A Modified Allochrome Procedure for Demonstrating Mycobacteria in Tissue Sections[1]

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The carbol fuchsin stain is commonly used for demonstrating mycobacteria. Prior prolonged periodic acid oxidation enhances the staining affinity of carbol fuchsin for mycobacteria including leprosy bacilli ([2,3,6]). The possibility of replacing Schiff's reagent with basic fuchsin for demonstrating tissue aldehydes has been investigated by some authors ([1,4]). The periodic acid Schiff method is used for demonstrating collagen, reticulum and basement membranes. The allochrome procedure ([5]) is a useful variant, specifically valuable for the differentiation of the connective tissues. After many trials, we recommended the following modification of the allochrome procedure for selective demonstration of mycobacteria in tissue sections.

MATERIALS AND METHODS

The tissues used were human leprosy nodules, tuberculous lesions of human lungs and murine leprosy lesions. The tissues were fixed in 10% formol for 24 hours, dehydrated, blocked in paraffin and sectioned at 5μ in the usual manner. Tuberculous lesions containing chromophobic bacilli were obtained through the generosity of Dr. W. Nyka, V.A. Hospital, Baltimore, Maryland.

Staining procedure.

1. Bring sections to water.
2. Oxidize in 10% aqueous periodic acid for 24 hours (overnight).
3. Wash in running water, five minutes.
4. Treat with carbol pararosanilin (C.I. 42500), one hour. Carbol pararosanilin formula was prepared as follows: 1 gm pararosanilin HCl, C.I. 42500, Chroma 7007 dissolved in 10 ml absolute ethanol with 10 ml of aqueous 5% phenol added.
5. Rinse in water.
6. Differentiate in 1% HCl-70% ethanol, until the tissue sections turn faintly pink, usually in a few minutes.
7. Wash in tap water for five minutes.
8. Stain in Weigert's acid iron hematoxylin for two minutes (or omit this step).
9. Wash in tap water for five minutes.
10. Stain in 0.04% methyl blue (C.I. 42780) in saturated picric acid aqueous solution for six minutes.
11. Dehydrate and differentiate in two changes each of 95% and absolute ethanol.
12. Clear in xylene and mount in a resin (we used HSR).

RESULTS

Nuclei, gray to black; cytoplasm and muscle cells, grayish-green to greenish-yellow; collagen and most reticulum, blue; muscle reticulum and epithelial basement membrane, reddish-purple; PAS-positive material, red; mycobacteria, brilliant red. If the nuclear stain is omitted, mycobacteria are more brilliantly stained. With usual Ziehl-Neelsen or Fite stain, leprosy bacilli are found in the subcutaneous cellular infiltrate, particularly within the histiocytes and lepra cells in lepromatous skin lesions. With the modified allochrome stain, the bacilli are found in epidermis, dermis (containing nerves, blood vessels and arrectores pilorum muscles), and even subcutaneous fat as well as in the infiltrate. Leprosy bacilli are more intensely stained and more numerous than that with the usual Ziehl-Neelsen stain. The findings may indicate the presence of chromophobic bacilli. In a tuberculoid leprosy lesion, the infiltrate is composed of epithelioid cells with a few giant cells and a mild lymphocytic admixture. With carbol fuchsin, there is scarcity or absence of bacilli. With the modified allochrome stain, there are many acid-fast granules of various sizes and a few bacilli in the infiltrate. The chromophobic tubercle bacilli which did not stain with the carbol fuchsin stain could be strongly stained by this method.

DISCUSSION

Acid-fastness has been thought to signify the presence of acid-fast wax. Leprosy bacilli are usually weakly acid-fast. Therefore, in

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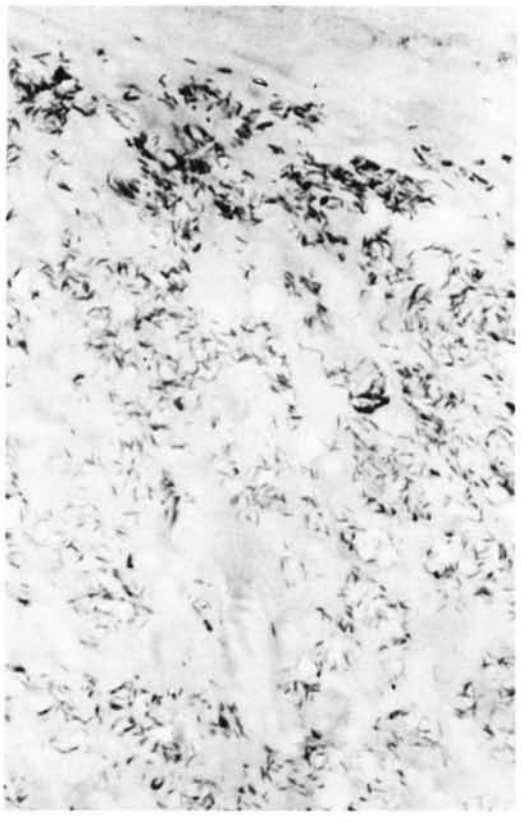

FIG. 1. Lepromatous nodules with the usual carbol fuchsin stain. ×250.

FIG. 2. Lepromatous nodules with the modified allochrome procedure in a sister section. ×250.

the usual carbol fuchsin procedure, the leprosy lesion should be decolorized slightly. Some authors recommend the oil fuchsin method for demonstrating leprosy bacilli in tissue sections ([7]). With this procedure the bacilli are sometimes not in their original position, but just as often they are observed to occupy new positions. The acid-fast and even chromophobic tubercle bacilli can be made fully acid-fast by prolonged periodic acid oxidation; special fixatives containing H_2O_2 or periodic acid are essential for this procedure ([6]).

The modified allochrome procedure can be used on formalin fixed tissues and both acid-fast and chromophobic mycobacteria tested can be made to stain a brilliant red in contrast with allochrome-stained background tissues. Moreover, the procedure is the most sensitive and reliable method for demonstrating weakly acid-fast and chromophobic bacilli such as leprosy bacilli in tissue sections. The principle mechanism of this stain is the prolonged periodic acid oxidation and subsequent staining with carbol pararosaniline.

Arylamines condense readily with aldehydes to form Schiff bases: thus, $RCHO + 2ArNH_2 \rightarrow RCH(NHAr)_2$. Schiff bases are quite resistant to acid ([4]). Aldehydes are regarded as being produced from 1-hydroxy-2-amino groups as in sphingomyelin ([8]).

Further studies of the mechanism of this procedure and its practical use in leprosy lesions are continuing.

SUMMARY

A modified allochrome staining procedure is presented as being the most reliable and sensitive method for demonstrating mycobacteria in tissue sections. The technic is as follows: Deparaffinize formalin fixed sections, oxidize in 10% periodic acid for 24 hours, differentiate in 1% HCl-70% ethanol, stain in Weigert's iron hematoxylin nuclear stain, and counterstain in picro-methyl blue. Mycobacteria stained brilliant red in contrast with the allochrome-stained background tissues, and apparently otherwise chromophobic bacilli are demonstrated.

RESUMEN

Se presenta un procedimiento modificado de tinción alocrómica como el método más confiable y sensible para la demostración de micobacterias en cortes histológicos. La técnica consiste en la desparafinación de los cortes fijados con formalina, la oxidación con ácido peryódico durante 24 horas, la diferenciación con una mezcla de etanol (70%) y ácido clorhídrico (1%), la tinción con hematoxilina férrica de Weigert, y la tinción con picro-azul de metileno como coloración de contraste. Las micobacterias se tiñen de un color rojo brillante que contrasta con el fondo teñido por el alocromo.

RÉSUMÉ

Un procédé modifié de coloration allochrome est présenté ici. Il est considéré comme la méthode la plus fiable et la plus sensible pour mettre en évidence des mycobactéries en coupes de tissus. La technique est la suivante: déparaffinisation des coupes fixées au formol, oxydation dans l'acide periodique à 10% pendant 24 heures, différenciation dans une solution d'HCl à 1% dans l'éthanol à 70%, coloration par le colorant nucléaire à l'hématoxyline et au fer de Weigert, et coloration de contraste au bleu de picro-méthylène. Les mycobactéries se colorent en rouge brillant, sur le fond constitué par les tissus colorés par l'allochrome. Des bacilles qui autrement résistent à la coloration peuvent être mis ainsi en évidence.

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