CHOLINE ESTERS AS LOCAL HORMONES

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The identification of the active substance in the ciliary epithelium of *Mytilus edulis* illustrates the steps which are essential for a complete proof that this substance is acetylcholine, and not a mixture of choline esters.

Other choline esters, if present in extracts or perfusates, may confound detection. For, as pointed out by Chang and Gaddum (4), parallel quantitative assay on as many as 5 different test objects may fail to differentiate pyruvyl- from acetylcholine, so that unless one uses the distinguishing test object mentioned by the previous speaker, i.e. the frog heart, the presence of a proportion of pyruvylcholine in admixture with acetylcholine might easily escape detection.

Until recently this has been considered a minor objection since the only choline ester to be identified chemically in tissues was acetylcholine. The position today has changed largely as the result of the work of Banister, Whittaker and Wijesundera (2), who have demonstrated chromatographically the presence in *ox* spleen of 3 choline esters: (1) acetyl- (2) propionyl- (representing 20% of the total activity) and (3) an unidentified ester (named F) of higher R_F, probably of a keto-acid (representing 30% of the activity). On the other hand, in Dale and Dudley's (5) experiments on *horse* spleen a purified fraction obtained after precipitation with mercuric acetate was equiactive with respect to acetylcholine on 3 different test objects, and was therefore identified as acetylcholine. This discrepancy may be due either to (a) a species difference between horse and ox spleens, (b) loss of the other two esters in a discarded fraction, or (c) loss during the decomposition of the mercuric acetate precipitate by means of H_2S (which was known to hydrolyse 30% of the activity of the extract).

Similar doubts may arise with regard to other tissues. "Acetylcholine" (A.Ch.) is a substance of wide occurrence. Its presence in many tissues (e.g., spleen and placenta) remains completely unexplained. In many cases there is the further uncertainty of whether the substance really is A.Ch., because its identification has been based on bioassay upon a single test object (see also 8, p. 225).

For instance, in *Electrophorus electricus* the cholinesterase of the electric organ has the same optimum substrate concentration for propionyl- as for acetylcholine (1), so that either ester could equally well be the natural substrate for this enzyme*. On the other hand the active substance present in this electric tissue, or synthesized by it, has so far been examined on a single test object, namely the frog rectus, on which, incidentally, propionyl- is 2 to 4 times as active as acetylcholine.

* An even more interesting example of this is given in a recent paper by Myers (11) where it is shown that the true cholinesterase of chicken brain hydrolyses propionyl- more rapidly than acetylcholine.

The second example relates to the product of choline acylase reactions. Korey, de Braganza, and Nachmansohn (9) have shown that purified choline "acetylase" can utilise propionic acid to synthesize an active choline ester, and Gardiner and Whittaker (7) have now produced more conclusive chromatographic evidence that the product of this enzymatic reaction is propionylcholine. These results show that the nature of the substrate can predetermine the path of the acylating reaction; in most cases we have no knowledge of the actual path of this reaction in the living tissue, and the identification of the product(s) has been based on a single test. For instance, the presence of an acylating enzyme in the intestinal mucosa (6), resembling that found in the enteric plexus, need not necessarily signify that the choline ester synthesized *in vivo* is the same in the two layers. One would perhaps expect that, in the mucosa, other fatty acids, absorbed from the intestinal lumen, would esterify with choline.

As to the occurrence of other active substances besides A.Ch. in nervous tissue, there have been a few hints in the literature which seem worth following up. The first of these was given by Nachmansohn, Hestrin and Voripaieff (12), who have reported the synthesis by a brain enzyme, in the absence of choline, of a substance resembling acetylcholine pharmacologically but not chemically. The results of Middleton and Middleton (10) suggest that this substance may have less activity (relative to A.Ch.) on the frog rectus than on the cat's blood pressure and frog heart.

Bearing these points in mind, an attempt has been made (Ambache and Robertson, unpublished) to detect any differences, by parallel assay on the guinea-pig ileum and the frog rectus, in extracts of guinea-pig and rabbit brain prepared by the method of Bentley and Shaw (3). In this method acetylcholine is extracted with trichloracetic acid and precipitated by ammonium reineckate in the presence of a 100 to 1 excess of choline. The precipitate is redissolved in 60% ethanol with warming and dilutions of this reineckate solution are made in saline and assayed directly. The method appears to suffer from the disadvantage that the reineckate precipitate is insoluble unless it is first warmed in alcohol, and it is possible that at this stage some esters may undergo alcoholysis. Of eight extracts examined by this method, five have assayed identically on the two test objects and only three have shown a difference in favour of the frog rectus.

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