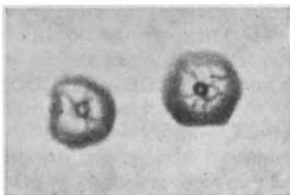
Crystallization of Arginase

ATTEMPTS to obtain arginase in a pure state were only partly successful and resulted in an approximately twenty-fold purification of the enzyme from liver extracts[1,2,3].

While engaged in research on the role of arginase in the metabolism of various types of malignant tissue, I attempted a purification of the enzyme from ox liver with the view of using a purified solution for metabolic experiments on malignant tumours. A high degree of purification was eventually reached, and from solutions above a certain activity of the enzyme, protein crystals were obtained, before or after dialysis, in five different cases and by different methods of precipitation. The hexagonal crystals, as shown in the accompanying reproduction, were found, however, to be too unstable to separate them from the mother liquor in order to test their activity, and even during the short time necessary for taking a microphotograph, the crystals showed signs of resolution. The shape of the crystals was found to be hexagonal whichever the method of purification.

There is a linear relation between activity and concentration of the enzyme; the purest fraction was almost colourless and showed a Q_{CO_2} of 67,000 at 37°.



(× 330.)

The use of the greater part of the purest fractions for metabolic experiments and the instability of the crystals have so far prevented further investigations of the pure enzyme. However, some information obtained during the process of purification is given below, as well as an outline of the procedure.

Activity test for enzyme fractions. 0·1–0·05 ml. of the enzyme solution is incubated for 15 minutes at 30° with 2 ml. 0·1 *M* pyrophosphate, *p*H 9·0 plus 0·2 ml. 1 per cent cobalt chloride, after which period 0·5 ml. 2 per cent *l* (+) arginine hydrochloride is added and the incubation continued for exactly 10 minutes. The enzyme action is then stopped by addition of 0·8 ml. 3 *M* acetate buffer *p*H 4·65 and the urea estimated manometrically as carbon dioxide by addition of urease. Activity is expressed as $\frac{\mu l \text{ urea} - CO_2}{\text{mgm. } N}$ in 10 minutes, which corresponds approximately to the *Q* value $\frac{\mu l \text{ urea} - CO_2}{\text{mgm. protein} \times \text{hour}}$.

Outline of purification. (1) Preparation of acetone powder: 880 gm. fresh ox liver are minced and treated with 5 volumes of acetone at room temperature. The suspension is filtered, the residue treated with another 2 volumes of acetone and air dried. Yield, 300 gm. The powder, stored *in vacuo*, in the ice chest, keeps most of its activity two to three months. Activity of suspension, approximately 200.

(2) Extraction: 100 gm. acetone powder are extracted at room temperature with 2 l. *N*/500 potassium hydroxide for 100 minutes, the suspension is centrifuged, the turbid, dark-red solution (1,770 ml.) contains the enzyme (Sol. A). Activity, 300–500. With fresh acetone powder, higher activities are obtained.

(3) Heating: Sol. A, at a temperature of 25°, is adjusted to *p*H 6·1 (Brom-Cresol-Purple) and heated in 600 ml. portions with vigorous shaking to 51°; the temperature is maintained for 75 sec., the mixture cooled rapidly and the precipitate centrifuged off. The turbid supernatant fluid (1,650 ml.) is adjusted to *p*H 7·2 and contains the enzyme (Sol. B). Activity, 600–800.

(4) Acetone precipitation: To Sol. B 1·2 vol. acetone are added at +3° with vigorous shaking in 400 ml. portions. The precipitate, which contains the enzyme, is centrifuged off in cooled cups and re-suspended in a third of the original volume of distilled water. The insoluble part is separated and discarded. The solution is adjusted to *p*H 6·3 (Brom-Thymol-Blue) and left standing in the ice chest until a bulky precipitate is formed, which is separated and discarded. The pink, fairly clear solution (1,650 ml.) is adjusted to *p*H 7·2 and treated with a cobalt chloride solution of 0·02 per cent final concentration (Sol. C). Activity, 1,500–2,000.

(5) Heating: Sol. C, at a temperature of 25°, is placed in a water bath at 75° and heated with vigorous stirring to 54°. The temperature is maintained for 3 minutes, the solution cooled and the bulky precipitate separated and discarded. Supernatant fluid: Sol. D (570 ml.). Activity: 4,000–6,000.

(6) Precipitation with zinc sulphate: By using a weak solution of zinc sulphate, the greater part of the coloured material can be removed. To Sol. D, zinc sulphate crystals are cautiously added until the solution becomes turbid and precipitation of coloured material begins. At this point the addition of the zinc salt is stopped. The *p*H during this procedure should be kept at 7·5–7·8, and the final concentration of zinc sulphate should not exceed 0·1 per cent. Cobalt chloride salt is added to a final concentration of 0·01 per cent to the supernatant fluid (550 ml.), the *p*H of which is adjusted to 7·2 (Sol. E). Activity, 8,000–14,000.

(7) Ammonium sulphate fractionation: Sol. E is dialysed in succession against 20 vol. ammonium sulphate solution of 50, 55, 60 and 65 per cent saturation at *p*H 7·2 for 7–10 hours at room temperature. The precipitate after each dialysis is removed, taken up in 1/10 vol. distilled water and tested for activity. The fractions showing an activity exceeding 10,000 (mainly with 50, 55 and 60 per cent saturation) are united and the dialysing procedure is repeated. In this way fractions of an activity of 20,000–50,000 are obtained. They are adjusted to *p*H 6·6 and dialysed against 44 per cent saturation; the precipitate is discarded and the supernatant fluid is finally dialysed against 47 per cent saturation, when crystallization sets it.

Stability: The enzyme is stable for 1–2 days in Sol. A and for 1–2 weeks in Sol. B. It is less stable in Sol. C and becomes somewhat unstable in Sols. D and E. The ammonium sulphate fractions are fairly stable for one month. The enzyme is most stable between *p*H 7·2 and 8·0, and least stable at *p*H below 5·5.

Activation by metal salts. The enzyme solution is activated by cobalt and nickel salts in stages A and B, but not by manganese salts, while colloidal iron inhibits the enzyme. In stages D and E activation by cobalt salts was observed, while in the purest stages (ammonium sulphate fractions) no significant activation was seen by any of the metals mentioned.

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Aug. 6.

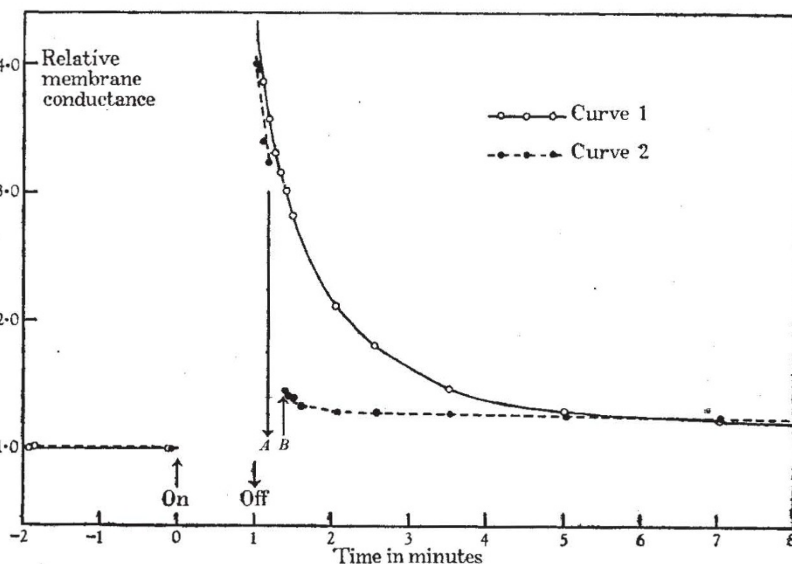
[1] Richards, M. M., and Hellerman, L., *Biol. Chem.*, **134**, 237 (1940).
[2] Van Slycke, D. D., and Archibald, R. M., *Federation Proc.*, **1**, 139 (1942).
[3] Mohamed, M. S., and Greenberg, D. M., *Arch. Biochem.*, **8**, 349 (1945).

Potassium Leakage from an Active Nerve Fibre

ACCORDING to the membrane theory of nervous action, a minute quantity of potassium ions should leak out of a nerve fibre each time that an impulse travels along it. There is now general agreement that prolonged stimulation may cause a loss of potassium from nerve and muscle[1,2,3,4,5], but there is no certainty that activity is normally and invariably accompanied by such leakage. Nor is there any clear information about the time course of the leakage of potassium.

We have recently devised an indirect but very sensitive method of recording the loss of potassium from an isolated axon and have applied it to the 30 *μ* non-medullated axons from *Carcinus mænas*[6]. The method depends upon a previously unpublished observation. *Carcinus* blood contains 11–12 m.mol. potassium per litre[7], and isolated *Carcinus* axons survive for 24 hours in sea-water containing 9·8 m.mol. potassium per litre. If the potassium chloride content of sea-water is increased by 20 m.mol./l., the fibres continue to transmit impulses satisfactorily, but their membrane conductance increases approximately threefold. On the other hand, addition of a similar quantity of sodium chloride produces no measurable change in membrane conductance. The difference between the effects of potassium chloride and sodium chloride is probably connected with the difference in mobility or solubility of potassium and sodium ions in the surface membrane, and provides some support for the view that this membrane is much more permeable to potassium than to sodium ions. It also provides a practical method of measuring the amount of potassium leaking from a nerve fibre.

When an isolated axon is immersed in oil, it is surrounded by a thin film of sea-water. Electrical and optical estimates give the cross-sectional area of this film as approximately 3×10^{-6} cm.^{2}. Hence if 3×10^{-11} mol. potassium were to leak out of 1 cm. length of nerve fibre, it would double the concentration of potassium in the external fluid and should produce a large increase in membrane conductance.



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