Short Communications

SC 11029

Estimation of choline acetyltransferase in small samples of nervous tissue

For accurate analysis of their content of choline acetyltransferase (Acetyl-CoA : choline–*o*-acetyltransferase, EC 2.3.1.6), tissue samples can be pulverised in acetone and extracted in saline, or they can be homogenized and treated with ether[1] which is necessary since untreated homogenates yield much lower values. Analysis of rabbit brain by the two methods gives the same values but they have the disadvantage that they cannot be used on samples smaller than about 50 mg. Otherwise small losses occurring during preparation become more significant as do errors of measurement introduced by the number of operations involved and the necessity of transferring the material from one receptacle to another.

The present communication describes three techniques of analysing small samples of tissue which avoid these difficulties and make it possible to estimate their choline acetyltransferase activity with considerably greater accuracy than was previously possible. All three methods with some applications are described in the text.

With each method the samples prepared for testing were incubated in small pyrex tubes (internal diameter 0.7 cm) to which 0.4 ml of the substrate solution previously incubated for 10 min was then added. 1 ml of this contained about 40 units of CoA and 0.32 mg of phosphate acetyltransferase (EC 2.3.1.8) in 0.21 M KCl, 0.17 M choline, 0.0125 M acetyl phosphate, 0.006 M $MgCl_2$, 0.03 M cysteine, 0.16 M sodium phosphate (pH 6.9) and 0.1 mM eserine sulphate. The amount of acetylcholine expressed as the chloride formed after 1 h of incubation at 38° was estimated by bioassay.

In the first method to prepare samples for incubation small volumes (0.01–0.05 ml) of brain homogenates or of particulate fractions obtained by differential centrifugation are pipetted onto Whatman No. 50 paper and then dried in a stream of cold air. The part of the paper impregnated with the enzyme preparation is cut into small pieces which when dropped into the bottom of an incubation tube occupy a relatively small volume that is completely covered by 0.4 ml incubation fluid.

The results with this method in tests of choline acetyltransferase activity of mitochondrial fraction of brain (rabbit) and of homogenized cerebrum (hen) were in good agreement with values obtained from ether-treated samples (see Table I), and were better than values from untreated samples tested in the usual way. Filter paper had no activating or potentiating effect on the enzyme extracted from acetone powders and incubation of an untreated particulate fraction or homogenate in a tube with filter paper did not increase its activity above the control level. The activating effect therefore appears to depend on some change in the state of the enzyme which occurs as the result of its application to the paper.

This technique has been valuable as a means of testing samples of homogenates too small in volume to treat with ether and in testing homogenates of certain tissues

(*e.g.* dogfish or hen brain) which, owing to differences in the state of the enzyme, are not always effectively activated by ether.

In the second method a piece of tissue, estimated by volume to be between 0.5 and 2 mg, is placed on one leaf of a small piece of folded cellophane (pieces of Visking tubing are suitable), weighed, then squeezed between the opposing leaves of cellophane until spread out on an area of about 1 cm^2. The cellophane is made into a roll, with the layer of tissue on the inside and introduced into the incubation

TABLE I

COMPARISON OF CHOLINE ACETYLTRANSFERASE ACTIVITY (μG ACETYL CHOLINE FORMED/G/H) IN TISSUE SAMPLES PREPARED BY DIFFERENT METHODS FOR INCUBATION

Tissue	Extracts of acetone-dried tissue	Homogenates			Fresh tissue	
		untreated	ether-treated	dried on paper filter	smears	sections
Rabbit caudate nucleus (head)		2700	6400	—	6300	—
Rabbit brain, large-granule "mitochondrial" fraction		190	615	620	—	—
Hen cerebrum	—	1300	2200	2750	—	—
Goat ventral roots	2000–2500	600–1200	1800–2500	—	—	2200–2400

tube. The volume of substrate solution, then added by pipette, is, with a little practice in the operations, enough to ensure that all of the tissue is completely covered but if necessary the volume can be increased by adding 0.2 ml of 0.9% NaCl. The smear, if prepared from mammalian tissues, can be left for several hours without loss of activity.

By this method, the choline acetyltransferase of the rabbit's caudate nucleus, the pigeon and hen's optic tectum (per gram of fresh tissue) was as active as that of ether-treated homogenates of the same tissues (Table I). The method, suitable only for relatively soft tissues, has been used to do a regional analysis of choline acetyltransferase activity within the caudate nucleus of the rabbit and the optic lobe of the chicken. In both, the ratio between regions of maximum and minimum activity was about 4. In the caudate nucleus of the rabbit its most rostral part had the highest activity and the tail part the lowest which agrees with findings on the caudate nucleus of larger animals in which, because the nucleus is big enough, it has been possible to do a regional analysis without recourse to the special methods now described.

In the third method fresh-frozen sections from 10 to 60 μ thick are placed on a small piece of cellophane within an area of about 1 × 2 cm, the cellophane is then put into the incubation tube as before and the substrate solution added. It is not essential for the sections to remain attached to the cellophane during the incubation; the cellophane is simply a convenient vehicle for their transfer to the incubation tube. If desired several can be placed one on top of the other; thin narrow pieces of glass (broken coverslips) can be used instead of cellophane.

The technique has been used for sampling peripheral nerves for which, because of the connective tissue present the tissue-smear method is unsuitable, but it produces equally good results on central nerve structures. In tests on (a) the hen's optic lobe (b) the rabbit's caudate nucleus and (c) the ventral and dorsal roots of the goat

the choline acetyltransferase activity observed was the same as in samples of these tissues prepared by conventional methods (Table I).

We originally had the idea of using cellophane simply as a convenient vehicle for inserting fresh tissue into a small test tube. It was an unexpected piece of good fortune to find that tissue smears and sections were active without further treatment. We had assumed that they would be only as active as untreated homogenates of nervous tissue and were proposing to activate them either by adding a surface-active reagent to the incubate, with dubious chances of success, or by applying ultrasonic vibrations (which effectively activate some preparations) to the incubation medium after the tissue had been immersed in it. As it turned out no further treatment was necessary. However, we do not as yet know by what means the activation of the tissue, on glass or cellophane, or of the homogenates on filter paper is effected.

It seems probable that the important factor in all three methods is that the enzyme is available to its substrates over a larger surface than is provided when homogenized tissue is added to the medium in the usual way. If so, the activating effect of ether may not be due to the release of occult enzyme, as is sometimes assumed, but to an effect on the enzyme-carrying particles which lead to their more even dispersal through the incubation fluid.

To summarize, three methods of preparing small samples of nervous tissue for estimating their choline acetyltransferase are described. The first method, suitable for all types of tissue, is to apply an homogenate in small volume to filter paper, dry it and incubate the impregnated paper in a solution containing the substrates required for acetylcholine synthesis. The second, suitable for samples of up to 2 mg of soft tissues, is to make a smear of the tissue on cellophane and incubate it in the usual way. In the third method, suitable for all types of tissue, fresh-frozen sections replace the tissue smear. With all three methods the choline acetyltransferase activity of the tissue tested was found to be equal to or in excess of the activity of the same tissues prepared by established procedures.

A.R.C. Institute of Animal Physiology, Babraham, Cambridge (Great Britain)

G. BULL
CATHERINE HEBB
DOBRILA RATKOVIC

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Received July 5th, 1962

Purification and properties of phosphoglycerate kinase from chicken breast muscle

SC 11024

Phosphoglycerate kinase (ATP: D-3-phosphoglycerate 1-phosphotransferase, E.C. 2.7.2.3.)activity has been demonstrated in a great variety of animal and vegetal tissues[1]. The enzyme has been crystallized from yeast by BÜCHER[2] and partially purified from pea seeds[3]. The purification and some properties of the rabbit-muscle