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]	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.

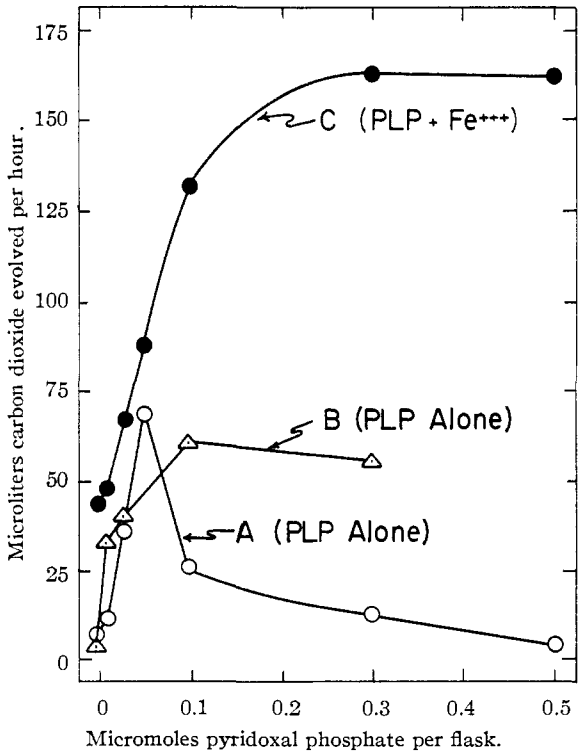

Fig. 1.—Reactivation of dialyzed histidine decarboxylase by pyridoxal phosphate and ferric ions: A and B, PLP alone (different days); C, PLP + 0.5 μatoms of Fe^{+++} per flask. A and C were run simultaneously. Assay conditions as in Table I, but with dialyzed cell-free enzyme (2γ of protein) in place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

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RECEIVED JULY 23, 1954

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).

That is

$$2NH_3 + 2NO_2 + H_2O \longrightarrow NH_4NO_3 + NH_4NO_2$$
$$NH_4NO_2 + NO_2 \longrightarrow NH_4NO_3 + NO$$

Hydrazine has not been found by us and must therefore be produced in an amount less than 0.1% of total nitrogen, if at all. The above stoichiometry was shown to exist at 25 and at 100° by standard chemical and physical techniques. In the range of 150 to 200° the pressure change agreed, to within a few per cent., with the change predicted.

The kinetics of this reaction has been studied between 150 and 200° by following the pressure change with time. Pressures up to 100 mm. of each gas were used and the ratio NO_2:NH_3 was varied from 4:1 to 1:4. An examination of initial rates showed a third order reaction—first with respect to ammonia and second with respect to nitrogen dioxide. The initial third order rate law was not followed throughout the entire course of the reaction but, instead, an acceleration took over. A large negative temperature coefficient was found—*e.g.*, at 151° the specific third order rate constant was shown to be 1.1×10^{10} cc.^{2} mole^{-2} sec.$^{-1}$, whereas, at 205° it has dropped to 2.2×10^9 cc.^{2} mole^{-2} sec.$^{-1}$. A plot of the log of the rate constant as a function of the reciprocal of the absolute temperature gave an excellent straight line over the 50° interval studied and led to a "negative activation energy" of -12.5 ± 1 kc.

The negative temperature coefficient and the "activation energy" of -12.5 ± 1 kc. leads to the proposition that N_2O_4 and not NO_2 is the reactive species since the ΔH of dissociation of N_2O_4 to NO_2 is just about that value—*i.e.*, 13.6 kc. at 25°.[5] Another possibility involves inhibition by NO since the dissociation of NO_2 to NO also has a ΔH of this order of magnitude.

The effect of NO on this reaction is now being considered in order to clear up this last point. In addition work is in progress to determine what effect, if any, excess O_2 and H_2O might have. Finally, a survey of rates from room temperature to 400° is being carried out.

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ACTION OF INTESTINAL EXTRACTS ON "BRANCHED" OLIGOSACCHARIDES[1]

Sir:

α-Amylotic hydrolysis of amylopectin produces maltose, glucose and a mixture of "branched" oligosaccharides.[2,3,4] In gastrointestinal digestion maltose is further hydrolyzed to glucose by maltase (α-glucosidase)[5] and the glucose absorbed. The fate of the branched oligosaccharides is unknown. We have isolated from small intestinal mucosa a new enzyme, which hydrolyzes the α-1,6-linkages of isomaltose,[6] panose[7] and the mixture of branched oligosaccharides remaining after extensive α-amylolysis of amylopectin. This new enzyme allows essentially complete digestion of starch to occur in the gastrointestinal tract. The name oligo-1,6-glucosidase[8] is accordingly proposed.

With isomaltose as substrate (0.0004 *M*), a quantitative activity assay has been set up measuring the time rate of reduction of TPN[9] in the presence of excess hexokinase,[10] Zwischenferment[11] and ATP. The system is buffered at *p*H 7.4 with 0.083 *M* glycylglycine in a final volume of 3.0 ml. When substrate is added there is an initial time lag of about one minute, after which the reaction proceeds linearly for at least 4–6 minutes provided TPN is present in excess. Rate of TPN reduction is proportional to extract added over a tenfold range of dilution. A unit of enzyme activity has been defined as that amount of enzyme which effects an increase in optical density at 340 mμ in the Beckman model DU spectrophotometer of 0.001 per minute under the stated conditions.[12] Table I lists specific activities (units/mg. protein) of extracts from four species. In the case of freshly prepared extracts of rabbit intestine, jejunum and ileum show much lower activities than extracts of duodenum.[13]

Oligo-1,6-glucosidase is differentiated from amylo-1,6-glucosidase[14] by the inability of the latter to hydrolyze (microenzymatic test) isomaltose, panose or the mixture of branched oligosaccharides.[15] Furthermore, although crude intestinal extracts rapidly liberate glucose from the phosphorylase limit dextrin (or glycogen), this activity is lost during subsequent purification. Starch-treated extracts[14] no longer liberate glucose from the phosphorylase limit dextrin (or glycogen) while hydrolysis of isomaltose continues. α-Amylase rather than amylo-1,6-glucosidase would therefore seem responsible for glucose production in the untreated extracts.[16]

α-1,6-Glucosidases have been described from

(1) Supported in part by a grant from the Graduate College, University of Illinois, Urbana.

(2) W. J. Whelan, *Biochem. Soc. Symposia* No. 11, p. 17 (1953).

(3) P. Nordin, G. Wild and D. French, Abstracts of 124th Meeting American Chemical Society, p. 53C.

(4) K. H. Meyer and W. F. Gonon, *Helv. Chim. Acta*, **34**, 308 (1951).

(5) J. P. Peters and D. D. Van Slyke "Quantitative Clinical Chemistry," Vol. I, Second Edition, Williams and Wilkins Co., Inc., Baltimore, Md., p. 103.

(6) We are indebted to Dr. Allene Jeanes, Northern Regional Research Laboratory, Peoria, for the sample of isomaltose, 6-(α-D-glucopyranosyl)-D-glucose.

(7) We are indebted to Dr. S. C. Pan, Squibb Institute for Medical Research, New Brunswick, N. J., for the sample of panose, 4-(α-isomaltosyl)-D-glucose. During intermediate stages of panose hydrolysis, maltose accumulates which is hydrolyzed by maltase (α-glucosidase) present to free glucose.

(8) If more than one α-1,6 splitting enzyme is present, the name would then apply to the class of enzymes.

(9) Abbreviations: triphosphopyridine nucleotide, TPN; adenosine triphosphate, ATP.

(10) Purified from yeast by an unpublished method of C. R. Park.

(11) A. Kornberg, *J. Biol. Chem.*, **182**, 805 (1950).

(12) The over-all reaction has been followed also by increase in reducing power, and by paper chromatography.

(13) Bacterial contamination as a major source of activity has been ruled out by filtration through a bacteriological filter (sintered glass), centrifugation at top speed of Sorvall centrifuge, Model SS-1 for 30 minutes, and direct bacteriological counts. We are indebted to J. R. Stamer and I. C. Gunsalus, of the Department of Bacteriology, for performing these counts.

(14) G. T. Cori and J. Larner, *J. Biol. Chem.*, **188**, 805 (1950).

(15) Unpublished experiments, J. Larner and L. H. Schlisefeld.

(16) Glucose production in the untreated extracts could occur by α-amylase action alone as well as by the action of oligo-1,6-glucosidase on the "branched" dextrins remaining after α-amylase action. Starch-treated extracts still contain maltase (α-glucosidase).