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Determination of Acid Phosphatase Activity in Nervous Tissue

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High levels of acid phosphatase activity in nerve cells allow methods for its detection to be used in morphofunctional studies of nervous tissue elements. Using neurons in the stellate ganglion in cows as an example, the bodies and processes of nerve cells are shown to have different levels of enzyme activity. Nerve endings have consistently high levels. This method provides for detailed morphological investigation of neurons and assessment of their interactions. This report provides a detailed description of an improved method for identifying acid phosphatase activity in nervous tissue structures.

Keywords: nerve cell, nervous tissue, acid phosphatase.

Recent decades have seen wide use of novel study methods allowing various physiological and functional characteristics of biological objects to be studied at the cellular level [10]; histochemical methods have almost disappeared from use, though in a number of cases they have clear value. These include the Gomori method, which allows acid phosphatase (AP) activity to be detected in tissues; AP is an important enzyme in the phosphomonoesterase group and is involved in phosphorus metabolism in tissues. This enzyme splits macroergic ester bonds in phosphoric acid monoesters to form free orthophosphate, with release and transfer of large amounts of energy [4]. AP has been shown to take part in cell metabolism and organ regeneration and to be activated in organs in compensatory states and in muscle contraction [2]. Most studies of AP have been directed to detecting its specificity and potential for diffusion into surrounding structures. Investigations have demonstrated that AP is widely distributed in nature [4, 8]. It is found in virtually all tissues in animals and plants (including microorganisms). High levels of AP activity are found in nervous tissue [3, 6, 12], where it is present in lysosomes, Golgi complexes, pinocytic vesicles, and secretory vacuoles, and large quantities are seen in macrophages and osteoclasts. Genetic impairments to the biosynthesis of phosphatases lead to disease associated with impaired glycogen metabolism. A test for estimating its activity is used in clinical practice in the treatment of oncological and other diseases, and also in forensic medicine. Methods of assessing its status in hydrobionts to address ecological problems have been developed [8]. Large quantities of AP are present in nervous tissue. It has roles in protein, fat, and carbohydrate metabolism [1, 9].

The aim of the present work was to demonstrate that the value of the rarely used Gomori method comes from the fact that apart from the histochemical estimation of activity and locations of one of the most important enzymes in structural elements of nervous tissue, this method provides for studies of their structure.

The method for detecting AP in tissues using histological sections was developed by Gomori in 1941 [13], after which a number of investigators made various changes, which improved the results. The modification introduced by Wolf et al. [14] was the most suitable for studies of elements of the nervous system. The essence of this histochemical reaction is the need to convert the precipitate formed at the location of AP first to an insoluble state and then to a visible form.

An advantage of this method of detecting AP activity is the ability to use thick, frozen histological sections and total preparations, providing for studies not only of neuron structure, but also their interactions (Fig. 1, *a*). One benefit of the method is the rapid production of results using unlimited quantities of specimens.

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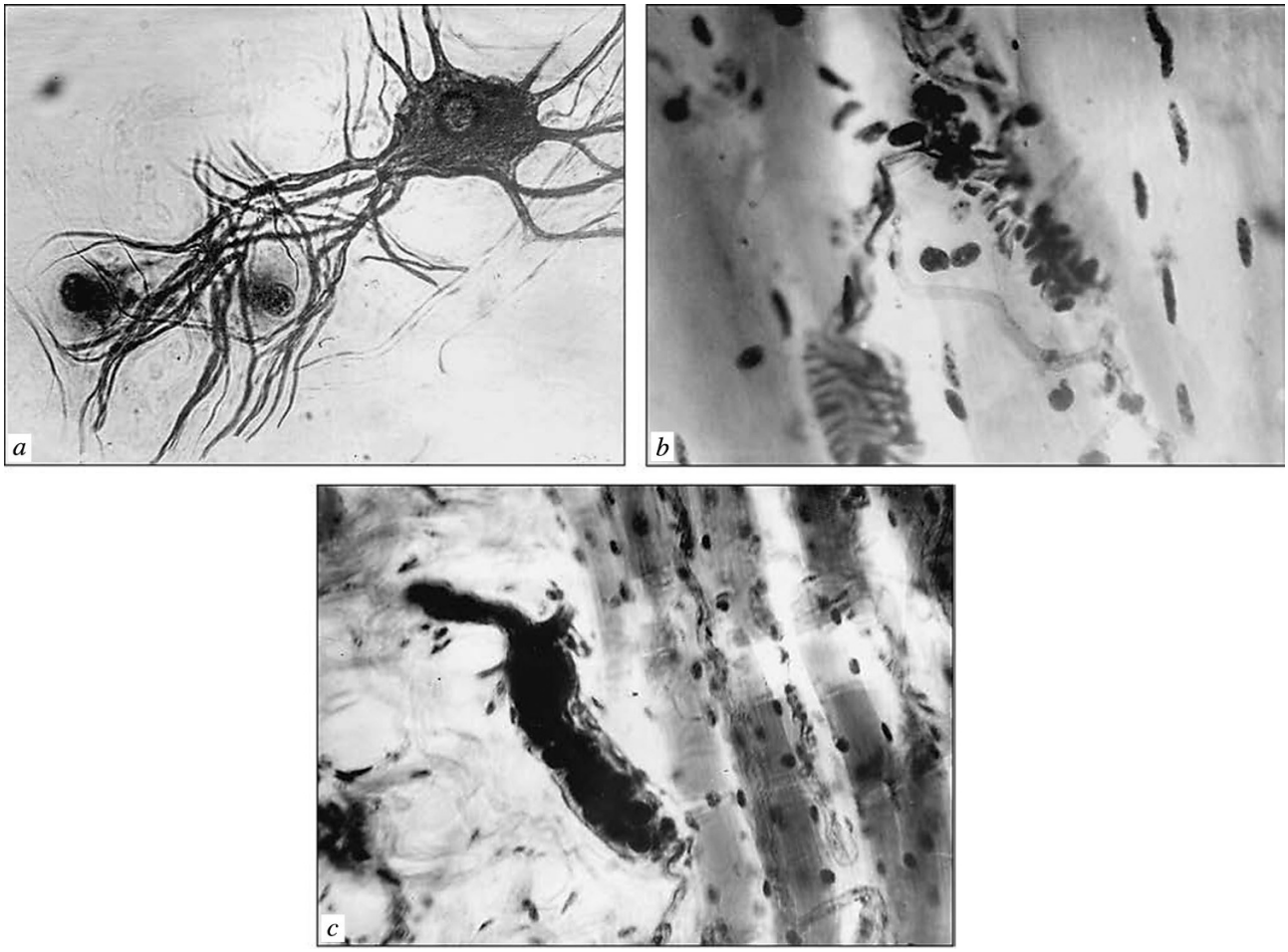

Fig. 1. Structural elements of neurons in the stellate ganglion. *a*) large neuron with moderate acid phosphatase (AP) activity, whose processes form a plexus around a neuron with a weakly positive reaction for AP; *b*) a neuromuscular spindle with high AP activity, a motor plate in cat gastrocnemius muscle; *c*) encapsulated receptor in cat tongue. Fixed with 80° alcohol. Gomori method. Objective ×40. Ocular ×7.

Drawbacks of the method are that it uses only fresh material (no more than 12 h post mortem), which is due to the quite rapid inactivation of the enzyme. Some inconvenience comes from the duration of incubation required for sections (4 h) and the need to prepare specimens immediately after incubation is complete. Possible difficulties can also arise in relation to selecting the substrate on which the enzyme acts (sodium β-glycerophosphate or sodium α+β-glycerophosphate).

When performing uncomplicated monitoring [1], along with assessment of AP activity in the structural elements of neurons (bodies, processes, synapses, growth cones, neurofibrils), the same sections can be used for studies of the structure of nervous tissue (see Fig. 1). Apart form synaptic connections between neurons, which can be detected by the Gomori method as highly active structures, histological preparations clearly show interactions between neurons in the form of diverse types of plexuses between processes. The level of AP can be used to evaluate the functional status of these formations. The Gomori method can also be used to study the afferent and efferent components of neurons (see Fig. 1, *b*, *c*), and, in some cases, the neuroglia.

In morphological studies, the Gomori method has been used successfully in studies of the structure of nervous tissue in humans and domestic animals (cattle, dogs, cats, chickens) [1–3, 5, 11] and has been used with positive results in experimental studies, especially on sciatic nerve regeneration, spinal cord trauma, and others [5, 7, 12]. Unfortunately, these studies have not been developed further.

Specimens for the studies reported here were of the stellate and celiac ganglia of the autonomic nervous system in cows, obtained from the Moscow Meat Combine from 30 animals 30 min after slaughter. Specimens from three cats were also used, collected after sacrifice by ether anesthesia.

Considering that AP is an enzyme which loses its activity at room temperature, all specimens were immediately placed in a thermos containing cooled 80° alcohol and were then transferred to fresh 80° alcohol and fixation was con-

tinued at a temperature of –4°C in the freezer compartment of a refrigerator for 1–2 days. Improved fixation of material provides for maximum preservation of enzyme activity and yields more precise information on its locations and a clear morphological picture of the structural elements of nervous tissue (see Fig. 1).

Specimen processing:

1. Specimens are immediately placed in cooled fixing fluid. The most suitable for this are acetone and 80° alcohol. As alcohol provides better preservation of nerve cell structure but produces some inactivation of enzyme, fixation should be performed in a refrigerator at very low temperature (to –4°C). Specimens are fixed for 1–2 days in two changes of solution.

2. Specimens are rinsed in cold distilled water.

3. Sections are cut on a cryomicrotome (cryostat), thickness about 30 μm, and are immersed into cold distilled water.

4. Sections are incubated in previously prepared medium ("substrate") in an incubator at 37–38°C for 4 h.

5. After incubation, sections are carefully rinsed with distilled water at room temperature and immersed in freshly prepared 0.1% sodium sulfide solution, in which the insoluble precipitate at enzyme location sites is converted to a visible form (sections acquire a brown color).

6. After rinsing in distilled water at room temperature, sections are placed on slides, dehydrated, clarified, and embedded beneath a cover slip.

All manipulations (except incubation) with each section are performed separately. It must not be forgotten that the whole process of detecting AP (as in the preparation and storage of ready solutions) must be performed in chemically clean vessels (boxes, beakers). Glass hooks needed for transferring sections should be constantly rinsed in distilled water.

The whole of the process for detecting AP activity can also be performed on paraffin sections if material is embedded rapidly. However, the result will be more restricted in terms of section area, and enzyme activity is lower. Very good results have been obtained using total preparations obtained by delaminating the walls of the hollow organs (such as the intestine).

Composition of incubation medium:

1. Acetate buffer mix, pH 4.7, 12 ml.

2. 1/10 M lead nitrate, 10 ml.

3. 2% sodium glycerophosphate solution (sodium β-glycerophosphate or sodium α+β-glycerophosphate), 6.5 ml.

4. Distilled water is added to 100 ml.

The resulting precipitate is removed by filtration.

Preparation of incubation solution:

1. Acetate buffer mix (if ready solution is not available), pH 4.7:

1) distilled water, 200 ml;
2) glacial acetic acid, 12 ml;
3) distilled water, 300 ml;
4) sodium acetate, 39.9 g;
5) the solutions are mixed and the pH is checked.

2. 1/10 M lead citrate:

1) distilled water, 100 ml;
2) lead citrate, 3.3 g.

Monitoring the histochemical reaction:

1. Inactivation of sections at a temperature of 80°C for 10 min or 90°C for 5 min. Then incubation etc. as per recipe.

2. Add 0.01 M sodium fluoride solution to the incubation mix.

3. Exclude substrate (sodium glycerophosphate) from the incubation medium, replacing it with distilled water.

Modifications to the Gomori method proposed by Chilingaryan should particularly be noted [9] to detect submucosal and intermuscular plexuses. Before incubation, prepared external longitudinal layer of muscle sheath of the intestine (along with the intermuscular nerve plexus) should be immersed in distilled water warmed to 72°C for 5 min, followed by transfer to distilled water at room temperature. This approach provides for the maximum possible demonstration of neuron composition, including small neurons, which are otherwise not detected or are incompletely detected. In addition, inactivation of enzyme in tissues surrounding the intestine provides for clearer differentiation of the structural elements of neurons and intraganglion interactions. This method has also been used successfully by other authors [11].

Thus, the Gomori method for detecting AP activity as modified by Wolf et al. [14] is a valuable method allowing not only detection of activity in the structural elements of neurons when specimens are fixed correctly, but also complete studies of both the central and peripheral nervous system. The potential of this method for morphological studies is far from exhausted.

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