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*.

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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.

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