MODIFICATION OF GOLGI'S METHOD USING SODIUM TUNGSTATE

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In 1873 Golgi described a method of chromate–silver impregnation based on the production of a complex group of salts of chromic acid with silver nitrate and capable of staining ganglion cells. Like other methods of impregnation of nerve tissue with silver, this method does not yield constant results. The mechanism of action of its individual components has not been adequately studied, and the importance of the concentrations of the substances used, the time relationships, and the reaction temperature is uncertain. The classical Golgi method is time consuming and it gives best results when used to study the brain tissues of young animals. Several modifications have been suggested for making the Golgi method more reliable [3, 4, 5, 7].

Using the basic reaction of the Golgi method (the reaction between potassium bichromate and silver nitrate) as the starting point, the author has investigated the possibility of adding various reagents precipitating the proteins and lipids of nerve tissue to the fixing fluid. For this purpose the action of certain chemical reagents in various concentrations, for different times, and at different temperatures on brain tissue was analyzed.

One of the components of the fixing fluid in the author's modification was sodium tungstate (Na_2Wo_4 $2H_2O$), which was used to coagulate the proteins.

A mixture of neutral formalin and potassium bichromate was used to give simultaneous oxidation, chromation, and fixation of the proteins and lipids. The reaction with proteins takes place under the influence of formalin. It is claimed that formalin unites the peptide chains of the proteins into a network [2, 6]. In the course of fixation the readily soluble lipids are washed out; these are evidently not components of the lipoprotein complexes, and the results of the reaction are thereby improved.

After fixation, the material was treated with osmic acid, together with potassium bichromate and chloral hydrate. The reaction of reduction of the osmium tetroxide (OsO_4) in dilute aqueous solutions is used to detect lipids. The method of demonstration of lipids by means of osmic acid gives a clearly defined morphological picture. In this reaction the osmic anhydride (OsO_4) is reduced to OsO_4, and the lipids thereby become visible. Pure OsO_4 causes blackening of all the structural elements of nerve tissue, interfering with the precise staining of the neurons. If, however, oxidizing agents are present along with the OsO_4, this generalized blackening does not occur. It has been shown that normal myelin has an affinity for water because of the presence of phospholipids, and it is therefore permeable to oxidizing agents dissolved in water [6].

For this reason osmic acid was used in a mixture with potassium bichromate and chloral hydrate. In this case the aldehyde groups liberated as a result of the oxidation of chloral hydrate (in a medium with OsO_4 and $K_2Cr_2O_7$) formed a complex with silver and thus enabled the structures of the neuron to be rapidly demonstrated.

Success in this reaction is dependent on the degree of coagulation of the proteins achieved by the action of the sodium tungstate.

In brain tissue treated in this way the nerve cells and fibers are impregnated reasonably uniformly, and the time required for impregnation is considerably shortened.

Treatment of material for testing by the author's modification of the Golgi method is carried out as follows.

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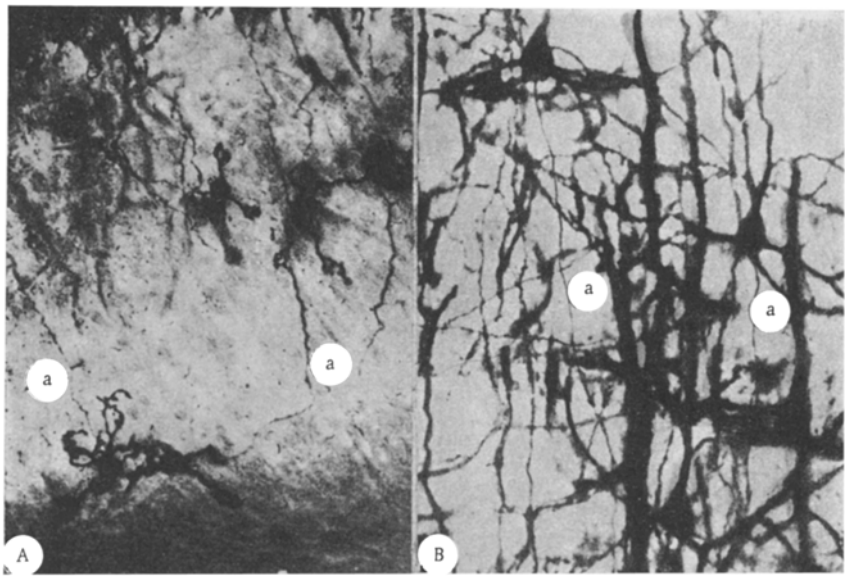

Granular cells of the cerebellar cortex of a monkey (A) and pyramidal cells and vessels of the motor cortex of a rat (B). a) axon. Impregnation with silver (modification of Golgi's method using sodium tungstate). Objective 20×, ocular 10×.

Fresh material is taken, irrespective of the animal's age. The thickness of the pieces must not exceed 5 mm, and their area 2×1.5 cm.

The fixing fluid consists of neutral formalin (12 ml), sodium tungstate (0.8 g), potassium bichromate (3 g), and tap water (88 ml); temperature 18-20°. The time of fixation varies from 2 to 6 days. During the first day the solution is changed twice, and thereafter once daily. After fixation of the cerebellum for 2-3 days it is possible to distinguish the Purkinje cells, the stellate cells, and the large Golgi cells, while after fixation for 4-5 days the granular cells and small Golgi cells can be seen (see Figure, A). The pyramidal and stellate cells of the cerebral hemispheres can be detected after fixation for 2-3 days, and the nerve cells of the subcortical structures after fixation for 5-6 days (see Figure, B).

After fixation, the pieces of tissue are rinsed once or twice in 4% potassium bichromate solution in distilled water, and transferred to a solution of the following composition: 1% osmic acid (20 ml), potassium bichromate (4 g), chloral hydrate (2 g), distilled water (80 ml). The pieces remain in this solution for 1-2 days (not more than 24 h in the case of the cerebellum). They are then dried with filter paper, washed a few times in 1.5% silver nitrate solution, and then placed in the same solution for 2 days. They are then passed through alcohols of increasing concentration and embedded in celloidin. Sections cut to a thickness of 80-120 μ are cleared in eucalyptus oil and mounted in balsam. If the nerve cells and blood vessels are to be stained at the same time, the amount of sodium tungstate is increased to 1.5 g. The whole process of fixation and impregnation in the author's modification takes from 5 to 8 days.

This modification of the Golgi method thus enables the different types of neurons of nerve tissue to be fully demonstrated, while requiring shorter times of fixation and impregnation. A satisfactory level of reproducibility of the results is observed.

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