A MODIFIED NAUTA METHOD

NAKAMURA, Kazushige

(Citation)
The Kobe journal of the medical sciences,9(3):135-138

(Issue Date)
1963

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/0100489228

Kobe J. Med. Sci.
9, 135-138, Sep. 1963

A MODIFIED NAUTA METHOD

Kazushige NAKAMURA

*Department of Anatomy, Division I**

Kobe Medical College

Formalin fixed brains (rabbit, rat and cat) containing surgical lesions were frozen and sectioned. Subsequent procedure is as follows: soak in 0.5% aqueous phosphomolybdic acid solution for 20~45 min.; wash and treat with 0.1% pyrogallol solution for 20 min.; wash thoroughly and soak sections in 1.5% silver nitrate for 20~40 min.; Wash (2 changs) each 1 min.; place in ammoniac silver nitrate for 2~4 min.; reduce in Nauta-Gygax fluid for 2 min.; wash and pass through 1% sodium thiosulfate for 2 min.; wash thoroughly and cover with glycerin.

An entirely new method for silver impregnation of degenerated axons was reported by Nauta in 1952. Since then the method has been improved by Nauta and Gygax (1954), Glees and Nauta (1955), Nauta (1957), Albrecht and Fernstrom (1959).

The methods that have been most often used by researchers are the Nauta-Gygax's (1954) and Nauta's one (1957) which employs Laidlow's solution as a reducing fluid. Difficulties to carry out the experiments by these methods are the time to soak the sections in potassium permanganate solution and the procedure of step 10 (described below), "wash the section in distilled water". It is impossible to obtain a good result unless this procedure is performed with a delicate care. It requires the trained technique, moreover, it is hardly possible to stain a numerous sections at the same time.

Present report is a new modified method employing pyrogallol. This procedure can impregnate the numerous sections without precise care and study even a fine degenerated axon. Regarding the color tone, according to this new method, normal fibers can be seen as brown color, presenting a contrast with black color of the degenerated one which provides sharp differentiation of the degenerating axons.

Artifacts as noted by Glees and Nauta (1955) and Nauta (1957) are present in this modified method.

Received for publication September 16, 1963

*Chairman: Prof. Hajimu TAKEDA

The procedure are as follows :

1. Fix in 10% neutral formalin for 2 weeks to 6 months.
2. If the preservation of anatomical continuity is required, embed in gelatin after Albrecht's method (1954).
3. Cut frozen sections of 30～40 μ thickness. Assembled sections must be stored in 10% neutral formalin immediately.
4. Wash sections in distilled water.
5. Soak sections in 0.5% phosphomolybdic acid for 20～45 minutes.
6. Wash briefly in distilled water.
7. Transfer scetions to 0.1% aqueous pyrogallol for 2 min. Light brown color will appear.
8. Wash thoroughly in at least 3 dishes of distilled water, 1 minute each.
9. Soak sections in 1.5% solutions of silver nitrate for 20～40 min.
10. Wash in 2 changes of distilled water, 1 minute each. In the previous Nauta method, the procedure (10) must be treated briefly by well-trained laborants, but with author's method, this treatment would be done well even by beginners.
11. Place sections for 2～4 minutes in ammoniac silver nitrate solution, freshly prepared as follows;

Silver nitrae	3.6 gm
Distilled water	80.0 ml

When solution is complete, add:

Pure ethanol	40.0 ml
Strong ammonia	8.0 ml
2.5% aqueous sodium hydroxide	6.0 ml

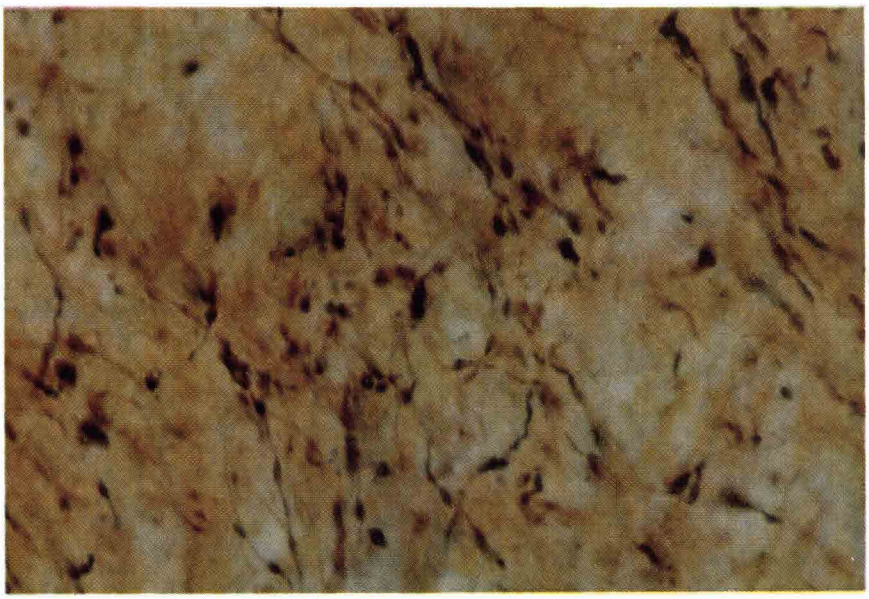
12. Without washing transfer the sections to Nauta-Gygax's reducing fluid (1954) for 2 minutes. Brown color will appear within 10 seconds.
13. Wash in distilled water.
14. Pass sections through 1% sodium thiosulfate.
15. Wash again in 3 changes of distilled water.
16. Mounting is best covered with glycerin.

ACKNOWLEDGMENTS

The author wishes to thank Professor Hajimu TAKEDA, Department of Anatomy, Kobe Medical College for his continued interest and kind guidance during the course of this investigation. I am also grateful to Dr. Masao WAKABAYASHI, Department of Biochmistry, Kobe Medical College for his advice.

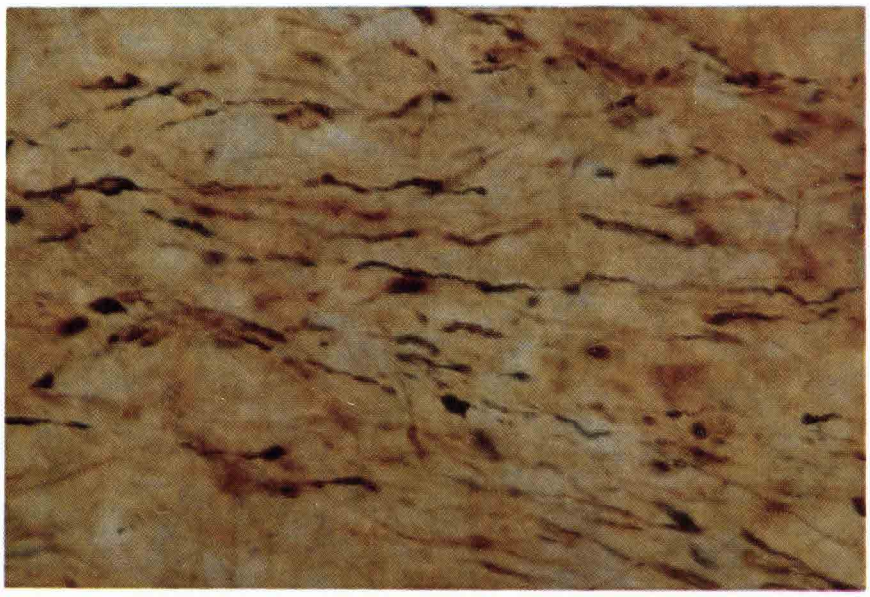
REFERENCES

1. Albrecht M. H. and Fernstrom, R. C. A modified Nauta-Gygax method for human brain and spinal cord. Stain Techn., 34: 91-94 (1959).
2. Albrecht, M. H. Mounting frozen sections with gelatin. Stain Techn., 29: 89-90 (1954).
3. Glees, P. and Nauta, W. J. H. A critical review of studies on axonal and terminal degeneration. Mschr. Psychiat. Neurol., 129: 74-91 (1955).
4. Nauta, W. J. H. and Ryan, L. F. Selective silver impregnation of degenerating axons in the central nervous system. Stain Techn., 27: 175-179 (1952).
5. Nauta, W, J, H, and Gygax, P. A. Silver impregation of degeneraitng axons in the central nervous system, A modified technic. Stain Techn., 29: 91-93 (1954).
6. Nauta, W. J. H. New research techniques of neuroanatomy. 17-26 (1957). C. C. Thomas Co., Springfield. Illinois.



Lateral nucleus of the cerebellum showing axon degeneration resulted from the lesion of the rat's cerebellar cortex (paraflocculus).

(Magnification, 650×)



Degenerating stem fibers in the rabbit's superior cerebellar peduncle following the lesion of the lateral cerebellar nucleus.

(Magnification, 650×)