THE EFFECT OF FIXATIVES ON STAINING PROCEDURES FOR LEPRA BACILLI IN TISSUES[1]

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An essential to the study of the histiogenesis of leprosy consists of reliable demonstrations of the bacilli in tissues. In the older days, when celloidin embedding was more widely used, fair results were often seen. Paraffin embedding, however, has almost totally replaced celloidin in common practice, and the problem has become that of determining the conditions of fixation, dehydrating, and staining calculated to produce the best results.

In order to determine the ideal fixative, four of the ones commonly employed were tested on biopsy specimens of lepromatous tissues, by varying only the fixative and using the same methods of preparation of blocks and of staining. The fixatives tested were:

1. Formalin (37% formaldehyde) ..... 20 parts
   95% alcohol ..... 80 parts
2. Formalin ..... 20 parts
   Water ..... 80 parts
3. Bouin's solution:
   Saturated (1.2%) aqueous picric acid ..... 75 parts
   Formalin ..... 25 parts
   Acetic acid, glacial ..... 5 parts
4. Zenker's fluid:
   Potassium bichromate ..... 2.5 gm.
   Mercuric chloride ..... 5.0 gm.
   Acetic acid, glacial ..... 5.0 ml.
   Water ..... 100.0 ml.

The results are shown in Table 1. It was observed that Bouin's fluid is an extremely poor fixative for acid-fast bacilli, while the results with Zenker's fluid were distinctly superior to those obtained with the other fixatives, irrespective of the staining procedure employed. It is concluded that the picric acid in Bouin's fluid is responsible for the poor results which it gave, and that no fixative containing this substance should be used for leprous tissues.

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TABLE 1.—*Results of staining of acid-fast bacilli in paraffin sections after different fixatives.*

Fixative	Staining Method	
	Ziehl-Neelsen	Oil-fuchsin
Formalin-alcohol	Fair	Fair
Formalin	Fair	Good
Bouin's solution	Poor	Poor
Zenker's fluid	Very good	Excellent

Dehydration of the tissues employed in this study was carried out in graded alcohols, followed by clearing in benzene. As with other histologic procedures, it cannot be over-emphasized that thin blocks which require a minimal amount of time in the various fluids are ideal; with them the shrinkage is least and the staining effect best.

It has been found that leaving a small residuum of oil in the tissues during the staining procedure greatly improves the results. This "oil-fuchsin" method (1), developed from Faraco's (2) suggestion, produces brilliant staining of bacilli. Under ideal conditions the staining of the organisms takes place rapidly, and every one in the section can be stained. Hallberg's suggestion (3) of using Nachtblau, or its American equivalent Victoria Blue, has been tested but has not been found as satisfactory as the use of basic fuchsin.

CONCLUSIONS

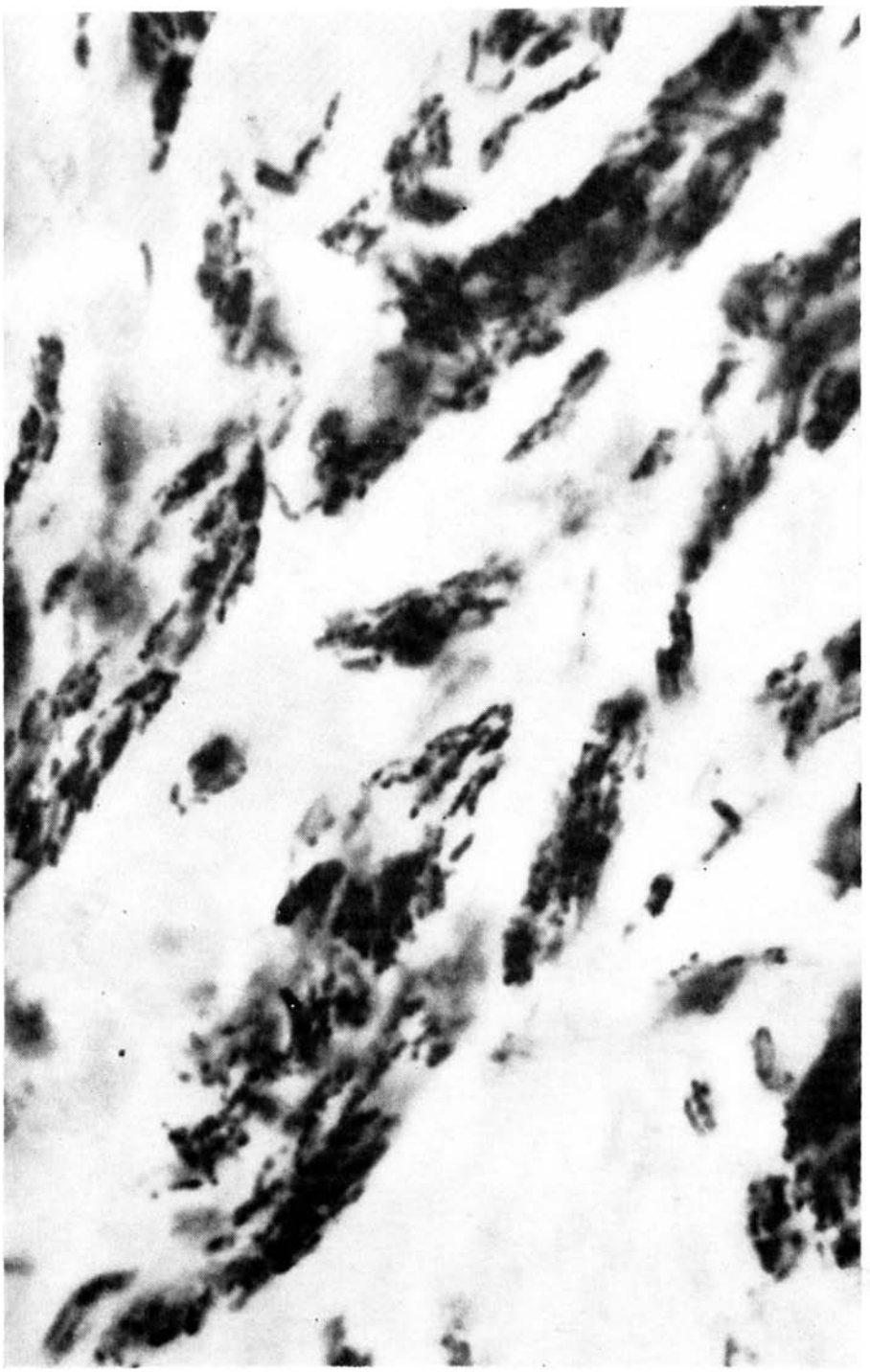
For the demonstration of lepra bacilli in paraffin sections the following are recommended:—(*a*) Fixation in Zenker's fluid; (*b*) use of thin blocks, with minimal time in dehydrating and clearing fluids; (*c*) staining by the oil-fuchsin method, and (*d*) mounting in one of the modern synthetic mediums, "clarite" or "permount," never in balsam.

REFERENCES

1. FITE, G. L., CAMBRE P. J. and TURNER, M. H. Procedure for demonstrating lepra bacilli in paraffin sections. Arch. Path. **43** (1947) 624—5.
2. FARACO, J. Rev. brasileira leprol. **6** (1938) 177.
3. HALLBERG, V. A new method for staining lepra bacilli. Internat. J. Leprosy **14** (1946) 67—73.

DESCRIPTION OF PLATE

Leproma stained by the oil-fuchsin method, lightly counterstained to emphasize the bacilli rather than the cells. Photographed at 900 diameters with subsequent enlargement to bring out details of bacilli.



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