

## AN IMPROVED HISTOLOGICAL METHOD FOR EXAMINATION OF CUTANEOUS NERVES IN LEPROSY\*

M. BALASUBRAHMANYAN, A. PAUL JAYARAJ, and H. H. GASS.

The study of the changes in cutaneous nerves in leprosy was undertaken by us during the course of the last two years at this hospital. It was necessary to devise and perfect the histological methods, so that consistent results could be obtained and conclusions drawn. Davonport's method, Bodian's protargol method and the Bielchowsky's technique were tried for the demonstration of axons. None of these original methods gave entirely satisfactory results in our hands. The following silver impregnation technique is a modification of the previously well known techniques and has given uniformly consistent results. The study of the myelin sheath of the nerves was also undertaken and a method for the simultaneous demonstration of acid fast bacilli and myelin on the same section has been devised. This has proved to be a valuable technique to study the exact location of the bacilli. The object of our recording these techniques below is to enable, if possible, other workers in this field to undertake the study of the nerves in leprosy

### TECHNIQUE

1. Pieces of skin are taken and fixed immediately in 10% neutral formalin (neutralised with excess of calcium carbonate) for not less than 24 hours. It is important that the tissue is not crushed and the infiltration with the local anaesthetic does not distort the tissues.
2. Wash in several changes of water and cut frozen sections of 15-20 microns thickness.
3. Wash sections in three changes of distilled water.
4. Place in 60% alcohol containing a few drops of concentrated hydrochloric acid for half an hour. This step is important to produce a good contrast between collagen fibres and the axons.
5. Wash in 3 changes of distilled water.
6. Transfer to 20% silver nitrate in an amber coloured bottle for 20 to 30 minutes.

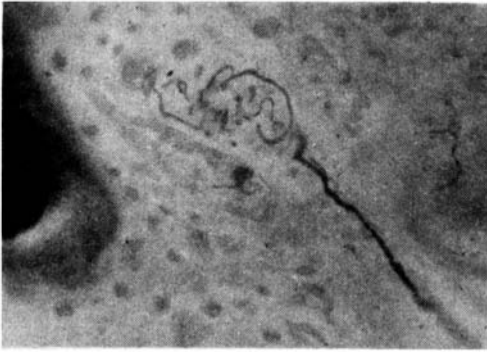


Fig. 1. Normal palmar skin showing Meissner's corpuscle.  $\times 470$ .

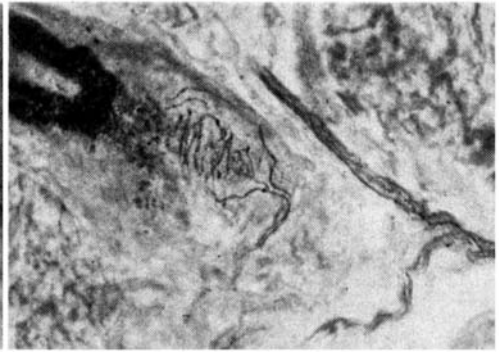


Fig. 2. Normal hair follicle showing network of fibres around.  $\times 100$ .

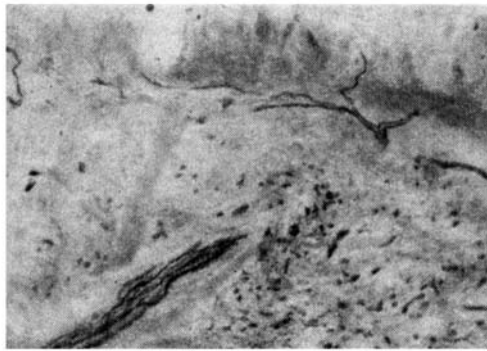


Fig. 3. Fine nerve endings inside the epithelium.  $\times 100$ .

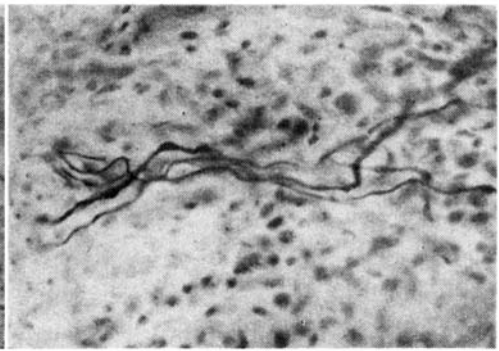


Fig. 4. Lepromatous leprosy showing ballooning and swelling of axons inside inflammatory infiltrate.  $\times 470$ .

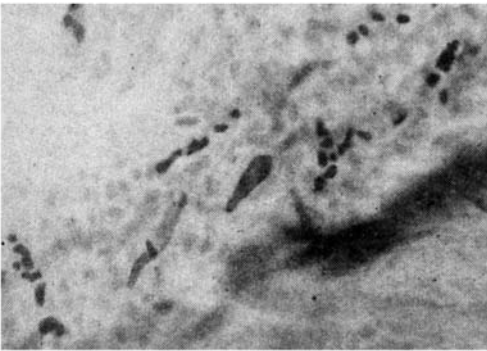


Fig. 5. Lepromatous leprosy myelin stain showing ballooned up myelin sheath inside inflammatory infiltrate.  $\times 470$ .



Fig. 6. Same as above showing acid fast rods and granules inside the myelin sheath.  $\times 900$ .

7. Transfer sections without washing to ammoniacal silver solution for two minutes and keep moving the section in it to avoid precipitate.
8. Pass the section through three changes of developer with constant agitation. The time in the third change of developer is controlled by the desired intensity of brown colour imparted to the section.
9. Wash well in distilled water and place in 5% liquor ammonæ till the section becomes translucent.
10. Wash, dehydrate in alcohol, clear in xylol and mount in canada balsam.

#### AMMONIACAL SILVER SOLUTION

To 5 cc. of 20% silver nitrate add 5 drops of 40% sodium hydroxide. Dissolve the precipitate that is formed by adding liquor ammoniæ drop by drop. Make up to 50 c.c. with distilled water. For staining purposes take 2 cc. of this solution and add 5 drops of liquor ammoniæ. The amount of liquor ammoniæ added should be determined by trials till the desired depth of impregnation of the axons is obtained.

#### DEVELOPER

Prepare 10% formalin using tap water. The pH of this solution is adjusted to 7.6 to 7.8 with tenth molar citric acid and fifth molar disodium phosphate buffer. A precipitate will form and it does not interfere with the staining process. We have tried other buffers but failed to get the same results. Different pH levels were tried to develop the sections. It is possible to vary the intensity of the background stain and of the axons by altering the pH level. At pH 7.6 to 7.8 it is possible to produce an even impregnation of axons and the inflammatory infiltrate and Schwann's cells. Thus a detailed histological examination is possible.

Since the developer is made with tap water, the results of analysis of the water used in this laboratory is given below.

Total solids 90, total hardness 30, permanent hardness 10, temporary hardness 20, chlorine 20.8, parts per 100,000. Free ammonia trace, albuminoid ammonia 0.002, oxygen absorbed 0.022, nitrate 1.75 parts per 100,000. Nitrate, phosphate and iron nil. Sulphates marked. pH 7.2.

**RESULTS**

The axons are stained in different shades of brownish black. The background is stained yellow with the nuclei of cells light brown. (Fig. 1 to 4.)

**Demonstration of Acid Fast bacilli and myelin**

1. Frozen sections prepared in the same way as for the demonstration of axons are washed in several changes of distilled water.
2. Stain sections in cold Ziehl Neelson's carbol fuchsin for 15 minutes.
3. Treat section with 50% alcohol slightly acidified with 1 or 2 drops of concentrated hydrochloric acid for 2 minutes. This step is not meant to decolourise the section.
4. Mordant sections in 4% ferric alum for 5 minutes.
5. Transfer without washing to 1% alcoholic haematoxylin solution diluted 50 times with distilled water. Stain for 4 hours.
6. Wash in distilled water and decolourise in 4% ferric alum solution, controlling under the microscope till the desired contrast between bacilli and the myelin sheath is obtained.
7. Place in 50% alcohol containing 1% liquor ammoniæ for 2 minutes.
8. Wash in distilled water, dehydrate, clear and mount in canada balsam.

**RESULTS**

The bacilli stained red while the myelin sheath takes a black colour. (Fig. 5 and 6.)

**SUMMARY**

1. An improved method of silver impregnation for demonstration of axons in the skin in leprosy is described.
2. A new method of staining for demonstrating lepra bacilli inside the myelin sheath of nerves is described.