fast bacteria, especially tubercle bacilli. The suitability of this method for cultures of *Myco. leprae* has been confirmed by Küster[3] and for leproma smears by Kline and Leach.[4]

Recently one of us (H.J.H.) has investigated the separation and concentration of acid-fast organisms from the tissues of leprosy patients. To cryochemed spleen† distilled water was added and direct impression smears were made from a piece of the tissue. One set of smears was stained with auramin O and examined according to the fluorescence procedure described by Richards and Miller.[5]

A representative field is shown in the accompanying photomicrographs. The faint material in the background is non-acid-fast splenic cellular substance. The acid-fast bacilli have a characteristic granular appearance.

Although no accurate comparison was made with Ziehl-Neelsen stained smears, the fluorescence technic was clearly superior for demonstrating globi and leprosy bacilli. It is of interest to note that Richards, Kline and Leach[6] believed more tubercle bacilli could be demonstrated in the same microscopic fields by fluorescence than by the conventional method.

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A Method for Separating Intact Leprosy Bacilli from Leprous Tissue.*

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In a recent investigation the author[1] attempted to obtain from spleens of lepers an antigen or product analogous to tuberculin which might be used in the diagnosis of leprosy. The product, obtained

2 Hagemann, P. K. H., *München. med. Wchnschr.*, 1938, **85**, 1066.

3 Küster, H., *Deutsche med. Wchnschr.*, 1939, **65**, 92.

4 Kline, E. K., and Leach, R. E., *Proc. Conf. State and Prov. Lab. Directors*, 1940.

† Obtained through the courtesy of Dr. John Hanks, Culion, P.I.

5 Richards, O. W., and Miller, D. K., *Am. J. Clin. Path.*, 1941, **11**, 1.

6 Richards, O. W., Kline, E. K., and Leach, R. E., *Am. Rev. Tuberc.*, 1941, **44**, 255.

* Aided by a grant from the Leonard Wood Memorial for the Eradication of Leprosy (American Leprosy Foundation).

1 Henderson, H. J., *Internat. J. Lep.*, 1940, **8**, 271.

by buffer solution extraction of finely ground leprous spleens rich in acid-fast bacilli was tested on leprosy patients by a committee in Manila, P. I.[2] Specific tuberculin-like action could not be demonstrated. There are at least two possible explanations for this negative result. One is that there is no hypersensitiveness to leprosy bacilli. The other is that the acid-fast bacilli in the spleen after the grinding were adsorbed or protected by the preponderantly great amount of normal splenic cells present, and therefore not well extracted.

It seemed preferable, if possible, to separate the bacilli from leprous tissues before grinding and extraction. Previously, attempts have been made to separate acid-fast bacilli from the tissue of the lesions in which they are found. Cowdry[3] and his coworkers dissected bacilli-laden cells from tissues in rat leprosy and then ruptured them with a Mueller press. The expressed tissue fluid, cytoplasm, nucleoplasm and some unruptured cells were ground with sand and centrifuged at low speed. The supernatant fluid, which contained acid-fast bacilli, was then removed and recentrifuged at high speed. The sediment contained a large number of acid-fast organisms. In the experiments here reported advantage was taken of homogenizing chambers[4] for tissue cells and the interface studies of Mudd and Mudd[5] which showed that certain acid-fast organisms, including stock strains of leprosy bacilli, pass readily from the watery into the oil phase.

Leprous splenic tissue was obtained through the courtesy of Dr. John Hanks of the American Leprosy Foundation, Culion, P. I. In order to prevent autolysis in transit the spleens were minced and "cryochemed" to remove practically all the moisture and sealed *in vacuo*.

Sixty-one grams of dehydrated leprous spleens were ground with mortar and pestle, suspended in 0.25% phenolized distilled water, and homogenized at a pressure of 3,000-3,500 lb. Smears of the homogenized material showed that practically all of the splenic cells were disrupted and that very few, if any, of the acid-fast bacteria were injured. The homogenized suspension was then mixed with equal parts of neutral "virgin" olive oil in a shaking machine for one hour. After centrifugation for 15 minutes there were 4 separate distinct layers in the bottles (Fig. 1). Layers 1, 2, and 3 were decanted from layer 4 and allowed to reform in the course of 2 hours

[2] Joint Committee on Leprosy Skin Tests, *Internat. J. Lep.*, 1940, **8**, 263.

[3] Cowdry, E. V., Ravold, A., and Packer, D. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 341.

[4] Chambers, L. A., personal communication.

[5] Mudd, S., and Mudd, E. B. H., *J. Exp. Med.*, 1927, **46**, 167.

standing in a separatory funnel. Layer 2 was then separated from the oil (layer 1) and watery (layer 3) layers. Stained smears of all 4 layers were then made, and showed the following: Layer 1, the top layer, was clear olive oil containing no acid-fast bacteria. Layer 2 consisted chiefly of acid-fast bacilli, with a few unruptured tissue cells and some debris. Layer 3 was a cloudy, watery layer and contained an occasional acid-fast bacillus. Layer 4, the bottom layer, consisted of whole tissue cells, debris, and an occasional acid-fast bacillus.

After layer 2 had been separated mechanically from the other layers it was washed several times with acetone and cryochemed. Eight hundred and forty-eight milligrams of dry residue, consisting almost entirely of acid-fast bacilli, were recovered from the 61 g of dried spleen. Studies with these organisms are in progress.

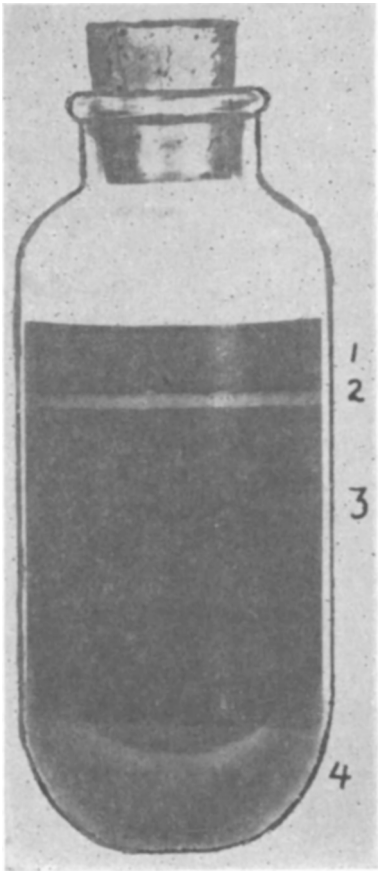

FIG. 1.