The container is an ordinary 46-ounce tomato juice can cut down to a height of 13 cm. A tin cover is fabricated just large enough to telescope over the top of the can (Fig. 1). Also illustrated is the separator which slips inside the can and which consists of a central tin cylinder, with an inside diameter of 31 mm., surrounded by 7 vanes, each 3 cm. in width. The cylinder and vanes are 14 cm. in length.

A 50-cc. centrifuge tube, containing the sputum-sodium hydroxide mixture, is slipped into each compartment. Thus, 8 specimens can be shaken at one time. Two wide rubber bands (cut from an automobile inner tube) encircle the separator to help keep the tubes from rattling; a disc cut from rubber belting covers the bottom of the can.

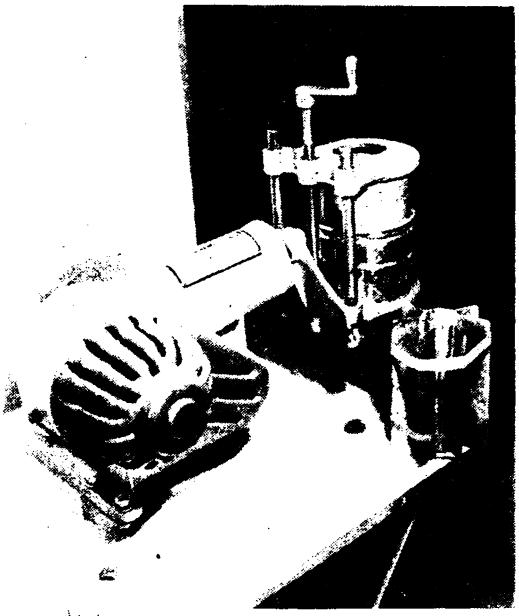

FIG. 1

The centrifuge tubes are of the round-bottom variety. They should be straight edge with no "pourout" lip. The tubes are closed with cork stoppers; rubber corks are not satisfactory. There should be an air space of at least 1 cm. between the top of the sputum-sodium hydroxide mixture and the bottom of the cork. When the corked tubes are in place in the can, there is always some variation in the total height of the various tubes with their corks. To compensate for this, a supply of cork discs of varying thickness is kept on hand, a disc of proper thickness being laid on top of each of the lower tubes. This leveling process does not have to be exact, since the telescoping cover, when screwed down tightly, will take up slight unevenness.

When the specimens are shaken in the paint-conditioning machine, complete homogenization is secured in 5–10 minutes. The same centrifuge tubes are used for spinning down the sediment.

Reference

STEENKEN, W., and SMITH, M. M. *J. lab. clin. Med.*, 1942, **27**, 1582.

Estimation of Coenzyme I Through the Uptake of Oxygen

P. S. KRISHNAN[1]

Department of Biochemistry,
New York State College of Agriculture,
Cornell University, Ithaca, New York

A new method for the estimation of Coenzyme I has been worked out in which washed, dried brewer's yeast is used as the source of the enzymes, aldolase and triose phosphate apodehydrogenase. The substrate is hexose diphosphate, in the form of the potassium salt. Aldolase splits the hexose diphosphate into dihydroxyacetone phosphate and 3-phosphoglyceric aldehyde; the latter reacts with inorganic phosphate to give 1,3-diphosphoglyceric aldehyde. When Coenzyme I and methylene blue are now added, oxidation to diphosphoglyceric acid takes place, resulting in oxygen absorption, which is measured in the Warburg manometer. Oxygen absorption is steady for a period of 15–20 minutes. When different concentrations of Coenzyme I are employed, we find that if oxygen absorption in microliters during the first 15 minutes is plotted against the concentration in gamma of the coenzyme, the points lie more or less on a straight line (Fig. 1).

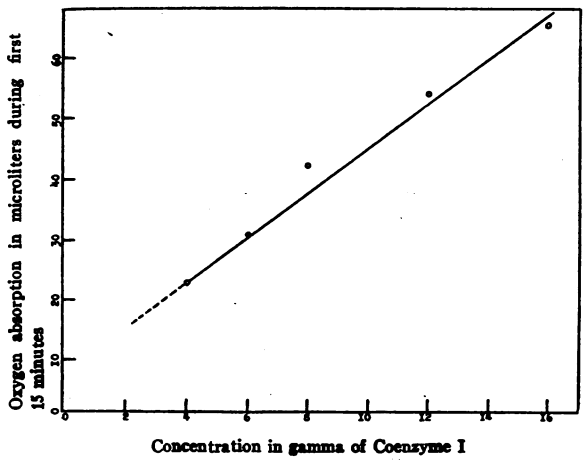

FIG. 1

It has not been possible to wash out the coenzyme completely from dried bottom yeast, but by the use of alkaline phosphate buffer we have succeeded in reducing blank oxygen absorption to a minimum, at the same time retaining maximal potency. There is no induction period, and the reaction is not altered appreciably by the absence of magnesium and manganese ions.

[1] The author's thanks are due to Prof. J. B. Sumner for his interest in the investigation and to the Haberle Brewing Company, Syracuse, New York, for generous supplies of bottom yeast.