OXYGEN INDUCED ELECTROREDUCTION OF HYDROGEN PEROXIDE AT THE ROTATED PLATINUM ELECTRODE

Sir:

Based upon the following considerations the effect of hydrogen peroxide upon the diffusion current of oxygen has been investigated. It is assumed that the electroreduction of oxygen to hydrogen peroxide occurs in two steps

$$O_2 + e^- \longrightarrow O_2^- \quad (1)$$
$$O_2^- + e^- \longrightarrow O_2^{=} \quad (2)$$

At the surface of the electrode the O_2^-—or its corresponding acid HO_2—may react according to the Haber–Weiss[1] mechanism with hydrogen peroxide

$$O_2^- + H_2O_2 \longrightarrow OH^- + OH\cdot + O_2 \quad (3)$$
$$HO_2 + H_2O_2 \longrightarrow H_2O + OH\cdot + O_2 \quad (3a)$$
$$OH\cdot + H_2O_2 \longrightarrow HO_2 + HO_2 \quad (4)$$

Reactions (1) and (3) account for an exalted oxygen wave in the presence of hydrogen peroxide. Reactions (1), (3) and (4) also account for the same effect.

Terminators of the above chains are reaction (2) and

$$OH\cdot + e^- \longrightarrow OH^- \quad (5)$$

No information on the rate of electroreduction of $OH\cdot$ and HO_2 under the experimental conditions being available, the effect of hydrogen peroxide upon the oxygen reduction was studied empirically. Experimentally it was found that hydrogen peroxide causes a very large increase in the limiting current of oxygen at the rotated platinum electrode. Figure 1 illustrates the effect in 0.1 M sodium perchlorate solution. The oxygen concentration of the original solution was less than 10^{-6} M and the solution yielded a diffusion current of the order of 0.2 microampere at 25°. In the presence of 3.2×10^{-4} M hydrogen peroxide the limiting current became 2 μ and in 16×10^{-4} M hydrogen peroxide, 10 μ. Under the experimental conditions the exaltation was proportional to the hydrogen peroxide concentration. Analytical application of this exaltation for the determination of traces of oxygen is now being made.

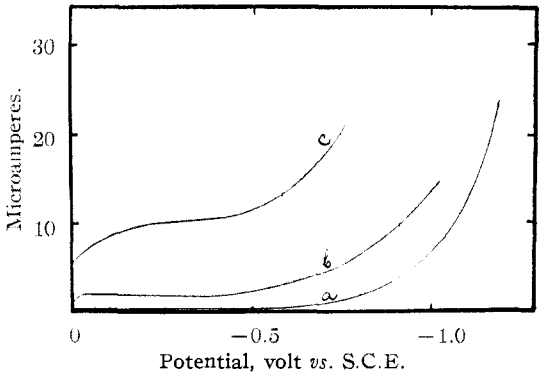

Fig. 1.—Current–voltage curves in 0.1 M sodium perchlorate: $[O_2] < 10^{-6}M$; (a) no H_2O_2, (b) $3.2 \times 10^{-4}M$ H_2O_2, (c) $16 \times 10^{-4}M$ H_2O_2.

(1) F. Haber and J. Weiss, *Naturwiss*, **20**, 948 (1932); *Proc. Roy. Soc. (London)*, **A147**, 332 (1934).

The effect was also observed at the stationary platinum electrode. However, at the dropping mercury electrode the exaltation was obscured by maxima.

Substances which react very rapidly with $OH\cdot$, like acrylonitrile, allyl acetate, and other monomers did not affect the exaltation at the rotated electrode. From this it was concluded[2] that reaction (4) does not occur to a measurable extent and that reaction (5) is the main terminating reaction. Thus the electroreduction of oxygen induces the electroreduction of hydrogen peroxide, at potentials at which hydrogen peroxide is not (or very slowly) reduced, according to the over-all reaction

$$H_2O_2 + 2e^- \longrightarrow 2OH^-$$

This was confirmed by electrolysis experiments with a large platinum cathode in which the decrease of the hydrogen peroxide concentration during the electrolysis was determined.

The effect of various factors, such as pH, kind of supporting electrolyte, concentration of oxygen and hydrogen peroxide and of the temperature are now being investigated. A detailed account will be given at a later date.

(2) See I. M. Kolthoff and E. P. Parry, THIS JOURNAL, **73**, 3718 (1951).

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RECEIVED DECEMBER 26, 1951

CRYSTALLINE PYRIDOXAMINE PHOSPHATE

Sir:

Phosphorylated derivatives of vitamin B_6 are believed to play an important role in a number of enzyme systems, including transaminases, amino acid decarboxylases, and tryptophanase. It appears probable that pyridoxamine phosphate (2-methyl - 3 - hydroxy - 4 - aminomethyl - 5 - pyridylmethylphosphoric acid,[1,2] which has been reported to occur naturally,[3,4] is involved in biological transamination. Although this system has received considerable attention, the cofactors have thus far been available only in impure condition and the mechanisms of their action have not been established. Very recently, Viscontini, *et al.*,[5] have reported the preparation of calcium pyridoxal phosphate of high purity. We report at this time the preparation of crystalline pyridoxamine phosphate which gives theoretical analytical values and a very high order of activity.

Commercial pyridoxamine dihydrochloride was converted to the free amine and purified by recrystallization from 50% ethanol. To the colorless amine was added 10 times its weight of anhydrous phosphoric acid (1 part P_2O_5 to 2.5 parts 85% H_3PO_4)[6] and the mixture was heated at 100° for

(1) D. Heyl, E. Luz, S. A. Harris and K. Folkers, THIS JOURNAL, **73**, 3436 (1951).
(2) A. N. Wilson and S. A. Harris, *ibid.*, **73**, 4693 (1951).
(3) J. C. Rabinowitz and E. E. Snell, *J. Biol. Chem.*, **169**, 643 (1947).
(4) W. S. McNutt and E. E. Snell, *ibid.*, **173**, 801 (1948).
(5) von M. Viscontini, C. Ebnöther and P. Karrer, *Helv. chim. acta*, **34**, 1834 (1951).
(6) R. H. A. Plimmer and W. J. N. Burch, *Biochem. J.*, **31**, 308 (1937).

24 hours. Nine volumes of absolute ethanol was added slowly with stirring to the cooled reaction mixture to yield a white precipitate, which, after being washed successively with absolute ethanol and ether, was dissolved in a minimal amount of water and brought to about *p*H 6 with concentrated ammonia. The mixture was applied to the top of an Amberlite XE-64 (a fine mesh, weak cation exchange resin) column in the washed free acid form. The effluent fractions from the column, on elution with water, were examined spectrophotometrically and by paper chromatography.[7] Two components, one of which was very small, gave positive ninhydrin tests. The major and slower moving of these, which showed a maximum absorption at 3250 Å., was concentrated *in vacuo* to a white residue which upon treatment with a small amount of water soon became crystalline. The product was sparingly soluble in water and was recrystallized by the addition of an equal volume of ethanol to the aqueous solution. The crystals appeared as systems of rhombic plates.

Anal. Calcd. for $C_8H_{13}N_2O_5P \cdot 2H_2O$: C, 33.8; H, 6.0; N, 9.9; P, 10.9. Found: C, 34.1; H, 5.9; N, 10.2; P, 11.1. When dried *in vacuo* (P_2O_5), the compound lost the theoretical amount of weight.

Ultraviolet absorption in 0.01 *M* buffers: $\lambda_{max.}$ (*p*H 2.0) 2935 Å., E_m 9600; $\lambda_{max.}$ (*p*H 7.2) 2535 Å., E_m 5200, 3265 Å., E_m 9400; $\lambda_{max.}$ (*p*H 10.0) 2440 Å., E_m 7500, 3120 Å., E_m 8300.

The crystalline pyridoxamine phosphate was oxidized with MnO_2[2] yielding NH_3 stoichiometrically. The oxidized preparation (pyridoxal phosphate) exhibited a high order of catalytic activity in a transaminase system of *Lactobacillus arabinosus*,[8] the tyrosine decarboxylase system of *Streptococcus faecalis*,[9] the aspartic acid β-decarboxylase system of *Clostridium welchii*,[10] and the tryptophanase system of *Escherichia coli*.[11] Within experimental error, the determination of relative purity yielded values of 100% based on assay with the tyrosine decarboxylase system[9] and by comparison with an impure preparation of pyridoxal phosphate assayed in another laboratory.[12] Assay of the crystalline pyridoxamine phosphate as described by Hendlin, *et al.*,[13] yielded similar results.

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RECEIVED DECEMBER 1, 1951

(7) Pyridoxamine and pyridoxamine phosphate were detected by the orange color formed after reaction with ninhydrin.

(8) A. Meister, *Federation Proceedings*, **10**, 223 (1951).

(9) W. W. Umbreit, W. D. Bellamy and I. C. Gunsalus, *Arch. Biochem.*, **7**, 185 (1946).

(10) A. Meister, H. A. Sober and S. V. Tice, *J. Biol. Chem.*, **189**, 577 (1951).

(11) W. A. Wood, I. C. Gunsalus and W. W. Umbreit, *J. Biol. Chem.*, **170**, 313 (1947).

(12) This preparation was generously provided by Dr. W. W. Umbreit.

(13) D. Hendlin, M. C. Caswell, V. J. Peters and T. R. Wood, *J. Biol. Chem.*, **186**, 647 (1950).

(14) Postdoctoral Fellow, National Cancer Institute of the National Institutes of Health, Public Health Service, Federal Security Agency.

BOOK REVIEWS

Progress in Chromatography 1938–1947. By L. ZECHMEISTER, California Institute of Technology, Pasadena, California. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1951. xviii + 368 pp. 14 × 22 cm. Price, $8.00.

Professor L. Zechmeister, well known as a superb experimentalist both before and after his migration from Hungary to the California Institute of Technology, performed a distinct service to the science by writing, with L. Cholnoky, the authoritative "Principles and Practice of Chromatography," published in 1941 as a translation of a second German edition of the original work.

In the present volume Professor Zechmeister has presented a progress report consisting of a survey of the literature on the technique and application of chromatography in the period 1938–1947. The field is currently expanding and developing at so rapid a pace that the author felt that at this time a supplement would be more appropriate than a revision of the original monograph. He has thus presented a meticulously prepared survey of literature on principles and methods of chromatography, and on specific applications.

The 57-page survey of advances in the principles and methodology is a worthy extension of the original book as far as it goes, but the long gap between literature coverage (through 1947) and publication (1950) is hardly excusable in a book dealing with this rapidly developing and enormously useful technique. Thus partition chromatography, particularly as applied in the paper strip method, has become so familiar a tool that the papers of 1941–1947 now seem like early history.

The major part of the book is devoted to a review of the literature on specific applications of chromatography to organic compounds of some twenty structural types and to inorganic compounds. In the preface, the author states that "even hints about methods, adsorbents or solvents which can be used within a certain class of compounds may be welcome and time-saving for the experimenter." The objective is worthy, and perhaps the plan of citing applications classified according to type of compound was the best expedient for this interim work. However, the citation of chromatographic experiences and accomplishments in various fields seems to me to fall somewhat short of the mark. One working in a given field will soon learn from the specific literature the methods of chromatography traditionally used for the class of compounds concerned and might derive more stimulation from a cross-sectional discussion that perhaps would suggest trial of methods found useful for separation of compounds of other types. Chromatographic processing of reaction mixtures and mother liquors by the empirical elution technique is now practiced in many laboratories so frequently—to an extent at least comparable to