Reports and Letters

Simple Method for Histochemical Detection of Esterase Activity

Several methods (*1*, *2*) have already been proposed for the histochemical demonstration of esterase activity. For acetylcholinesterase in the nervous system, the acetylthiocholine method of Koelle and Friedenwald (*2*) seems to be the most histologically accurate as well as biochemically specific. Unfortunately, the substrate is expensive and the procedure is hazardous (*3*).

On the other hand, the mechanism of enzymic hydrolysis has been studied thoroughly (*4*). Acetylcholinesterase has been shown to contain an anionic site capable of binding the cationic portion of acetylcholine and similar molecules and also an esterasic site upon which the enzymic activity is directly dependent. The theory involves an acetylated enzyme as intermediate. On this basis, it has been demonstrated that acetylcholinesterase catalyzes the hydrolysis of thiolacetic acid with liberation of acetic acid and hydrogen sulfide (*5*). The undissociated acid molecules are the direct reactants. The rate of reaction falls off rapidly with increasing *p*H and in the presence of prostigmine. This reaction could be the basis of a histochemical test for esterase, the evolved H_2S to be trapped *in situ* by either lead nitrate or acetate, thus forming a precipitate in areas of enzymic activity.

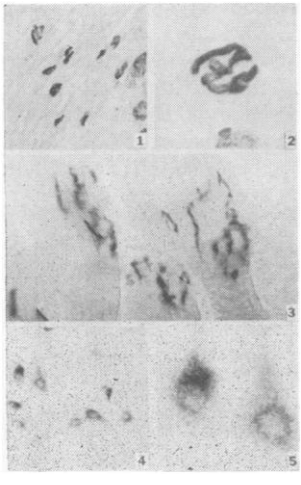

Fig. 1. Bouquet of motor end plates in the rat's tongue. Counterstained with basic fuchsin (×100). Fig. 2. Motor end plate in rat's tongue (×350). Fig. 3. Superficial arborization in frog's gastrocnemius. Glycerogel mount, unstained (×117). Fig. 4. Neurons of the dentate nucleus, cerebellum of rat. Formalin fixed, unstained (×100). Fig. 5. Two cells from the dentate nucleus (×350).

The tongue and central nervous system of the rat and also the gastrocnemius muscle of the frog were the objects of investigation. Portions of tissue 2 to 3 mm thick were either frozen immediately or prefixed overnight in 4-percent neutral, isotonic formaldehyde at 4°C, before freezing. Sections of 10 to 20 μ were mounted from saline onto clean glass slides precoated with 1-percent egg albumen in distilled water. They were dried rapidly with an electric fan or slowly by vacuum desiccation over $CaCl_2$ at 4°C, so that they became solidly attached to the glass slide and also that autolysis could be prevented.

The incubation medium was prepared extemporaneously by dissolving thiolacetic acid ($0.12M$) and lead nitrate ($0.001M$) in 83 ml of Na_2HPO_4 ($0.1M$) and adding 17 ml of McIlvaine phosphate-citrate buffer at *p*H 6.2.

The dried sections were placed in the incubation bath for periods of 30 to 60 minutes at room temperature. They were then washed for 5 minutes in slow-running water at 4°C. The rat tongue was counterstained 8 to 10 minutes in 0.02-percent basic fuchsin, then dehydrated rapidly in one bath of 95-percent alcohol and three baths of 100-percent alcohol. When counterstain was not used, the sections were passed through 80-percent alcohol and then through 95- and 100-percent alcohol. All sections were then cleared in three baths of xylene and permanently set in Permount (Fisher). Some were mounted in glycerogel from water. Other counterstains such as safranin were tried; they must be avoided because they destroy the integrity of the lead sulfide image or its support.

The results have been constant with each type of tissue utilized. Photomicrographs have been taken at different magnifications to illustrate various features. In the rat tongue at low power (Fig. 1), the reliability of the procedure is demonstrated on a group of adjacent motor end plates. The reticular appearance of the individual plate and its clear central channel reported by Couteaux after staining with Janus Green B (*6*) have been reproduced by this histochemical procedure (Fig. 2). The superficial arborization of the frog muscle has been well demonstrated also (Fig. 3).

In the formalin-fixed central nervous system of the rat, the precipitate occurred regularly along certain fiber tracts but especially over the neurons. These were grouped into four categories in relation to intensity: (i) least intense, the pyramidal cells of the cerebral cortex; (ii) the Purkinje cells of the cerebellum; (iii) the motor neurons of the spinal cord; and, most intensely reactive, (iv) the dentate nucleus of the cerebellum (Figs. 4, 5), the red nucleus, the motor nucleus of the fifth nerve and the diffuse nucleus of the pons. In individual cells, the precipitate occurred over the cytoplasm and along the proximal processes (Figs. 4, 5). The nucleus was always negative. Often a perinuclear localization of the precipitate was observed (Fig. 5).

Formalin fixation, as applied, did not seem to decrease the intensity of the reaction in the rat tongue, but it produced granulation and changes in the morphology of the motor end plate, as compared with the unfixed specimens. No image was obtained in frog muscle after fixation; only a diffuse, generalized precipitate was obtained.

Preincubation in prostigmine bromide ($10^{-6}M$) and also in tetraethylpyrophosphate at ($10^{-7}M$) have inhibited completely the activity of the motor end plate in the rat tongue, while a nonspecific esterase active site in the surface epithelium was still present after 15 minutes in either one of these inhibitor solutions.

It is proposed that this precise, constant, simple, and inexpensive procedure can be utilized, along with specific inhibitor control, for histological investigations and also for physiological, pharmacological, and pathological studies involving cholinesterase (*7*).

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References and Notes

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