

The fact that the enzyme is so intimately bound to the insoluble material of the cell makes it extremely difficult to study this problem by the ordinary methods of purification and isolation. In the circumstances, every possibility should be carefully examined and tested by suitable experimental methods, and of these possibilities the following three deserve special consideration :

(1) The enzyme may yet be a hæmatin-protein compound similar to cytochrome *a*, or even a protein sharing with cytochrome *a* its hæmatin nucleus. Owing, however, to its possible low concentration, it may still be spectroscopically invisible even in our strong enzyme preparations.

(2) The enzyme may be an iron-protein compound devoid of porphyrin and resembling some other iron-protein compounds known to occur in tissues.

(3) The enzyme may be a copper-protein compound. Although there is no direct evidence yet in favour of this supposition, it is nevertheless strongly supported by the following considerations :

(a) Copper salts are so far the only known simple metal salts which rapidly oxidize all the components of cytochrome. (b) The addition of copper to nutrient media for yeast and to copper deficient diet for rats produces not only an increase in the concentration of cytochrome *a* in the cells of these organisms but also greatly intensifies their indophenol reaction<sup>3,4,5</sup>. (c) There is a rough parallelism between the intensity of the indophenol reaction of various tissue preparations measured in terms of  $Q_{10}$ , (at 39° C. and *pH* 7.3 in presence of excess of cytochrome *c* and of *p*-phenylenediamine) and their copper contents. (d) The only other known intra-cellular enzyme which has many properties in common with cytochrome oxidase is the polyphenol oxidase of plants. This enzyme, as was recently demonstrated<sup>6,7</sup>, is a copper-protein compound. (e) Moreover, the inhibition by carbon monoxide of cytochrome oxidase in cells devoid of cytochrome *a* resembles that of polyphenol oxidase in being insensitive to light.

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<sup>1</sup> Keilin, D., and Hartree, E. F., *Proc. Roy. Soc.*, B, **125**, 171 (1938).

<sup>2</sup> Keilin, D., *NATURE*, **133**, 290 (1934).

<sup>3</sup> Elvehjem, C. A., *J. Biol. Chem.*, **90**, 111 (1931).

<sup>4</sup> Cohen, E., and Elvehjem, C. A., *J. Biol. Chem.*, **107**, 97 (1934).

<sup>5</sup> Yoshikawa, H., *J. Biochem. (Japan)*, **25**, 627 (1937).

<sup>6</sup> Kubowitz, F., *Biochem. Z.*, **124**, 10 (1937).

<sup>7</sup> Keilin, D., and Mann, T., *Proc. Roy. Soc.*, B, **125**, 187 (1938).

#### Preparation of Radio-Element $^{32}\text{P}$ in Weighable Quantity by Means of an Electric Field

In order to obtain large quantities of radio-phosphorus for certain experiments, we have attempted to find a method of concentrating the radio-element by means of an electric field. Some attempts of this kind have already been made with success by J. W. J. Fay and F. A. Paneth on bromine, iodine and arsenic<sup>1</sup>.

The radiophosphorus was produced by the neutrons from a source of 120 mgm. of radium + beryllium. Very pure carbon disulphide, prepared by shaking for two days with corrosive sublimate and distillation, was placed in a tall glass flask of about 1 litre capacity ; two brass rods with two rectangular brass electrodes

soldered at their extremities pass through the stopper ; the field applied was of 70 volts per cm. After nine days of irradiation we examined the electrodes with a Geiger-Müller counter and we measured, on the four faces of the electrodes, the following activities :

anode		cathode	
inside	outside	inside	outside
827	367	427	191

that is, a total of 1,800 kicks per minute. These figures show that the activity of the anode was about double that of the cathode.

The carbon disulphide was submitted to the chemical concentration described by O. Chievitz and G. Hevesy<sup>2</sup>. The result showed that during this first attempt a large part of the radiophosphorus escaped concentration. In order to be sure that this was really  $^{32}\text{P}$ , we followed the activity of the electrode and the resulting products of chemical concentration for 50 days, and we observed that the activities decreased with a period of 14–15 days, which is that of  $^{32}\text{P}$ .

Cleaning the electrode with emery paper removes all its activity but boiling it in distilled water does not affect its activity ; though this latter entirely disappears if one puts the electrode in dilute nitric acid for one minute ; in this case, practically all the activity is found again in the residue after evaporation of the acid.

The appearance of the electrodes after irradiation was totally changed, for they were darkened. Experiments done under the same conditions as above, but without neutrons, left the electrodes unaffected in appearance. From this we can conclude that the visible deposit is due to radiophosphorus. It is very probable that the  $^{32}\text{P}$  formed was combined with the copper or another element of the brass and formed a phosphide of this element.

When repeating the experiment but dissolving a little yellow phosphorus in carbon disulphide and without any irradiation we obtained the same alteration of the brass. In other attempts similar to these, we used pure copper electrodes ; we observed that the copper was altered and covered with a black layer. A qualitative chemical analysis showed that this layer contained phosphorus and copper : so there is very probably formation of phosphide of copper. This determined us to use pure copper electrodes in the following experiments.

These second attempts were conducted in a similar way. The irradiation lasted nine days ; the field was 70 volts per cm. during the first days and 90 volts per cm. during the last three. The electrodes were covered with a black deposit, and the anode alone showed an activity of 1,800 kicks per minute. The electrode weighed before and after the irradiation showed an increase of 3.7 mgm. The remaining carbon disulphide submitted to the chemical concentration had an activity so insignificant that we may conclude that practically all the activity has been collected on the electrodes.

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