupko, *Compt. rend.*, **225**, 941 (1947).

(5) D. M. Yost and H. Russell, "Systematic Inorganic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1946, p. 17.

(6) V. A. Klevke, *J. Chem. Ind. (Moscow)*, **13**, 164 (1936).