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Detection of Mycolic Acid in Leprous Tissue[1]

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The detection of α,ϵ-diamino-pimelic acid (DAP) in a caseous tuberculous nodule and in a sample of noncaseous tuberculous lung tissue was first described by Consden and Glynn ([2]). The bacterial residues, as measured by DAP, represented some 20% of the dry weight of the caseous lesion despite the absence of living tubercle bacilli in the nodule. Consden and Howard ([3]) improved the procedure and reported a lower limit of detection of DAP of 0.5 to 1.0 μg.

Nethercott and Strawbridge ([6]) extended the procedure by extracting a chloroform-soluble wax together with DAP from sarcoid tissue. They melted the wax on filter paper, stained the impregnated paper with hot carbol fuchsin and stated that because the spot was both acid- and alcohol-fast it contained mycolic acid. Since DAP and mycolic acid were non-mammalian substances, they concluded that sarcoidosis was a manifestation of tuberculosis.

Diaminopimelic acid is widely distributed among bacteria ([4, 7, 9]) and is not in itself indicative of mycobacterial origin. The detection of mycolic acid in a lesion, however, would be an additional indicator in the diagnosis of mycobacterial infections.

MATERIALS, METHODS, AND RESULTS

Preparation of standard mycolic acid. Mycobacterial peptidoglycolipid was prepared from *M. tuberculosis* strains C, DT, and PN by the method described by Stewart-Tull and White ([8]). Peptidoglycolipid (1.0 g) was dissolved in 5.0 ml boiling benzene, and 5.0 ml of 5% methanolic KOH was added. The mixture was boiled for two minutes, and the precipitate which formed was filtered off and washed with boiling methanol. After extraction with water to remove the glycopeptide moiety, the residue was extracted with boiling acetone until the extract was colorless. The residue was extracted with ether repeatedly, and the ether solution was evaporated to dryness to yield mycolic acid.

Chloroform extraction of tissue obtained *post-mortem* from a patient with lepromatous leprosy and from tuberculous animals. Samples of tissue were minced with scissors and homogenized in a high-speed multi-purpose laboratory mixer (Silverson Machines Ltd., London) in distilled water. An equal volume of spectroscopic grade chloroform (B.D.H. Chemicals Ltd., England) was added to the homogenate, and the mixing was continued for 3 to 5 minutes. The chloroform phase was decanted off, centrifuged at 3,500 rpm for 10 minutes to remove debris and evaporated to dryness. The chloroform-soluble material was resuspended in 0.2 to 0.5 ml spectroscopic grade chloroform.

Thin layer chromotography. Glass plates, 20 × 20 cm, were thoroughly cleaned with alcohol and placed in a Shandon Uniplan plate spreader. A slurry of 40 g silica gel G (Merck, Germany) and 80.0 ml distilled water was prepared, and the plates were coated using the spreading device. The thin layers were allowed to dry before activation in an oven at 105°C for 30 minutes. The plates were stored in a desiccator cabinet until required.

Commercial thin layer plates (Polygram Sil G: Camlab Instruments, Cambridge) were also used in this study for comparison.

The tissue extracts and mycolic acid in chloroform were spotted onto the thin layer plates using 5 μl Microcaps (Drummond Scientific Co., U.S.A.). The plates were developed with undiluted spectroscopic grade chloroform ($CHCl_3$) or di-isobutylketone ($C_9H_{18}O$) (Hopkins and Williams, England). When the solvent reached the 15.0 cm mark, the plates were dried, and the lipid spots were visualized either by

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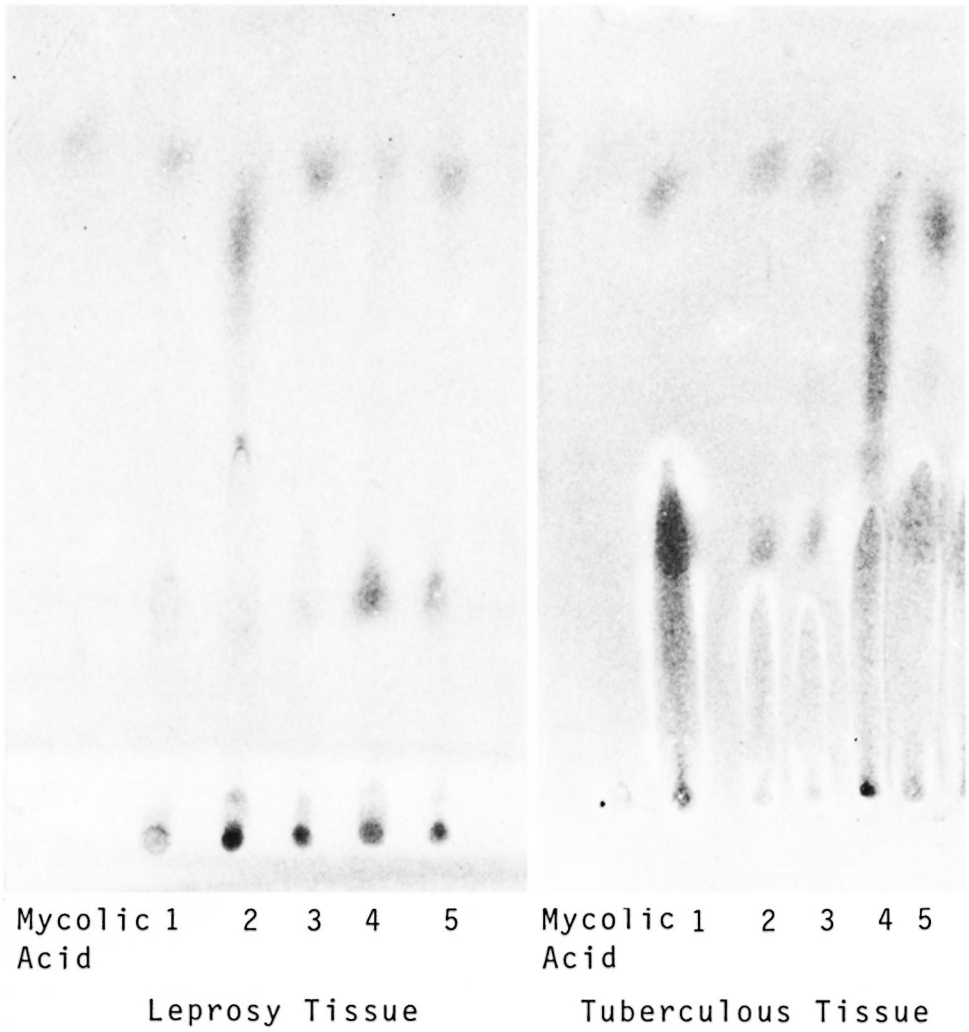

THE FIGURE. Thin layer chromatographs of 5 chloroform extracts of tissues from a lepromatous leprosy patient and 5 from tuberculous animals compared to mycolic acid.

charring with a spray of 50% H_2SO_4 or by spraying with 0.012% Rhodamine 6G (B.D.H. Chemicals Ltd.).

In two-dimensional thin layer plates it was noticed that mycolic acid produced a single spot with R_f values of 0.93 for $CHCl_3$ and 0.95 for $C_9H_{18}O$. In a unidimensional silica gel G plate it was found that spots from five $CHCl_3$ extracts of lepromatous tissue corresponded to the standard spot of mycolic acid (The Figure). A similar result was obtained with $CHCl_3$ extracts of tuberculous tissue. There was no significant difference between the prepared and commercial silica gel G plates.

Infrared spectroscopy. The sample of purified mycolic acid was scanned in an Infrascan H901 (Rank Hilger) over the wavelength range of 2.5 to 15.0 μm; the preparations were examined as smears between sodium chloride discs. For comparison, a thin layer plate was set up with 5 cm bar origins using a $CHCl_3$ extract of leprosy tissue and standard mycolic acid. A strip of the plate was developed with Rhodamine G to determine the position of mycolic acid and the corresponding spot for the leprosy chloroform extract. The remainder of the undeveloped area for these spots was scraped off the plate into a bijou bottle and extracted with $CHCl_3$. The extracts were dried down on sodium chloride discs and scanned in the Infrascan. It was noticed that similar absorptions were found for the

TLC chloroform extract of the mycolic acid spot from the tissue as shown for the mycolic acid standard except that there was some evidence of hydroxyl ions absorbing between wave numbers 3200 and 3600. It was concluded that this was due to moisture in the thin layer base since the absorption was found with both standard mycolic acid and the chloroform extract of lepromatous tissue.

DISCUSSION

The presence of a mycolic acid in tissue is not in itself specific and diagnostic for leprosy since mycobacterial, corynebacterial, and nocardial mycolic acids have been described ([1,5]). If used in conjunction with clinical evidence, the finding of diaminopimelic acid and mycolic acid in biopsy tissue could be a useful additional qualitative test in the diagnosis of leprosy, even though it would require TLC apparatus.

SUMMARY

A method is described for the extraction of mycolic acid from lepromatous leprosy tissues and from tuberculous tissues and its identification by thin layer chromotography. Verification of the mycolic acid extracted and separated by thin layer chromotography was accomplished by infrared spectroscopy.

Although the presence of mycolic acid in tissues is not, in itself, diagnostic for leprosy, its demonstration in biopsy tissues might be a useful additional qualitative test if used in conjunction with clinical evidence of the disease.

RESUMEN

Se describe un método para la extracción de ácido micólico a partir de tejidos lepromatosos y tuberculosos, y para su identifación por cromatografía en capa delgada. La verificación de los ácidos micólicos extraídos y separados por cromatografía en capa delgada se hizo por espectroscopía al infrarojo.

Aunque la presencia de ácido micólico en los tejidos no es en si misma diagnóstoco de lepra, su demostración en biopsias puede ser una útil prueba cualitativa adicional si se usa junto con la evidencia clínica de la enfermedad.

RÉSUMÉ

On décrit ici une méthode pour l'extraction d'acide mycolique à partir de tissus de lèpre lépromateuse et de tissus tuberculoides, et pour l'identification de cet acide par la chromatographie en couches minces. L'identification certaine de l'acide mycolique extrait et séparé par la chromatographie en couches minces a été effectuée par spectroscopie en infrarouges.

Malgré que la présence d'acide mycolique dans les tissus ne soit pas, en elle-même, un critère diagnostique de lèpre, la démonstration de celui-ci dans les tissus de biopsies peut constituer une épreuve qualitative complémentaire utile, lorsqu'on l'utilise conjointement avec les signes cliniques de la maladie.

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REFERENCES

1. Asselineau, J. *Les Lipides Bactériens*. Paris: Hermann, 1962.
2. Consden, R. and Glynn, L. E. Chemically identifiable bacterial residues in lung lesions. Lancet **1** (1955) 943–945.
3. Consden, R. and Howard, A. Improved methods for detecting α,ε-diaminopimelic acid in lung lesions. J. Clin. Pathol. **10** (1957) 178–181.
4. Cummins, C. S. and Harris, H. The chemical composition of the cell wall in some Gram-positive bacteria and its possible value as a taxonomic character. J. Gen. Microbiol. **14** (1956) 583–600.
5. Lederer, E. Chemistry and biochemistry of some biologically active bacterial lipids. Pure Appl. Chem. **2** (1961) 587–605.
6. Nethercott, S. E. and Strawbridge, W. G. Identification of bacterial residues in sarcoid lesions. Lancet **2** (1956) 1132–1134.
7. Salton, M. R. J. *The Bacterial Cell Wall*. London: Elsevier, 1964.
8. Stewart-Tull, D. E. S. and White, R. G. The influence of age of culture on the production of adjuvant-active peptidoglycolipids by saprophytic mycobacterium. Immunology **12** (1967) 349–359.
9. Work, E. and Dewey, D. L. The distribution of α,ε-diaminopimelic acid among various micro-organisms. J. Gen. Microbiol. **9** (1953) 394–409.