IV. ACETYLCHOLINE AND CHOLINESTERASES

ACETYLCHOLINE METABOLISM OF NERVOUS TISSUE

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Following the original discoveries of the cholinesterases and the choline acetylating enzyme system (for references to early work see (1, 5, 19, 21)) numerous studies of the distribution of these enzymes in animal tissues have been carried out. The usefulness of such measurements when they are applied to the nervous system is that they indicate the sites at which acetylcholine may have a role in the transmission of nerve impulses.

The distribution of the enzymes synthesizing acetycholine in mammalian nervous tissue has been studied systematically by Feldberg and his co-workers (6, 7, 8) using a method described by Feldberg and Mann (7). From their measurements it appears that the capacity to synthesize acetylcholine is limited to certain neurones while others including some sensory nerves (spinal and optic) are inactive. The neurones containing the enzymes include some in the central nervous system as well as the peripheral cholinergic nerves. The method, however, has been criticised and in a recent review Nachmansohn and Wilson (19) suggest that all nervous and conducting tissue can acetylate choline and that failure to find synthesizing activity in sensory nerves is due to inadequate techniques.

In the experiments which I am now going to discuss this possibility has been examined in detail. By measurements made on mammalian brain powder it has been possible to show that Feldberg and Mann's method is not quantitatively insensitive. Under optimal conditions extracts of acetone-powdered brain yielded between 2,000 and 2,800 μg. acetylcholine per gm. brain powder per hour, values which are of the same order as the highest values obtained by other methods. There remains then only one way in which the method might be considered inadequate when compared with the techniques used in other laboratories.

Some time ago Dr. Balfour and I carried out a series of experiments (2, 3) to determine the role of citrate in acetylcholine synthesis. We were then able to show that in the system devised by Feldberg and Mann citrate is the only effective acetyl donor present in the incubate whereas in the system described by Nachmansohn and others in the series of papers reviewed by Nachmansohn and Wilson (19) citrate is much less effective as an acetyl donor and, chiefly because of the large amount of coenzyme A added, the conditions are optimal for acetylation by acetate.

We showed also that citrate is brought into reaction by an enzyme fraction (obtained by $(NH_4)_2SO_4$ precipitation) which is not necessary for acetylation by

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acetate. When this fraction is present the two substrates can be made equally effective; but after its removal citrate has little or no effect while addition of acetate still yields large quantities of acetylcholine.

The demonstration that acetylcholine is formed in the absence of the citrate enzyme is of theoretical importance since it is not known if this enzyme is present in all mammalian nervous tissue; and if the method of test relies on citrate as an acetyl donor, failure to obtain synthesis in some tissues may be attributed to this cause. The criticism attains more weight in view of evidence that some invertebrate tissues which synthesize large quantities of acetylcholine in the presence of acetate appear to be incapable of utilising citrate (18).

Nevertheless, a re-examination of the mammalian nervous system has shown that the method used in Feldberg's laboratory does give reliable results for this tissue. In our experiments we have used an incubation system which is optimal for acetylation by acetate; and by this means have re-determined the rates of synthesis in a number of tissues which provide a representative sample of those previously examined by the citrate method.

Except for the fact that coenzyme A has been prepared from yeast, the incubation mixture employed is in all respects similar to that described by Nachmansohn and Wilson (19). With rabbit brain as a source of enzyme the yield of acetylcholine is between 1,750 and 2,650 μg. per gm. acetone-powdered tissue per hour. In parallel incubation tests the yields of other tissues have been in close agreement with those obtained by Feldberg and his colleagues (6, 7, 8) and by Banister and Scrase (4). Cholinergic nerves synthesize 1,000 μg. or more per gm. acetone dried tissue per hour. The retina is also highly active but the cerebellum only moderately so and the optic nerves (horse, bullock, pig and rabbit) have scarcely any activity. In contrast to the anterior spinal roots, classed as cholinergic nerves, the posterior roots (of the bullock) synthesize no acetylcholine.

The amounts of acetylcholine produced by the optic nerves have been found to be between 0 and 2 per cent of the control rate of synthesis by brain enzyme. De Roetth (20), on the other hand, reported values for this tissue of 5 to 10 per cent of the maximal rates of synthesis. His results, however, are open to certain criticisms, the most important of which is that the active substance has not been identified definitely as acetylcholine. At least one other substance having similar properties but which is not a choline ester can be formed under the same conditions of experiment (17). Its appearance is evidently an artifact depending upon the purity of the coenzyme A used (19). Nor can the production of other choline esters be excluded since at least two types of nervous tissue have been shown to synthesize propionyl choline (10, 15). This latter suggestion gains some plausibility from evidence, to be considered later, that the supporting tissue of mammalian optic nerves contains pseudocholinesterase in relatively high concentration; it is possible that at this site there is active metabolism of some of these other choline esters.

To conclude this account of the choline acetylating enzymes, the criticism made of Feldberg's work while valid in principle is not, in fact, of practical importance for the mammalian nervous system. Further, the suggestions that citrate

can only be utilised by crude brain extracts and that the enzyme which brings it into reaction is not normally concerned in acetylcholine metabolism, are not in fact borne out by experiment (2). Balfour and Hebb (3) concluded that in view of its low coenzyme requirement this might be the normal substrate for choline acetylation by mammalian tissue.

With the development of a simple histochemical technique, the distribution of the cholinesterases in the nervous system can be determined with greater precision than was possible with earlier biochemical methods of sampling. The method developed by Koelle in 1950 (12) has recently been used by Hebb, Silver, Swan and Walsh (11) in a study of the cholinesterases of the retina and optic nerve. The results of these experiments show:— 1. True cholinesterase is present in the cells of the bipolar layer, the tissue of the inner synaptic layer and is concentrated in the region of the ganglion cells. No true enzyme is present in any part of the optic nerve. 2. Pseudocholinesterase is found in the glial cells and septa of the optic nerve throughout its course. Small amounts of pseudo enzyme are also found in the ganglion and bipolar layer cells as well as in the supporting network and cells of the retina.

Subsequent experiments have shown a similar distribution of enzymes in the optic systems of the horse, ox and pig. It has also been shown that the pseudocholinesterase of the rabbit's optic nerve can be totally inhibited by injection of DFP into the living animal; and that this procedure does not interfere with the normal light reflexes of treated eyes over periods of 2 to 24 hours.

Francis (9) and Koelle *et al* (14) have found a more limited distribution of enzymes in mammalian retinas. Neither has reported the presence of pseudocholinesterase and both suggest that of the cells in the bipolar layer only the amacrines contain true enzyme. These authors have used in their experiments the modified histochemical procedure earlier recommended by Koelle (13), and the differences in results are due to that circumstance. The modified procedure is intended to prevent intracellular diffusion of enzyme, but it also seems to reduce penetration of the tissues by the substrate and when the precise intracellular position of the enzyme is not in question the original method appears preferable.

The choice of methods appears to be of more importance for some tissues than for others. Francis found no cholinesterases in the frog retina, yet homogenates of this tissue hydrolyse acetylcholine; and when we examined the retina and optic nerve by the original histochemical procedure we found high concentrations of cholinesterases to be present. It is unlike the mammalian eye in the important respect that true cholinesterase is found in the fibres and supporting cells of the optic nerve trunk as well as in the optic nerve layer. The enzyme is also present in the innermost cells of the bipolar layer and the inner synaptic and ganglion cell layers. Some pseudocholinesterase is found in the nerve and retina as well but this is much less prominent than in the mammalian eye. The distribution of the true enzyme suggests that in this case the amacrine cells and the third order nerves may both be cholinergic in the classical sense.

The evidence of these and other experiments (12) that, in the mammal, the distribution of true cholinesterase is similar to that of the choline acetylating en-

zymes re-emphasizes the importance of true cholinesterase for the destruction of endogenously produced acetylcholine. The terminology introduced by Mendel (17) may not now be considered an adequate description of the cholinesterases, since the pseudo type of enzyme may take part in the hydrolysis of other choline esters. Nevertheless, the distinction is still an important one for physiological theory and, for this reason, Mendel's classification of these enzymes has seemed most appropriate for the present discussion.

From the results which have been reviewed here certain general conclusions seem possible. The absence of the enzymes, both for the hydrolysis and the synthesis of acetylcholine, from the optic nerves of mammals shows that transmission along this pathway cannot depend upon the intracellular release and hydrolysis of this substance. The theory that axonal transmission in all nervous tissues depends upon these events (19) is therefore contradicted by the evidence. The same opinion has previously been expressed by Banister and Scrase (4) who have shown that certain adrenergic neurones are incapable of synthesizing acetylcholine and yet conduct impulses in the normal manner.

The absence of the choline acetylating enzymes from the posterior spinal roots has a similar significance. In addition it also means that acetylcholine can play no part in intercellular transmission in the case of monosynaptic reflexes. If, as has been recently suggested, stimulation of the anterior horn cells is due to the release of a specific chemical—or local hormone—some other substance must be responsible. The classical concept of transmission by acetylcholine may, however, be extended to other central synapses as Feldberg and Vogt (8) have pointed out. Their idea that cholinergic alternate with non-cholinergic neurones fits well with observations on the sensory pathways; the evidence for such an arrangement in other parts of the central nervous system (*e.g.*, the cerebellum) is less satisfactory.

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