Effect of decalcifying agents on the staining of *Mycobacterium tuberculosis*

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SYNOPSIS Lymph nodes from guinea pigs inoculated with *Mycobacterium tuberculosis* were fixed in buffered formalin, then treated for the recommended times in Gooding and Stewart's fluid, EDTA, aqueous nitric acid, von Ebner's fluid, and rapid decalcifier (RDC). The blocks were processed to paraffin wax and sections were stained by the Ziehl-Neelsen technique. Only in sections of the blocks treated with RDC were no acid alcohol fast bacilli demonstrable.

Hydrochloric acid is a known constituent of RDC and it was found that *Myco. tuberculosis* is altered by treatment with 2.5M solutions of hydrochloric acid and above and cannot subsequently be demonstrated by the Ziehl-Neelsen stain.

From these results it is recommended that calcified tissue from patients in whom there is a suspicion of tuberculosis should be decalcified with an agent other than RDC.

Rapid decalcifier (RDC)¹ is used in this laboratory for rapid routine decalcification of tissues before processing for histology. It was noted in a calcified cervical lymph node, showing the histological features of a tuberculous lymphadenitis, that after decalcification with this agent no acid and alcohol fast bacilli (AAFB) could be demonstrated in sections using the Ziehl-Neelsen technique. It was thought that RDC might impair the staining of *Mycobacterium tuberculosis* with carbol fuchsin.

Material and Methods

Lymph nodes from guinea pigs inoculated with *Myco. tuberculosis* were fixed in buffered formalin and processed to paraffin wax to act as a control, while other blocks were fixed and then treated for the recommended times in Gooding and Stewart's fluid, EDTA, aqueous nitric acid, von Ebner's fluid, and RDC (Culling, 1974). These blocks were then processed to paraffin wax. Other blocks treated with these agents were neutralized in 0.5M sodium sulphate overnight, then washed in running tap water for a further six hours before processing to paraffin wax.

Three sections from each block were stained by the Ziehl-Neelsen technique (Culling, 1974) and examined independently by both of us.

Results

Only in sections of the blocks treated with RDC were no AAFB demonstrable by the Ziehl-Neelsen technique; the bacilli in these sections were, however, stained with the methylene blue counterstain, showing that no basophilia had been lost.

Although the precise formula of RDC is uncertain, it is known that hydrochloric acid is a constituent. Therefore, RDC was diluted 1:10 with distilled water, and 10 ml aliquots were titrated against 0.1M sodium hydroxide using methyl orange indicator. The concentration of hydrochloric acid was found to be 4.06M. As von Ebner's fluid contains 0.152M hydrochloric acid and AAFB were demonstrable in tissue treated with this agent, further blocks were treated with a range of hydrochloric acid solutions for four hours. After treatment with solutions of 2.5M hydrochloric acid and above, no AAFB could be demonstrated.

A section from the control block was stained with hot carbol fuchsin, decolourized with RDC, and then counterstained with methylene blue. AAFB were stained, showing that RDC cannot alter the nature of these bacilli once they have been stained with carbol fuchsin.

Conclusions

Myco. tuberculosis is irreversibly altered by treatment with 2.5M solutions of hydrochloric acid and

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¹ RDC, Bethlehem Instrument Ltd, Hemel Hempstead.

above and cannot subsequently be demonstrated by the Ziehl-Neelsen stain.

Calcified lymph nodes are not uncommonly examined histologically in cases of suspected tuberculosis. From the results of the above experiments we would recommend that tissue from patients in whom there is a suspicion of this diagnosis should be decalcified with an agent other than RDC.

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Reference

Culling, C. F. A. (1974) *Handbook of Histopathological and Histochemical Techniques*, 3rd edition. Butterworth, London.

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