A standardized method for staining nerve structures: Modifications on the classic Bodian stain

This staining technique is from a publication by Morse that I copied as an undergraduate student at Bowdoin College from a sheet that Jim Moulton had. There was no citation. I have italicized my own additions. Protargol can be purchased from a number of sources.

Fixation: *I have used a number of fixatives including Bouins. I currently use AFA (alcohol-formalin-acetic acid). Mix 90ml of 80% ethanol with 5ml of formaldehyde and 5ml of glacial acetic acid. This is a fast fixative. You should fix for at least a week and then wash in 70% ethanol overnight and then continue the dehydration and embedding process. You can immerse the brain or immerse the whole, anesthetized animal after you have removed the skull over the brain. I also cut the heart and allow blood to drain out of the animal before placing it in the fix.*

Sectioning: *I routinely section the tissue at 15µm thickness. You may wish to try different thicknesses to better fit your needs.*

The slides on which you float the paraffin sections should be subbed *in a Chrom-Alum subbing solution:*

1. *Add 2.5gm of gelatin to 500ml hot (60°C) distilled water*
2. *Allow solution to cool and add 0.25gm Chromium Potassium Sulphate*
3. *Store at 4 degrees C. This solution can be made in advance, and can be used over again. Warm before using.*

Staining—Silver Impregnation Technique

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A standardized method for staining nerve structures: Modifications on the classic Bodian stain *(I don't have a citation for this; look at Amer.J.Med.Tech. 30: 355)

The Bodian stain has been used as the "routine stain" for studying axons and nerve cell bodies. After the supply of German-made protargol had become exhausted, the author sought a suitable substitute which would give repeated satisfactory results with a minimum of complicated steps.

The following method is a modification of the classic technique which has given good results with the new material.

Of the various brains of protargol used, we found that that produced by Rogues of Paris (Proteinate d'argent) gave the most consistent results when the following modifications were used:

METHOD

1. **Hydrate** paraffin sections to distilled water as usual.
2. **Incubate** slides in 2% Protargol (Rogues)** solution to which 5gm of polished copper shot (i.e., 5gm of polished copper shot for each 100ml of Protargol solution) has been added. Incubate the slides in the Protargol solution for 48 hours at 37 degrees C in the dark. (I rotate the slides after 24 hours).
3. **Rinse** in 3 changes of dH_2O
4. **Reduce** in 1% Hydroquinone for 10 minutes.
5. **Rinse** in 3 changes of dH_2O
6. **Tone** in 1% aqueous gold chloride for 10 minutes (15 grain [1 grain = 0.065gm] tube of gold chloride [Fisher] in 97ml of dH_2O with 3 drops of glacial acetic acid added). *(This solution must be stored in a brown bottle and kept out of contact with any metals. It should be saved because it can be used for months – ideally, it should be filtered before each use.)*
7. **Rinse** in 3 changes of dH_2O
8. **Develop** in 2% oxalic acid for 20 minutes
9. **Rinse** in 2 changes of dH_2O
10. **Fix** in 5% sodium thiosulfate for 5 minutes
11. **Rinse** in 5 changes of dH_2O
12. **Dehydrate and coverslip**

*From the Dept. of Experimental Surgery, Walter Reed Army Institute of Research, Washington, D.C.
**Available through Roboz Surgical Instrument Company, 810-18th Street, N.W. Washington, D.C. 20006

Axons and some neuronal elements should appear black, the cell cytoplasm should appear red.

COMMENTS

All glassware must be clean. It has been our experience that the usual cleaning technique for chemical apparatus is sufficient, thus obviating the need for acid cleanings. Metal equipment should be avoided. Forceps may be dipped in paraffin if used.

The protargol solution should be mixed on a warming plate without shaking. Use a flat dish (*I use a large Petri dish*) with the measured amount of distilled water and warm on a Ransom warming plate set at 58 degrees C or less. The protargol can be sprinkled on the surface of the water and allowed to dissolve.

If the copper shot is not well polished it should be cleaned under a fume hood in a mixture of nitric and hydrochloric acids (aqua regia). The cleaned shot must be thoroughly washed. Previously used shot can be recovered in this manner; however, about half the volume of copper will be lost during cleaning.

Care should be taken to prevent copper from touching the tissue sections. If this is not done these areas will overtone with gold and a purple-red discoloration will result. (*This is the reason I rotate the slide tray holder after 24 hours in protargol*).

The classic technique uses sodium nitrite in the reducing solution. Other modifications substitute formaldehyde for sodium nitrite. It has been our experience to

use just a pure hydroquinone solution. Indeed, there is less staining of the reticulum and other extraneous structures as well as more intense and uniform staining of the axons themselves.

Counterstaining may mask fine nerve fibers but is useful in routine work. If the above precautions are observed, this method will yield uniform results consistently.

SUMMARY

A standardized method for staining nerve cells and their processes is presented as a modification of the classic Bodian stain.

Comments are given from the author's experience to aid in giving consistent results.

REFERENCES

1. Bodian, David: A new method for staining nerve fibers and nerve endings in mounted paraffin sections. Anat. Rec. 65: 89-97, 1936
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