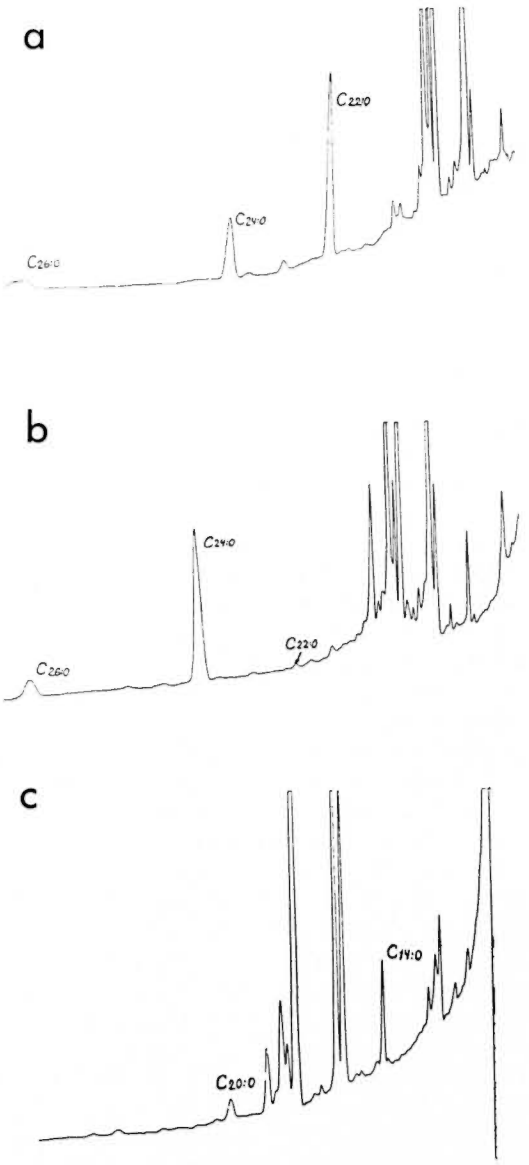
Assessment of the Purity of *M. leprae* Preparations from Tissues of Leprosy-Infected Laboratory Animals

TO THE EDITOR:

Mycobacterium leprae isolated from the tissues of experimentally infected animals must be checked for tissue contamination if they are destined for diagnostic or vaccine preparations. Available methods for assessment of *M. leprae* purity at different steps of their purification, such as light and electron microscopy ([4]), immunoblotting and ELISA (for detection of armadillo liver protein) ([7]), electrophoresis of *M. leprae* sonicates (determination of electrophoretic mobility of host malate dehydrogenase) ([9]), or skin tests on guinea pigs presensitized with tissues from intact animals ([3]), are either of low sensitivity ([9]) or are complicated, multistep and time-consuming ([3, 8, 9]).

We propose to assess the purity of *M. leprae* isolated from the tissues of experimentally infected animals by using a method of gas chromatography of copyrolysates of mycolic acids forming a part of mycobacterial cell walls ([1]). Esterified residues of the side chain of destructed mycolic acids extended by two carbon atoms eluate from the column. Previously we have shown ([6, 7]) that mycolic acid residues with 22, 24, 26, and 28 atoms of carbon were detected on copyrolytic chromatograms of *M. leprae* only; whereas on chromatograms of normal animal tissues mycolic acids and other long-chain components (with over 20 carbon atoms) were absent. Thus, the presence of short-chain components on the copyrolytic chromatograms of *M. leprae* undergoing purification may suggest host tissue contamination of the mycobacterial preparation. On copyrolytic chromatograms of *M. leprae* passed on mice and rats, tetracosanic acid (C24:0) prevails; whereas in *M. leprae* isolated from human lepromas and in *M. leprae* once passed on armadillos, behenic acid (C22:0) predominates among mycolic acids (The Figure).

For a quantitative assessment of the extent of tissue contamination of *M. leprae* preparations, we propose to determine the ratio between the amounts of the mycolic



THE FIGURE. Chromatograms of methyl ethers of fatty acids of *M. leprae* and foot pad tissue from mice: a) *M. leprae* once passed on armadillo and purified from infected spleen, peak of behenic acid (C22:0) prevails; b) *M. leprae* (3rd passage) isolated from mouse foot pads, peak of tetracosanic acid (C24:0) prevails; c) foot pads of intact mice, peak of tetradecanoic acid (C14:0).

THE TABLE. *Results of gas chromatographic assessment of host-tissue contamination of* M. leprae *isolated from leprosy-infected animals.*

Tissue	No. samples of purified *M. leprae* preparations	Ratio of mycolic acid : tetradecanoic acid	Method of purification
Mouse foot pad	190, 225, 240	2.0, 3.0, 2.8	Prabhakaran
Rat foot pad	260, 329, 333	4.0, 4.3, 3.7	Prabhakaran
Mouse foot pad	224, 299	9.2, 8.4	Draper 1[a]
Armadillo spleen	350	9.7[b]	Draper 1
Armadillo leproma	334, 349	8.6,[b] 7.0[b]	Draper 1
Rat foot pad	211	15.2	Draper 2[c]
Armadillo leproma	310	10.1[b]	Draper 2
Armadillo spleen	319	11.1[b]	Draper 2

[a] Variant 1: purification was ended at the step of separation of *M. leprae* suspension in Percoll gradient.
[b] Values of C22:0/C14:0 ratios; other values in this table express a ratio of C24:0/C14:0.
[c] Variant 2: purification was ended by separation of *M. leprae* in two-phase polymeric system.

acid prevailing in a given mycobacterial strain and tetradecanoic acid (C14:0), the content of which is approximately equal in animal tissue and in mycobacteria (1%–2% of total acids) and the ratio of tetradecanoic acid in mycobacteria to that in animal tissues therefore may be assumed as 1.

Infected foot pads of mice and rats and spleens and lepromas from experimentally infected armadillos were used. *M. leprae* were purified from the tissues according to the methods proposed by Prabhakaran ([5]) and Draper ([2]). Draper's purification of a part of the preparations was ended at the stage of separation in a gradient of Percoll (variant 1), while the remaining preparations were put through all the steps of purification including the two-phase polymeric system (variant 2).

The copyrolytic method ([1]) allows one to obtain methyl ethers of fatty acids without previous lipid extraction of the bacterial mass, thus cutting analysis time to 1–2 hr.

Purified *M. leprae* (5–10 mg) were placed in a glass ampoule into which 0.1–0.2 ml of a 7% solution of tetramethyl ammonium hydroxide in 80% aqueous methanol was added. The ampoule was soldered and heated at 120°C–130°C for 20–30 min. The sample took on the appearance of a transparent or slightly opalescent solution. Then 2–3 ml of the solution was introduced into the evaporator of a gas chromatograph with a flame-ionization detector and steel columns (1.5–2 m × 3 mm internal diameter), packed with celite 545 (60–80 mesh) and with 7% apieson L as a liquid phase. Analytical conditions were as follows: the temperature of the column thermostat was programmed from 170°C to 270°C at a rate of 5°C/min; the temperature of the injector was held at 360°C; argon was used as a gas carrier; argon and hydrogen flow was 2.4 l/hr, air flow was 25 l/hr.

The Table shows the results of gas chromatographic assessment of host tissue contamination of *M. leprae* as judged by the ratio of the prevailing mycolic acid to tetradecanoic acid. The degree of tissue contamination varied from 4.3 to 15.2, depending on the method of purification. The highest values corresponded to *M. leprae* preparations purified by Draper's method (variant 2), involving separation in a two-phase polymeric system. The low indices were attributed to *M. leprae* preparations purified by Prabhakaran's method.

It is important that a choice of optimal ratios of the prevailing types of mycolic acids (in our case, C24:0 and C22:0) to tetradecanoic acid (C14:0) depends on the tasks of the experiments and the demands for purity of the preparation.

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