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K-698, HIRANO-ZIMMERMAN METHOD FOR NERVE CELLS AND FIBERS

Fixation: 10% Buffered Neutral Formalin (F-113)

Sections: Paraffin @ 12-15 microns

Staining:

1. Deparaffinize and hydrate to distilled water.
2. **Silver Nitrate, 10%** (K-698-1) for 2 hours or longer.
3. Place sections directly into **Ammonia Water** (K-698-2) for 3 minutes.
4. Place sections directly in **Formalin Solution** (K-698-3) for 3 minutes. Tissue becomes black-brown. Rinse in distilled water, two changes.
5. Place sections again into **Silver Nitrate, 10%** (K-698-1) for 3-5 minutes.
6. Repeat steps 3 and 4. Tissue becomes darker in color.
7. Tone in **Gold Chloride Solution** (K-698-4) for 20 to 30 minutes.
8. Place sections directly into **Sodium Thiosulfate, 5%** (K-698-5) for 1 minute. Rinse in distilled water.
9. Dehydrate in 95% alcohol, absolute alcohol and clear in **Xylene** (C-120) two changes each.
10. Mount with **Permount** (M-18).

Staining Results:

Nucleolus and nuclear membrane	Black
Neurofibrils, dendrites and axis cylinders	Black
Cytoplasm of astrocytes and the cytoplasmic membranes of macrophages	Gray
Senile plaques	Black

Various lipid granules are unstained, but each granule is clearly outlined.

References:

AFIP Manual of Histological Staining Methods 3rd ed., Ed. L.Luna: New York: McGraw-Hill Publications, c 1968, p. 198.
Hirano , A. and Zimmerman, H.M., Arch.Neurol., 6:114-122, 1962.