A micro-scale procedure for the preparation of synaptosomal and mitochondrial fractions from cervical sympathetic ganglia and caudate nuclei of the cat

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The conditions which influence the preparation of synaptosomal fractions have been reviewed by Whittaker (1965). Two steps appear to be critical: (1) the conditions in which the tissue is initially homogenized, and (2) the fixation procedure used before electron microscopic examination.

The tough collagen capsule of sympathetic ganglia is extremely difficult to homogenize. An initial homogenization (3 × 3 sec) in 0.32 M sucrose with a modified Ultra Turax (dimensions 5 mm × 5 cm) followed by homogenization (3 × 5 sec) in a glass homogenizer produced a satisfactory homogenate (3 ml.). This was centrifuged (900 g × 8 min) and the supernatant recentrifuged (10,000 g × 20 min). The crude mitochondrial pellet was resuspended in sucrose (0.1 ml. × 0.32 M) and layered on a sucrose gradient prepared from 0.1 ml. each of 0.8, 1.0, 1.2, 1.4 and 1.6 M sucrose, which was then centrifuged (53,000 g × 120 min). The synaptosomal layers were then separated by means of a micro-suction device: the mitochondrial pellet was resuspended by gentle homogenization in a glass homogenizer. Synaptosomal and mitochondrial fractions were prepared from individual cervical sympathetic ganglia (12–16 mg) and from caudate nuclei (2 mg.). The initial Ultra-Turax homogenization was not used for caudate nuclei preparations. Even from such small samples of tissue, opalescent layers were clearly visible.

Both electron microscopic characterization and enzyme studies were carried out on the subcellular fractions thus prepared.

REFERENCE

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Decrease in cholinesterase activity of single motor end-plates after thiocholine staining

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Several authors have suggested that the thiocholine method for staining cholinesterase should not be used for quantitative estimation of cholinesterase activity, but Koelle (1963) has suggested that the motor end-plate is one situation where the method could be applied quantitatively. The results presented here suggest that caution is needed if valid results are to be obtained.

Fresh posterior latissimus dorsi muscles from chickens were stained for 15 min using a modified thiocholine method (Buckley & Heaton, 1968). After this initial staining small pieces of muscle containing groups of end-plates were dissected and stained for further periods up to 4 hr. At the end of the staining period single end-plates were dissected and cholinesterase measured by a radiometric method (Buckley & Heaton, 1968).

Figure 1 shows that staining for 2 or 4 hr caused a marked decrease of the cholinesterase activity of the end-plates. The mean decrease of the median cholin-

esterase activity after 4 hr staining was 51%. Incubation of the tissue for 4 hr in pH 6.4 phosphate buffer caused no loss of activity, whereas incubation for a similar period in the staining medium containing either no copper-glycine or no acetylthiocholine resulted in a 13% and 25 % decrease of the median cholinesterase activity respectively.

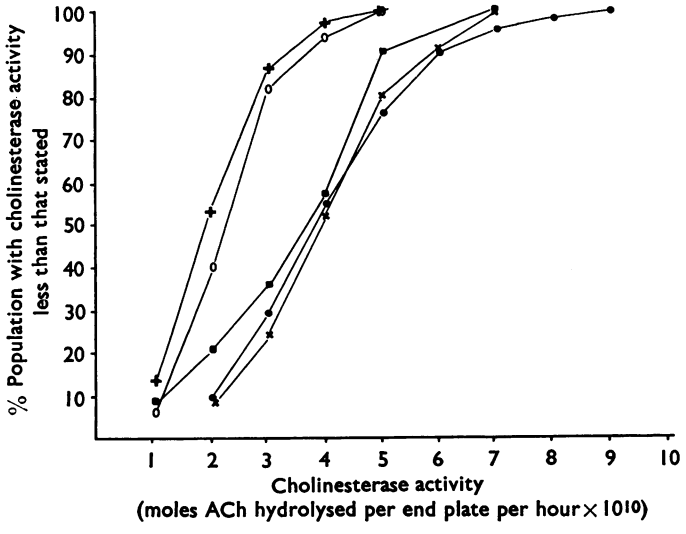

FIG. 1. Cholinesterase activity of single motor end-plates stained by a thiocholine method initially for 15 min (●) and for further periods to give a total of ½ hr (×), 1 hr (■), 2 hr (○) and 4 hr (+).

From this investigation involving the study of 500 individual end-plates it is concluded that staining with the thiocholine method may cause a marked decrease of the measured cholinesterase activity. The absence of either copper-glycine or acetylthiocholine causes only a slight decrease so it is likely that the precipitate of copper thiocholine is responsible for the marked decrease after staining for 4 hr.

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Measurement of gastric acid secretion by conductivity

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Ghosh & Schild (1958) developed a method for the assay of stimulants and inhibitors of gastric secretion, in which the acid output was measured by pH determination in a continuous flow system. Rosenoer & Schild (1962) perfused a buffer