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Collection Method for *Mycobacterium leprae* from Infected Armadillo Liver[1]

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The method of collection of *Mycobacterium leprae* is an important problem in the study of leprosy. Many researchers have tried to collect a purified leprosy bacillus fraction. Elliston ([1]), Draper (1980, personal communication), Rees ([6]), and Prabhakaran ([5]) reported collection methods using their own special procedures. Miller, *et al.* ([2]) reported that protease, which was contained in the tissue extract, and added trypsin, destroyed some protein fractions of *M. leprae*. We followed Draper's rather complicated collection method and collected a bacillary fraction that was not quite free from tissue contaminants. We have now devised a simple and effective collection method for *M. leprae* from infected armadillo liver.

MATERIALS AND METHODS

M. leprae-infected armadillo liver was supplied to our laboratory by IMMLEP with the help of the Sasakawa Health Foundation. Twenty grams of armadillo liver were homogenized with a twofold volume of distilled water in a Waring blender for 2 min at 20,000 rpm. The homogenate was filtered through gauze. Each Percoll solution of 40–100% concentration was layered slowly into a cellulose nitrate tube (Figure 1) and 6.5 ml of liver homogenate was slowly added. These tubes were centrifuged for 1 hr at 100,000 × *g* (27,000 rpm) in a Beckman Ultracentrifuge, Swing Rotor Type 27. Leprosy bacilli which distributed in the Percoll gradient were collected, through a hole made in the bottom of the tube, and divided into four fractions. Each fraction was washed with distilled water and centrifuged at 20,000 × *g* to remove Percoll. The tissue contaminants in the bacterial fraction free from Percoll were estimated by Ziehl-Neelsen and Ziehl-Nile blue ([4]) staining, and a bacterial count was carried out to calculate the yields by a modified method of Shepard ([3]).

RESULTS

A centrifuged tube is shown in Figure 2 and schematically in Figure 3. The bottom of the tube is a zone of concentrated Percoll. This zone is highly viscous and could not be taken out through the hole at the bottom of the tube. In the middle zone, Fraction 1 is pure leprosy bacilli, free from tissue contaminants by Ziehl-Neelsen and Ziehl-Nile blue staining, and the yield is 46.7% of the leprosy bacilli in the liver homogenate as shown in The Table. In the upper zone, Fraction 2 contains a few tissue contaminants; in the borderline zone, Fraction 3 contains many tissue contaminants. Fraction 4 is a tissue fraction. Bacterial counts of Fractions 2, 3, and 4 are shown in The Table. We tried to separate the tissue contaminants from Fractions 2, 3, and 4 by repeated Percoll gradient centrifugation.

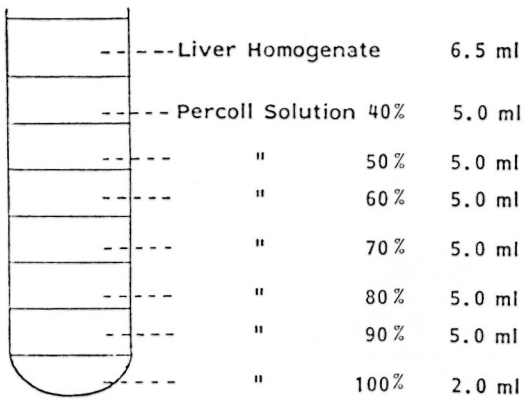

FIG. 1. Schematic drawing of preparation of Percoll gradient.

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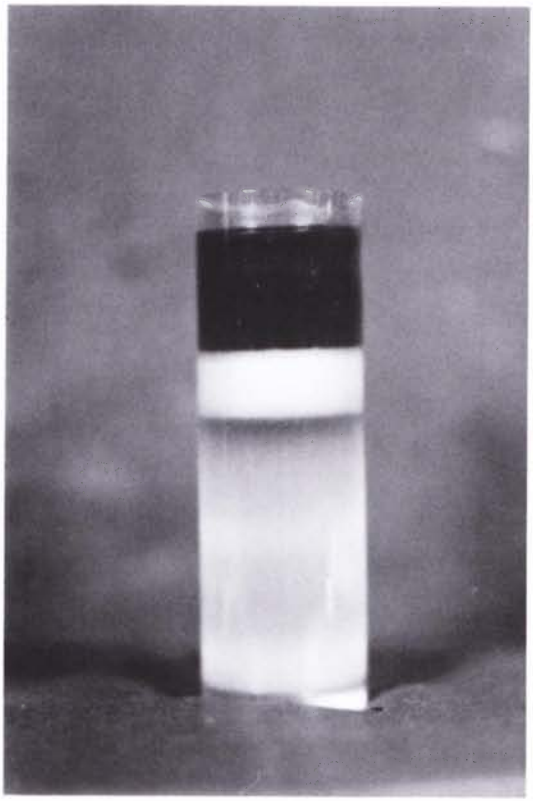

FIG. 2. Photograph of preparation after ultracentrifugation.

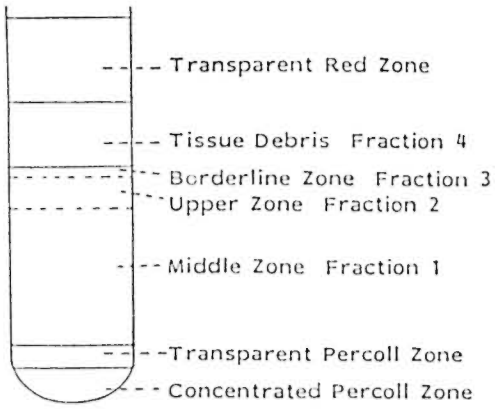

FIG. 3. Schematic drawing of preparation after ultracentrifugation.

However, the second centrifugation was not effective, and we could not obtain a pure leprosy bacillus fraction from either Fraction 2 or Fraction 3.

DISCUSSION

Tissue contaminants in the bacterial fraction could be measured enzymatically ([4]). AMPase, one of the lysosome enzymes, was measured but both the activities of the bacterial fraction and the liver homogenate were very weak. The armadillo liver used in this experiment seemed to have been stocked frozen for a long time, so we were not able to measure the tissue component by the enzymatic method. We could not use the Percoll gradient method in the case of infected foot pads from nude mice because of polymerization of the Percoll solution by fresh mouse homogenate. Since the polymerized Percoll and the leprosy bacilli could not be separated again, valuable leprosy bacilli had to be discarded. We do not know if fresh armadillo liver polymerizes Percoll solutions or not. Nevertheless precautions against polymerization of Percoll with fresh tissue extracts must be taken.

THE TABLE. *Yield of* M. leprae *from leprosy-infected armadillo liver.*

Material	No. bacilli per 1 g liver	Yield
Liver homogenate	4.5×10^9	100.0%
Fraction 1	2.1×10^9	46.7%
Fraction 2	1.1×10^9	24.4%
Fraction 3	1.9×10^8	4.2%
Fraction 4	4.4×10^8	9.8%
Total		85.1%

SUMMARY

Leprosy bacilli were separated from infected armadillo liver almost free from tissue contaminants by a Percoll gradient centrifugation. The yield of bacilli was 46.7%. This is a very simple and effective method without enzyme treatment.

RESUMEN

Por centrifugación en gradiente de Percoll se separaron bacilos de la lepra a partir de hígado de armadillo en forma casi libre de contaminantes tisulares. La recuperación de bacilos fue del 46.7%. Este es un método muy simple y efectivo que no utiliza tratamientos enzimáticos.

RÉSUMÉ

Des bacilles de la lèpre ont été isolés à partir de foie infecté de tatou, quasiment sans aucun contaminant

tissulaire, grâce à une centrifugation sur gradient de Percoll. Le taux de recouvrement de bacilles a été de 46.7%. Cette méthode, qui ne nécessite pas un traitement enzymatique, est fort simple et efficace.

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