deviations are not ruled out. Such small deviations cannot be regarded as likely, however.

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ISOLATION OF CRYSTALLINE PYROPHOSPHATASE FROM BAKER'S YEAST

Sir:

The presence in yeast of an enzyme capable of catalyzing the hydrolysis of inorganic pyrophosphate to orthophosphate has been established by Bauer[1] in 1936. The enzyme was named "pyrophosphatase." Several attempts have been made by various workers to purify the enzyme. The most notable advance in the purification of the enzyme was made in 1944 by Bailey and Webb.[2] They have been unsuccessful, however, in their attempt to obtain the enzyme in crystalline form. The enzyme has now been crystallized from Fleischmann's baker's yeast, in the form of fine needles and thin rectangular prisms.

The method of isolation consists essentially of the following steps: 1. Plasmolysis of compressed yeast with toluene at 38–40°, followed by extraction with water, at 5°. 2. Concentration and fractionation between 0.5 and 0.7 saturation of ammonium sulfate. 3. Removal of inert components by autolysis at 5°, accompanied by precipitation of the enzyme with ammonium sulfate. 4. Further removal of impurities by adsorption on $Ca_3(PO_4)_2$ gel, followed by precipitation of the enzyme with ammonium sulfate. 5. Removal of electrolytes by dialysis against distilled water at 5°. 6. Crystallization in dilute ethyl alcohol solution at −8°.

Crystalline pyrophosphatase is a soluble, colorless protein of the albumin type, free of phosphorus (C, 54.5; H, 7.4; N, 16.2; S, 0.14; ash, 0.36).

Details of the method of isolation, also a description of some of the physico-chemical and catalytic properties of the newly isolated crystalline enzyme, are to be submitted for publication in the *Journal of General Physiology.*

(1) E. Bauer, *J. Physiol. Chem.*, **239**, 195 (1936).
(2) K. Bailey and E. C. Webb, *Biochem. J.*, **38**, 394 (1944).

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RECEIVED JANUARY 25, 1951

PAPER CHROMATOGRAPHY OF HYDROXY AND KETOACIDS[1]

Sir:

Paper chromatography has been applied in the last few years to the detection of small amounts of various types of organic acids. Various procedures have been reported for the saturated aliphatic acids,[2–6] and Lugg and Overell[7] have developed an excellent method for the paper chromatography of polycarboxylic and other non-volatile acids. They employed butanol–water or other solvents, in combination with a volatile organic acid, such as formic or acetic, in order to decrease the ionization of the test acids and thus prevent streaking or "tailing."

In connection with an investigation of fatty acid metabolism in this laboratory, it was necessary to develop a technique for the separation and identification of small quantities of certain hydroxy and ketoacids of intermediate chain length. In attempting to apply a paper chromatographic method, it was found that the solvent system of Lugg and Overell was not suitable for most of these acids: they exhibited very high R_f values and poor resolution; furthermore, their moderate degree of volatility limited the length of time permissible to carry out the procedure.

It has been found, however, that a solvent system composed of toluene–acetic acid–water provides an excellent method for the analysis of many hydroxy and ketoacids. A mixture of 100 cc. of toluene and 5 cc. of acetic acid is equilibrated with 60 cc. of distilled water; after separation of the layers, an additional 4 cc. of acetic acid is added to the toluene layer. Whatman No. 1 filter paper is used without prior washing or other treatment. The papers are run in the descending manner for several hours, depending on the particular acids being chromatographed. After the removal of the paper from the chamber, it is dried several hours in a current of air. The test acids are located in a novel manner: the dried papers are exposed a few minutes to ammonia vapor in a closed chamber, the excess ammonia is removed by allowing the paper to stand 30 minutes, and the spots of ammonium salts are then located by dipping the paper in Nessler solution. Small, intensely orange spots against a light background result.

Since the mobile solvent is allowed to overrun the paper during the chromatographing, R_f values do not apply, but the distances the acids move from the starting point are equally characteristic. The excellent resolution obtained is indicated by the following data from a 6-hour chromatogram: α-hydroxyvaleric acid and α-hydroxycaproic acid move 5.3 cm. and 14.5 cm. from the starting point, respectively; β-hydroxycaproic acid moves 10.5 cm.; and α-ketovaleric acid moves 8.6 cm.

Preliminary experiments indicate the method of color development described above may be used for quantitative estimation of the test acids: After exposing to ammonia, the material can be eluted with water, Nesslerized, and the intensity of color determined in a photoelectric colorimeter or spectrophotometer.

(1) This work was supported in part by funds granted by the National Dairy Council on behalf of the American Dairy Association, and by the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

(2) K. Fink and R. M. Fink, *Proc. Soc. Exp. Biol. Med.*, **70**, 654 (1949).
(3) E. R. Hiscox and N. J. Berridge, *Nature*, **166**, 522 (1950).
(4) F. Brown and L. P. Hall, *ibid.*, **166**, 66 (1950).
(5) F. Brown, *Biochem. J.*, **47**, 598 (1950).
(6) L. A. Liberman, A. Zaffaroni and E. Stotz, in press.
(7) J. W. H. Lugg and B. T. Overell, *Australian J. Scientific Res., Ser. A*, **1**, 98 (1948).

Further details of the method and some biological applications will be published elsewhere.

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RECEIVED JANUARY 30, 1951

PHASE BOUNDARY POTENTIALS OF NICKEL IN FOREIGN ION SOLUTIONS[1]

Sir:

An investigation was started early in 1947 concerning the phase boundary potentials of inert metals in contact with solutions initially free from the common metal ions. The study of such systems, which remain relatively free from common ions, might contribute to a better understanding of the initial processes which induce corrosion of the metal. Our approach aims at very careful control of all experimental factors, since most metals show a greater or lesser tendency to interact with electrolyte solutions.

In a recent publication by El Wakkad and Salem,[2] the behavior of the potentials of mercury in buffer solutions initially free from mercury ions is discussed. An earlier article by Tourky and El Wakkad[3] dealt with an analogous investigation of the potentials of copper in foreign ion solutions.

Since in these laboratories work already has been done on several inert metals,[4] we believe that a preliminary account of our measurements on nickel may be of interest. Stable potentials could be obtained within 5 to 15 hours in a series of potassium hydroxide solutions, and in a series of phosphate buffer solutions covering the entire *p*H range. The stationary potentials, calculated against the standard hydrogen electrode, are plotted as a function of the *p*H of the solutions (Fig. 1).

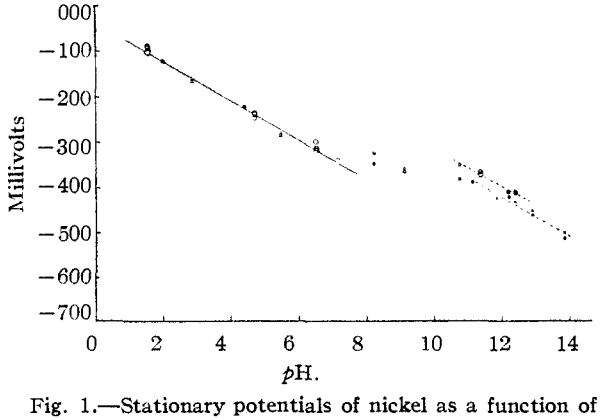

Fig. 1.—Stationary potentials of nickel as a function of *p*H in potassium hydroxide (× runs 4 and 5) and in 0.100 *M* phosphate solutions (O run XI, □ run XII, △ run XIV).

(1) This paper was presented before the General Meeting of the Division of Physical and Inorganic Chemistry of the American Chemical Society, Chicago, Ill., September, 1950.

(2) S. E. S. El Wakkad and T. M. Salem, *J. Phys. and Coll. Chem.*, **54**, 1371 (1950).

(3) A. R. Tourky and S. E. S. El Wakkad, *J. Chem. Soc.*, 740, 749 (1948).

(4) J. J. Singer, Jr., Ph.D. Dissertation, Clark University, 1949; J. H. Rosenbaum, Ph.D. Dissertation, Clark University, 1950.

Since aeration greatly accelerates the corrosion of nickel in acid media, oxygen was excluded from the half cells. Lengths of nickel wire of high purity were thoroughly cleaned and pretreated. The samples were freed from oxides and gases by induction heating first in hydrogen and then in a high vacuum. The solutions were freed from oxygen before making contact with the nickel samples. More details of the apparatus and the procedure will be given in a more extensive article. After each run the solutions were tested for nickel which might have gone into solution. The results were always negative, even in the acid phosphate solutions, unless oxygen had been admitted. The potentials were measured with a Leeds and Northrup K-2 potentiometer, using a Coleman electrometer as null point indicator.

Our graph of the nickel potentials in the phosphate buffers shows analogy with the curves obtained for copper[3] and mercury[2] in contact with a set of buffer solutions. In a more complete article we hope to discuss the interesting aspects both of an experimental and a theoretical nature.

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RECEIVED JANUARY 29, 1951

AN INTERMEDIATE IN THE CONVERSION OF FIBRINOGEN TO FIBRIN[1]

Sir:

When bovine fibrinogen and thrombin react in the presence of 0.4 *M* hexamethylene glycol (at *p*H 6.3, ionic strength 0.45), no clot is formed. However, the fibrinogen, whose sedimentation constant is about 9 *S*, appears to be gradually replaced by a new molecular species with a sedimentation constant of 25 *S*, which is evidently an intermediate polymerization product.[2] (All sedimentation constants given here are extrapolated to zero protein concentration.) We have now found very similar behavior with urea instead of glycol as the inhibitor. At *p*H 6.3 in 1.0 *M* urea, or at *p*H 7.5 in 2.35 *M* urea (ionic strength 0.15), sedimentation diagrams of a fibrinogen–thrombin system show, after 24 hours, two peaks; the sedimentation constant of one corresponds to that of unaltered fibrinogen, and the other is about 25 *S*.

Urea, unlike hexamethylene glycol, can in concentrated solution dissolve fibrin clots prepared in the absence of calcium and an unidentified serum factor.[3] A solution of fibrin in 3.5 *M* urea at *p*H 7.5, ionic strength 0.15, shows a single component in the ultracentrifuge with a sedimentation constant of 8 to 9 *S*; and its intrinsic viscosity is the same as that of fibrinogen, so that the fragments appear to

(1) This is paper 4 of a series on "The Formation of Fibrin and the Coagulation of Blood" from the University of Wisconsin, supported in part by research grants from the National Institutes of Health, Public Health Service. Grateful acknowledgment is made also of a grant from Eli Lilly and Company.

(2) S. Shulman and J. D. Ferry, *J. Phys. Coll. Chem.*, **55**, 135 (1951).

(3) E. Mihályi, *Acta Chem. Scand.*, **4**, 344 (1950); L. Lóránd, *Nature*, **166**, 694 (1950).