MODIFICATION OF BIELSCHOWSKY'S METHOD FOR DETECTION OF SENILE PLAQUES AND INTRACELLULAR NEUROFIBRILS

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UDC 616.899-053.9-07:[616.831-091.823+616.831.004]-091.5

KEY WORDS: senile plaques; Alzheimer's disease; impregnation; selective staining

In the autopsy department of psychiatric hospitals Bielschowsky's method [2, 3] is widely used for the microscopic diagnosis of various forms of senile dementia. The diagnosis is based on morphological pictures obtained as a result of silver-gold impregnation: specific formations stand out clearly against the pale gray background of the section, namely senile plaques (senile dementure plaques), and in Alzheimer's disease, neurons with characteristic changes in the neurofibrils. However, only the reticulo-fibrillary corona of the senile plaque can be seen in preparations of this kind, without its amyloid core, and as a rule, most of the neurons are not impregnated. The drawbacks mentioned above are difficulties in the way of qualitative and quantitative analysis of the picture obtained.

For the more complete and reliable demonstration of the various elements of nerve tissue, a very promising development is to combine silver-gold impregnation by Bielschowsky's method with selective staining by Nissl's method [2], i.e., a combination of impregnation and cytoarchitectonics methods. The specific feature of this modification is that sections impregnated with silver and gold are stained with a concentrated solution of fast cresyl violet, acidified with acetic acid. The writer has also developed a scheme for obtaining pieces of cerebral cortex with an exact profile section.

By the suggested modification it is possible to obtain accurately oriented survey sections with relatively good preservation of the nerve tissue, and with reliable demonstration of all its elements. This latter aspect is particularly important in histopathological investigations and in quantitative analysis. Sections of this kind are suitable for the study of the fine structure of the specific changes (senile plaques, neurofibrils, changes of Alzhemier type) and also for determination of the cytological characteristics of nerve and glial cells in different pathological states (Fig. 1).

For completeness of impregnation of the specific lesions and subsequent successful staining of the different elements of nerve tissues with the selective dye, the essential factors in Bielschowsky's method is fixation: choice of fixing fluid and the optimal time of keeping the brain fragment in it. Instead of a 10% solution of neutral formalin, as is used in Bielschowsky's method, it is better to use a 10% solution made up in physiological saline. This fixative can be successfully used in several impregnation methods and, in particular, in Nauta's method [4] and its various modifications.

The optimal time for keeping brain fragments in this fixative has been shown to be from 15 days to 2 months. However, brain fragments can be kept up to a year, if the fixative is changed periodically. Long periods of keeping of the fragments may adversely affect the process of silver impregnation of nerve tissue: its various elements are unevenly impregnated, and the background has a dirty gray appearance. This is a disadvantage if the section has to be stained subsequently with fast cresyl violet.

Considering that additional staining of the silver-impregnated preparations is considerably more difficult [1] and requires a more concentrated solution of the dye than Nissl's method (a 0.1% solution of cresyl violet), I chose a solution of the dye of the following composition: to 100 ml of a 0.3% or 0.4% solution of fast cresyl violet, 0.1 ml or 0.12 ml

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 0007-4888/87/0103-0138$12.50

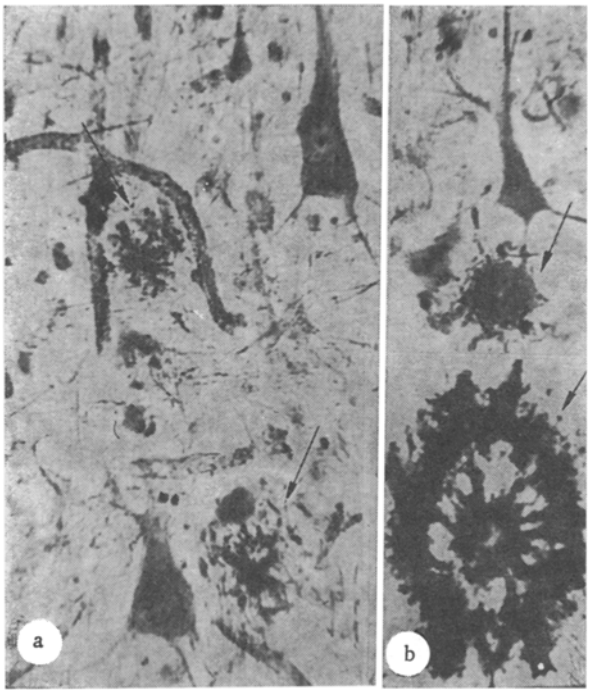

Fig. 1. Senile dementia. Human motor cortex, layer III 3. Arrows indicate senile plaques. Modification of Bielschowsky's method. Magnification: a) 200; b) 600.

of 10% acetic acid is added accordingly. The solution of the dye is used repeatedly and filtered from time to time.

To obtain accurate profile sections of the cerebral cortex, on which the quality of the survey pictures of the preparation to a large extent depends, the following procedure is recommended. Pieces of cortex are excised so that the direction of the scalpel (razor) is strictly perpendicular to one of the gyri in the desired region of the cerebral cortex. If the section is made in this way a piece of brain is obtained in the form of a disc with the pattern of the petal of the gyrus. This pattern must be the same as far as possible on both sides of the disk. If the brain fragment is cut on a freezing microtome (an essential condition of the method), it must be oriented so that the white matter of the gyrus is toward the knife blade, for in this way the upper layers of the cortex are better preserved. If the pia mater is present the brain fragment must be cut from the side of the gray matter. The thickness of the sections is 8-20 μ.

The formula for Bielschowsky's method, by the suggested modification, is given below.

1. After fixation the brain fragment is rinsed in running water for 1-1.5 h, after which it is placed on the stage of a freezing microtome. The sections are collected in a 10% solution of neutral formalin, made up in physiological saline, in which they are fixed for 2-3 h.

2. The sections are then quickly rinsed in distilled water and immersed in a 2% solution of silver nitrate for 20-24 h.

3. After rinsing in distilled water the sections are transferred for 2-4 min into an ammonical alkaline solution of silver nitrate (one drop of 40% caustic soda solution is added to 4 cm^2 of 10% silver nitrate). To the precipitate thus formed 4 or 5 drops of a 25% ammonia solution are added, and all the precipitate is dissolved with careful shaking. If the precipitate does not dissolve a further 1-3 drops of 25% ammonia solution are added. Next, 30 ml of distilled water is added to the transparent solution.

4. The sections are washed in two portions of distilled water and transferred into a 20% solution of neutral formalin (in tap water), where they turn brown in the course of 30 sec.

5. The sections are washed in distilled water and transferred for 2-4 min into a 0.5% solution of gold chloride, where they turn plane gray, with a steel gray tinge.

6. The sections are transferred from distilled water into a 5% solution of hyposulfide for 2-4 min, then washed in two portions of distilled water.

7. The sections are then glued to a slide, smeared with a mixture of albumin and glycerol, and allowed to stand for 10-20 min (overnight will do) for drying.

8. After drying the slides with the section is placed in a solution of cresyl violet of the above-mentioned concentration for 3-5 min and then washed in distilled water.

9. The sections are passed through a series of alcohols of increasing strength: 70% (quickly), 96%, and 100%, after which they are placed in eucalyptus oil, where differentiation of the stain takes place, and a clear difference in cover between the gray and white matter of the cerebral cortex ought to develop in the section. The sections are then quickly immersed in acetone, then in toluene, and are mounted in balsam beneath a coverslip.

This modification of Bielschowsky's method can be recommended for extensive use.

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