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The Use of Non-Deparaffinized Tissue Sections for Staining Leprosy Bacilli [1]

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The Ziehl-Neelsen stain is universally used for demonstrating mycobacteria. The acid-fast staining quality of mycobacteria, especially of leprosy bacilli, in paraffin embedded tissue sections is reduced or lost upon deparaffinizing the tissue sections in xylene and alcohols; the older and more decrepit bacilli being especially affected ([7]). The acid-fastness which has been extracted by ordinary xylene deparaffinization can be restored by heating and oil treatment ([1]). Fite *et al* ([3]) used a mixture of xylene and oil during dewaxing of paraffin sections to prevent diminution of the acid-fastness of leprosy bacilli. Wade ([7,8]) recommended the use of a mixture of turpentine and paraffin oil, since the xylene in Fite's mixture apparently accounted for some of the loss of acid-fastness of leprosy bacilli.

The method here presented permits intense staining of leprosy bacilli in non-deparaffinized sections when these bacilli were nonstaining or weakly acid-fast with the usual carbol fuchsin stain in deparaffinized sections.

MATERIALS AND METHODS

Materials. The tissues used were human leprosy biopsied nodules where leprosy baccilli were found to be few or absent with the usual Ziehl-Neelsen procedure. The tissues were fixed in 10% formol for 24 hours, dehydrated, blocked in paraffin and sectioned at six microns.

Staining procedures.

1. Non-deparaffinized sections are stained in carbol fuchsin, carbol night blue or carbol Victoria blue B for 60 minutes at room temperature. Carbol fuchsin solution is prepared in the usual manner; to 10 ml of saturated alcoholic solution of diamond fuchsin (C.I. 42510, Chroma lot No. 7002) add 90 ml of 5% aqueous phenol solution. Carbol night blue (C.I. 44085, Chroma, 5008) or carbol Victoria blue B (C.I. 44045, Chroma, 0703) solution is prepared as 0.5 gm of the respective dye dissolved in 10 ml of 95% ethanol with 90 ml of 5% phenol added in each instance.

1a. Alternatively, non-deparaffinized sections are treated with 10% periodic acid aqueous solution for 24 hours at room temperature, rinsed in tap water and then stained in carbol pararosanilin for one hour. The carbol pararosanilin solution is prepared as follows: 0.5 gm of pararosanilin HCl (C.I. 42500, Chroma, 7007) dissolved in 10 ml of ethanol with 90 ml of 5% phenol added.

2. Tap water rinse.

3. Differentiate in 1% HCl-70% ethanol, until the tissue sections turn faint pink. This requires a few minutes.

4. Wash in tap water.

5. Dip sections in Löeffler's methylene blue diluted 1:9, or in 0.5% aqueous malachite green or, if a blue dye is used as the primary stain, dip in dilute aqueous pyronin Y for a few minutes.

6. Rinse in tap water.

7. Blot with filter paper and dry in the air.

8. Clear in xylene (in this step, paraffin is removed from the sections) and mount in a resin (we used HSR).

RESULTS

The section of lepromatous leprosy in which leprosy bacilli are few or absent with the usual carbol fuchsin stain (Fig. 1A) shows many strongly stained bacilli (Fig. 1C). After prolonged periodic acid oxidation, more strongly stained and more numerous bacilli are demonstrated by carbol fuchsin, especially carbol pararosanilin, than without such oxidation (Fig. 1E).

The results of these procedures, however, tend to be irregular and patchy in staining. The non-deparaffinized sections of leprosy tissue can, alternatively, be stained by periodic acid-methenamine silver stain for demonstrating mycobacteria (Fig. 1F) ([4,5]). With this procedure, leprosy bacilli are stained black as rod forms. Elastic fibers are

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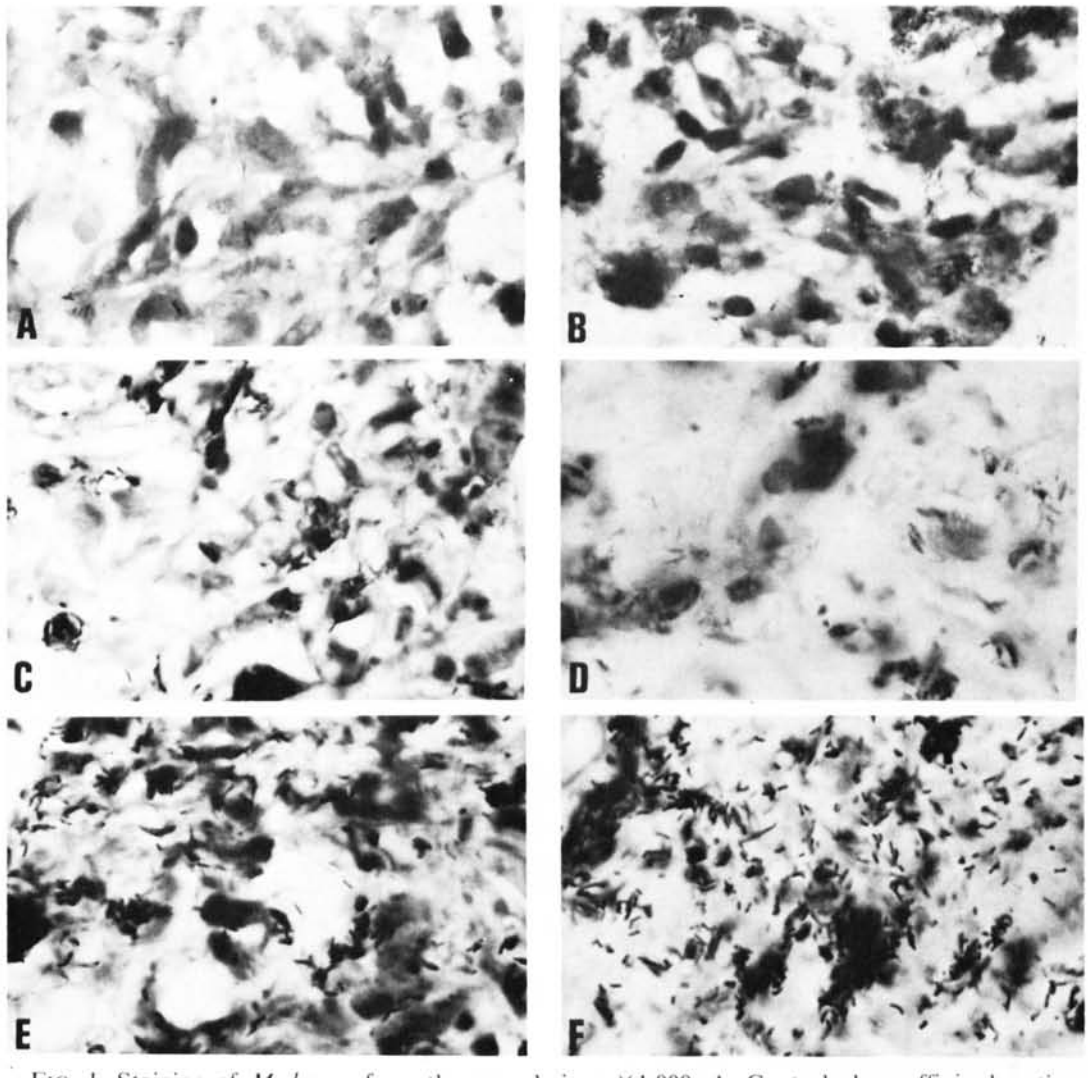

FIG. 1. Staining of *M. leprae* from the same lesion, ×1,000. A. Control, deparaffinized section, carbol fuchsin stain. B. Control, Fite's oil-carbol fuchsin stain. C. Non-deparaffinized section, carbol fuchsin stain. D. Non-deparaffinized section, carbol Victoria blue B stain. E. Non-deparaffinized section, periodic acid-carbol-pararosanilin stain. F. Non-deparaffinized section, periodic acid-methenamine silver stain.

also stained black in contrast to the unstained connective tissues.

DISCUSSION

Acid-fastness has been thought to signify the presence of unsaturated fatty acids of high molecular weight ([2]). These lipids account for the acid-fastness of the cells and are bound or complexed with the cellular components.

Leprosy bacilli, especially older and more decrepit bacilli, sometimes fail to stain by the usual carbol fuchsin stain. Moreover, it is apparent that, at least in fixed paraffin embedded tissue sections, the acid-fast lipids, especially of leprosy bacilli, are removed to some extent by xylene ([7]).

These experiments demonstrate that the carbol fuchsin procedure with non-deparaffinized sections reveals many more bacilli than are demonstrated by the same method in deparaffinized sections. This fact indicates that the protection of acid-fast staining is relatively complete in non-deparaffinized sections. However, the protection is not always complete in the oil Ziehl-Neelsen

method ([3]), for some decrepit bacilli may be extracted (Fig. 1B).

Aqueous night blue or Victoria blue B can be substituted for carbol fuchsin in the acid-fast stain ([4,5]). However, it is necessary to add phenol to the staining solution when using this stain on non-deparaffinized sections, because the use of aqueous night blue or the Victoria blue B methods alone give no staining or only inferior results in the demonstration of mycobacteria (Fig. 1D).

Prior prolonged periodic acid oxidation enhances the staining ability of carbol fuchsin for mycobacteria ([6]) and gives positive methenamine silver stain ([4,5]).

The experiments showed that periodic acid carbol pararosanilin or methenamine silver stain can be used on non-deparaffinized sections. Study of the effects of prior periodic acid oxidation in association with the carbol pararosanilin or methenamine silver methods are in progress. These procedures are equally effective with *M. lepraemurium* and also provide good staining of tubercle bacilli.

SUMMARY

Reduced acid-fast staining of leprosy bacilli occurs during the dewaxing of paraffin sections by xylene and alcohols; the older and more decrepit bacilli being especially affected. By the use of non-deparaffinized sections, the leprosy bacilli which could not be stained with the usual carbol fuchsin are strongly stained. Moreover, non-deparafinized sections can be used for the periodic acid-carbol pararosanilin stain or methenamine silver stain for demonstrating mycobacteria.

RESUMEN

Una reducción de la tinción ácido-resistente de los bacilos leprosos ocurre durante la desparafinización de las secciones con xilol y alcoholes; los bacilos mas viejos y decrépitos son especialmente afectados. Mediante el uso de las secciones no-desparafinizadas, los bacilos leprosos que no pueden ser teñidos con carbol-fuchsina se tiñen intensamente. Además, las secciones no-desparafinizadas pueden ser usadas para la tinción con el ácido peryódicocarbol pararosanilina o con el método argéntico para la demonstración de micobacterias.

RÉSUMÉ

Lorsque l'on procède au déparaffinage de coupes histologiques par le xylène et par l'alcool, il se produit une réduction de la coloration acido-résistante des bacilles de la lèpre. Les bacilles âgés et morphologiquement décrépits sont particulièrement affectés. En utilisant des coupes non-déparaffinées, on peut colorer de façon très forte des bacilles de la lèpre qui n'avaient pu être colorés avec la fuchsine habituelle. De plus, des coupes non-déparaffinées peuvent être utilisées pour procéder à la coloration par de la pararosaniline périodique acide carbonique ou par de la méthénamine argentique, et ceci en vue de mettre en évidence les mycobactéries.

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