K-697, HOLMES' METHOD FOR NERVE CELLS AND FIBERS

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

**Sections:** Paraffin @ 6 microns.

**Staining:**
1. Deparaffinize and hydrate to distilled water.

2. Treat with **Silver Nitrate, 20%** (K-697-1) for 2 hours. Rinse in 3 changes of distilled water for 10 minutes.

3. Place in **Impregnating Solution** (K-697-2) overnight at 37°C in a covered vessel.

4. Remove slides, drain excess solution and place in reducing solution for at least 2 minutes.

   To prepare reducing solution mix:
   - **Hydroquinone** (K-697-3) 1.0 gm
   - **Sodium Sulfite** (K-697-4) 10.0 gm
   - Distilled water 100 ml

   Prepare just before use - reducing solution will only keep a few days.

5. Wash in running water for 3 minutes, then rinse in distilled water.

6. Tone in **Gold Chloride, 0.2%** (K-697-5) for 3 minutes. Rinse briefly in distilled water.

7. Develop in **Oxalic Acid, 2%** (K-697-6) under the microscope until axons are thoroughly blue-black. Rinse in distilled water.

8. Place in **Sodium Thiosulfate, 5%** (K-697-7) for 5 minutes, then wash in running water for 5 minutes.

9. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes.

10. Mount with **Permount** (M-18).

**Staining Results:**

<table>
<thead>
<tr>
<th>Axis cylinders</th>
<th>Blue to black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerves and nerve endings</td>
<td>Black</td>
</tr>
<tr>
<td>Background</td>
<td>Gray to rose</td>
</tr>
</tbody>
</table>

**References:**

