Acta neuropath. (Berl.) 25, 161—162 (1973)

Kurze Originalmitteilungen · Short Original Communications
Communications originales brèves

Silver-Permanganate Method to Demonstrate Axis Cylinders and Myelin Sheaths on Frozen Sections

Walter Berger

Department of Pathology and Division of Neurology,
University of Colorado Medical Center, Denver, Colorado

Received January 30, 1973

Summary. A simple technique for demonstrating the axis cylinders and myelin sheaths on formalin-fixed frozen sections is described.

Key words: Silver-Permanganate Method — Demonstration of Axis Cylinders — Myelin Sheaths.

A new silver-permanganate method of demonstrating axis cylinders and myelin sheaths is described below. It is simple and can easily be reproduced on material well fixed in 10% formalin from which frozen sections can be made.

Cut sections to be stained 20—30 μ thick and placed in a container of distilled water. Sections should be carried one at a time to the staining solution by means of a glass rod. Only a few sections should be stained in a given solution to avoid precipitate from forming in the tissues.

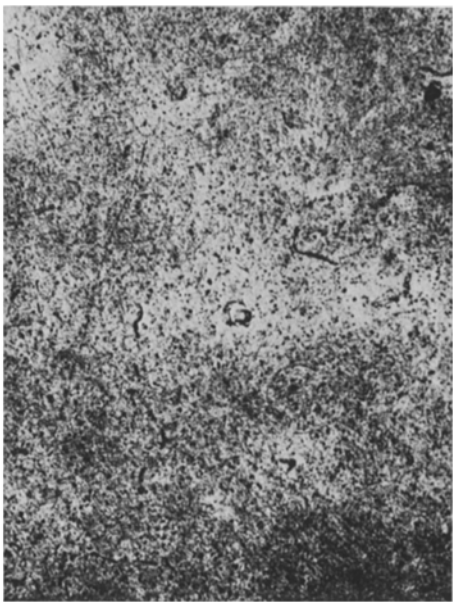
1. Impregnate the sections in the following silver solution with the aid of a thermostat at 60°C centrigrade for 45 min. The first silver solution is composed of 20% silver nitrate to which 3 drops of pyridine have been added to each 100 ml.
2. Wash well in two changes of distilled water.
3. Place in 20% formalin solution for 3 min. (Use distilled water to dilute formalin.)
4. Wash well in three changes of distilled water.
5. Impregnate sections at room temperature for 30 min in the following solution: To 5 ml of 10% silver nitrate solution, add 5 drops of 40% potassium hydroxide, agitating to form a precipitate. Then add ammonium hydroxide drop by drop until all the precipitate has disappeared. Then add distilled water until the total volume of solution is 100 ml.
6. Wash well in two changes of distilled water.
7. Place sections in a new 20% formalin solution for 10 min.
8. Wash briefly in distilled water.
9. In a shallow container of 1/4% potassium permanganate, place a small porcelain sieve. Place one section in the sieve for 1—2 min. The section should resemble tobacco in color when differentiated.
10. Wash in two changes of distilled water.
11. Dehydrate in 3 changes of 95% isopropyl alcohol.
12. To avoid shrinkage of the tissue do not place in absolute alcohol.

To mount tissue on slide, blot lightly with 3 pages of bibulous paper. Then dip the slide with the section in place into isopropyl alcohol until the section is without wrinkles or bubbles. Dip the slide in xylene, blotting in much the same manner as before until cleared of alcohol. Then cover slip with mounting media.

Results

The axis cylinders are black. The myelin sheaths will appear light to dark brown in color.

1



2

Fig. 1. The normal structure of the axis cylinder and myelin sheath in the medulla oblongata 770×, oil immersion, 20 μ

Fig. 2. Loss of axons and myelin sheaths in the periventricular white matter in a case of multiple sclerosis × ∅, 30 μ

Comments

This method used to demonstrate the axis cylinders is based on an impregnation of the axon by metallic salts. Silver from the silver nitrate is bound to the axis cylinders causing a light brown color at first. The concentrated pyridine prevents the over-impregnation of the fibers and cells. When treated with formalin solution the color of the axis cylinders is intensified to black. An additional impregnation with potassium permanganate renders the structure of the myelin sheaths a light brown color. This color is also seen staining the glial elements and cells.

This impregnation procedure is unique in that both the axis cylinders and myelin sheaths of the nerve fibers are readily demonstrated using high power lens and immersion oil.

Acknowledgements. The author wishes to thank Stuart A. Schneck, M.D. and Rosalie R. Donley for their kind help and advice.

References

Berger, W.: Eine Silber-Permanganat-Methode am Paraffinschnitt. Schweiz. Arch. Neurol. Neurochir. Psychiat. **92**, 292 (1963).

Walter Berger
Department of Pathology and
Division of Neurology
University of Colorado Medical Center
4200 East Ninth Avenue
Denver, Colorado 80200, U.S.A.