the great advances in orthopaedic surgery and plastic surgery," but I would like to make the point that it is the *subject* of the use of modern drugs that alone had made this advance possible, giving the surgeon much fodder.

In response to the request of WHO for data on mutilations, in connection with a world-wide rehabilitation scheme I am now . . . re-examining all our out-patients, and it is already certain that my statistics will show that, in a field campaign where practically 100% of patients are getting treatment regularly, there is no need for such a costly scheme as there would be so few to rehabilitate.

This optimistic picture brought a comment from Dr. H. W. Wheate, of Tanganyika (British Medical Journal 1 (1961) 75).

See Dr. Spencer Reed's letter on the treatment of leprosy was of great interest. He rightly emphasizes that sulphone treatment not only prevents the advance of the disease to mutilation but also prevents the social and economic complications of the disease. One cannot, however, ignore two facts: in some cases sulphone therapy precipitates reactions which lead to permanent nerve damage; and the very long period of treatment required presents serious social and administrative problems.

Dr. Reed has confined his remarks to Bali, and he would, I am sure, agree that they do not apply universally. In Tanganyika, for example—a vast country with 100,000 cases of leprosy—we cannot be so sanguine about reactions and cannot possibly agree with Dr. Reed that "all these can quickly be brought into a central ward" (my italics). On the other hand, there are parts of the country where the local problem is on a similar scale to that in Bali and has been tackled by a similar concentrated effort. Here, we can say with Dr. Reed, "(They have) seen with (their) own eyes the results of dapsone." But it must be emphasized that one cannot achieve results like this on more with only an effective drug. Staff, money, and a good organization are even more important. It may be true, in Bali, that "no reaction case properly treated should develop permanently damaged nerves," but if we in Tanganyika wait until the reaction has occurred we are often too late. Further, our experience is that "properly treated" usually means "treated with a drug other than dapsone"—and even then we are not always successful.

Finally, I think we must avoid undue optimism about the long-term effects of sulphone therapy. I am seeing leprosy cases who have been under apparently effective sulphone therapy for five, six, or seven years suddenly develop ulcers. "Properly treated," permanent nerve damage can be avoided, but they tend to relapse and one can never be quite sure that they will not end up with some degree of deformity.

CAPSULES OF PATHOGENIC MYCOBACTERIA

In this issue there appear the last three of a series of five short articles on this subject by Dr. J. H. Hanks, who has dealt with it more broadly and intensively than has been done before, and from a different point of view. The first two articles were in the preceding issue. It seems desirable to condense this important work, to make a continuous story of the findings with a minimum of technical details.

1. The first article 1 summarized studies of the factors which lessen the penetrability of pathogenic mycobacteria by certain dyes. In order that the properties of the bacilli should be as natural as possible, drying and heating of the smears were avoided; they were exposed to the dye after only 3 seconds.

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Staining of *M. phlei* (with safranin 0) occurred quickly, accelerating with time, whereas the staining of pathogenic (crystal violet found heat for these) proceeds progressively more slowly, and the impermeabilities of individual cells differ very greatly. The marine leprosy bacilli gave the slowest staining rate because of the microcapsules which enclose them uniformly. However, modification of the capsules of the latter, even by brief heating, increased the rate of staining tremendously.

Although *M. phlei*, representing the saprophytic mycobacteria, has in culture an extracellular matrix which promotes clumping, but capsular halos were not demonstrated by the surface coatings of the cells used; they can be demonstrated, however, provided the suspensions are exposed briefly to fresh substrates at 37°C. In the intermediate class represented by BCG (avirulent tubercle bacilli), most of the rods stain readily, but the others may exhibit outlines of capsular material after Congo red coating, and clumps may be so penetrated by matrix that the individual organisms cannot be delineated.

Leprosy bacilli from untreated patients which stain readily are narrow; those which resist dye penetration look larger because of capsular halos; and globi may be outlined by the surface matrix as entirely unstained masses. No such appearances are seen in smears from sulfone-treated leprosy patients, in which the bacilli present no capsules and are readily permeable to dyes. The difference may be seen even in ordinary carbolfuchsin-stained smears; the bacilli in the clumps from untreated patients are usually in contact with each other, while those from sulfone-treated patients lie closely side by side.

The rugged microcapsules of *M. leprae merio* are associated with infectiousness. The first demonstrations of actual capular structures were made by electron microscopy of ultrathin sections, but microcapsules can be demonstrated by light microscopy after proper preliminary treatment.

The extracellular components are very resistant to modification, and alcohol-like formalin—seems to have a fixing and toughening effect. On the other hand they are promptly modified by heating to 98°C, or by shaking in 5-10% per cent chloroform in aqueous suspension.

The differences of penetrability of dyes among the mycobacteria are due to surface characteristics associated with pathogenicity, and these properties are attributable to true capsules and capular matrices. The bacterial cells which stain readily are devoid of capsules, *M. leprae* may have large capsules, but the nonsulfonlated state in sulfone-treated patients is held to indicate sclerosis rather than lack of viability or a specific effect of the drugs.

II. This paper calls attention to the significance of capsules on *M. leprae* demonstrated by electron microscopy. Certain authors are cited who observed electron-transparent halos surrounding the rods in ultrathin sections, and in direct views of unsectioned clumps an amorphous matrix which tends to keep the rods separated. These are to be seen in material from untreated patients, but are lacking in material from treated patients.

In electron microscopy the electron-transparent zones are demonstrated by negative outlining with the surrounding materials (proteins and debris, and sectioned substances of the cells in which they occur). In light microscopy they can be demonstrated by negative outlining with surface coats. There are, it is suggested, certain advantages in the latter procedure and in the assay of dye permeability, these being simple procedures not requiring an electron microscope.  

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2 A distinction is made between macrocapsules which can be demonstrated by light microscopy, and microcapsules which are less than 0.5 μ wide and hence not visualized in light microscopy.


4 Halos may also be well demonstrated in fresh smears from lesions after exposure to ozone vapor until the background material is darkened.—H. W. W.
III. This article 3 deals briefly with the old moot question of the origin of the global matrix of glob of M. leprae—whether it arises from the bacillus, or by interaction between the bacillus and host. The electron-transparent zones in ultra-thin sections are interpreted by the author as capsules and matrices which are synthesized solely by the mycobacteria, and which disappear after sulfone treatment—this interpretation being in frank disagreement with those of certain other authors but not of all. Although chloroform condenses tissue components, it nevertheless promptly penetrates and disperses the globs, showing that chloroform-soluble waxes are the major bonding substance of the electron-transparent material. Treatment with a bile-penicillin mixture digests and disperses tissue components other than collagen, but it does not disintegrate the bacilli, and it does not alter the dye-impenetrability of the mycobacteria. Tissue components are absorbed to the capsular surfaces of tissue-grown mycobacteria such as the marine bacilli, and are retained after washing, but they are removed by penicillin with change of the iso-electric point of the suspension (from pH 4.5 to pH 1.5), without changing the viability and other properties of the bacilli. It is concluded that the tissue components occur only on the outermost (capsular) surfaces of the mycobacteria.

IV. Here 6 is discussed the problem of preserving the internal structure of pathogenic mycobacteria by fixation, which is interfered with by the impermeability of their inert capsules and matrices. These structures may prevent penetration by conventional fixatives, and the fixatives themselves may increase the impermeability. For example, exposure of smears to the fumes of strong formaldehyde produces excessive hemoglobin of most of the materials in the smears, but such exposure of the marine bacilli makes them less permeable to dyes than before.

Fixation of tissues by osmium tetroxide for electron microscopy is conventionally brief, and it suffices for the demonstration of internal structures of ordinary mycobacteria. With pathogenic mycobacteria, however, more time is needed (e.g., 2 days for M. arctis, 5 days for the H-37Rv tubercle bacilli, and 6 days for the marine leprosy bacilli), much longer fixation than is suitable for tissue cells. This leads to the question of how tissues containing such mycobacteria are to be fixed for study of their structure without over-fixation of the cells and other tissue elements. It is suggested that treatment, as with heat or chloroform, to reduce the impermeability of the bacilli is indicated, but there remains the problem of avoiding destruction of the capsules and distortion of internal structures, and that of simultaneous preservation of the tissue components.

V. In this final article 7 is a discussion of the demonstration of capsules on the leprosy bacilli in smear preparations after carbol-fuchsin staining. Unheated smears, after staining in carbol-fuchsin at 37°C for 5 minutes (and applying the ligroin coat), showed staining of about 2/3rd of the free bacilli with capsules evident, whereas heating at 60°C for 1 minute and staining in hot carbol-fuchsin for 1 minute showed all of the bacilli stained red but without capsules. Heat and the dilute phenol were found to be the primary capsular solvents in the Ziehl-Neelsen procedure.

Significant as the phenomena reported by Hanks doubtless are, there are workers who hold views that are not in accord with his conclusions regarding the sources of the capsular matrix of the leprosy bacillus. It is true that as far back as 1918 Mitsuda, 8 on the basis of

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work with fat stains, was inclined to regard the lipoidal substance of
vacuolated lepra cells as a product of, or due to, the bacilli—ascribable
mostly to its degeneration. However, he pointed out that the lepra-
cell colonies found in the visera show very few bacilli, and that "in
such cases lipoid transformation of the leprosy bacilli is unthinkable."

Most recently among the Japanese investigators, Fukushi has
concluded from histologic studies that the mechanism of the lepra-cell
formation is phagocytosis of lipids from outside the cell. The absorbed
lipid envelopes the bacilli and interferes with their metabolism, so
that they degenerate and finally disappear. The formation of the foam
cells has no connection with the age of the leproma, or with the amount
and destruction of the bacilli, nor is it due to degeneration of the lepra
cell itself.

Fukushi is also one of those concerned in studies of fixation of
mycobacteria for the demonstration of the inner structures. He
described a 3-layered cell wall on the tubercle bacilli as seen in ultra-
thin sections, but the "slime layer" demonstrable about unsectioned
bacilli, especially by metal shadowing, were not seen in the sections.

Other immediately available reports of interest are those of
McFadzean and Valentine and of Rees, Valentine and Wong about the
evidences of viability of leprosy and rat-leprosy bacilli shown by
electron microscopy (the latter group reporting no evidence that the
bacilli form capsules); by Malfatti of a study of the role of granules
of the leprosy bacilli; and by Chattjee et al. who found that in electron
micrographs at least not all globular substance is lost from globi in
sulfone-treated patients. The vacuoles around groups of bacilli in
Virchow cells contain what was thought to be cell debris.

It would seem that the final answers to some of the questions
involved are still to be reached. Certainly the matter is a complicated
one, especially if one tries to correlate the loss of capsules in the
lesions of treated patients with the ordinary histological picture of
paraffin sections of such lesions.—H. W. WARD