

DEMONSTRATION OF THE LEPROSY BACILLUS IN THE LEPRIDES

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BY DR. H. P. LIE

Bergen, Norway

In cases of ordinary nodular leprosy the demonstration of the bacillus is a comparatively simple matter, whether *from* the lesions by ordinary smears or *in* them histologically. They are typically so numerous that large numbers can usually be shown in tissue sections even by methods that are not especially favorable for their demonstration.

It is quite otherwise, however, in dealing with the ordinary spots of macular leprosy. Because as a rule bacilli could not be found in them, these have been looked upon, at different times and by different men, as being due to (a) toxins produced by the infection, or (b) disturbances of the nerves supplying the areas affected. But in time it came to be realized that by the use of proper technique and sufficient patience bacilli could be found in these macules, and I suppose that now there is scarcely anyone who is not of the opinion

that they are due to the presence of the bacilli, at least so long as the process is an active one.

The same question is met in connection with the lesions of tuberculoid leprosy. In a few reports on this variety of the disease the finding of bacilli has been recorded, but usually they have not been found. However, in view of what is known about ordinary macules, it is to be assumed that bacilli should regularly be present in active tuberculoid changes.

My personal experience with this kind of lesion has been limited. Though many histological examinations have been made in Norway for many years, only one typical case has been found, that reported by Bruusgaard (1). Personally, I have on a couple of occasions found a microscopic picture that was suggestive of this form, but none that was typical. At the suggestion of Wade I got into communication with colleagues in South Africa, and they¹ were kind enough to send me specimens from ten patients. Four of these must be looked upon as typical examples of tuberculoid leprosy. In all of the specimens, including those that were tuberculoid, I have been able to demonstrate bacilli. This is contrary to the experience of Wade, who was unable to demonstrate them in Zenker-fixed tissues from South African cases in ordinary phases (2), though he did find them, both in smears and sections, in certain cases in a state of chronic lepra reaction (3). As the differences of results in this examination may be due to questions of technical nature, I shall describe in some detail the technique which we have employed here in recent years.

It goes without saying that we refer here only to the examination of skin specimens. Furthermore, these specimens to be of any value must be from parts that show infiltration or other signs of an active process. In by far the greater number of cases this will be in the edge zones; in the atrophic centers of the macules there is nothing characteristic to be found, and certainly no bacilli. I take it for granted that the ordinary histological methods of examination are known, and that the general rules for these are followed; I will here mention only the special methods of procedure which are employed.

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The pieces of skin are fixed in a 10 per cent formalin solution, or in Müller's fluid.² The first-mentioned is the simpler and handier, and possibly the better for the demonstration of bacilli; the other fixative is more suitable for the examination of cell- and tissue-structure. The pieces can remain in the fixing fluid for 24 hours, after which they are well rinsed in water and then hardened in alcohols of increasing concentration. If tissues are left in the Müller fluid very long they may become so hard that it will be difficult to obtain thin and good sections. The tissues are generally embedded in paraffin, though one may also use celloidin, which affords greater protection of the tissue structure.

The sections are stained in carbol-fuchsin at 37°C. for 20 to 60 minutes, though there is rarely any advantage in staining for longer than 45 minutes. One first examines a few sections that have been stained for 20 minutes; if bacilli are not found in these the staining must be prolonged. After rinsing the sections in water, decolorization and counter-staining is effected with Gabbet's fluid³ for 30 seconds or a little more, according to the thickness of the sections. They are again rinsed in water, cleared in xylol, and mounted in Canada balsam. The contrast color is always somewhat faint, but that is of little significance for the finding of bacilli; in fact, a faint counterstain is often preferable to a deep color. However, if one wishes the contrast color strengthened, one can add carefully a little Loeffler's alkaline methylene blue. Sulphuric acid is absolutely preferable for work with leprosy bacilli, as it decolorizes them less than the other acids generally used. These organisms are undoubtedly somewhat less acid-fast than the tubercle bacilli.

The search for the bacilli must be made very carefully in all of the infiltrated parts of the tissue, and in the nearby nerves and vessels. The search should cover the entire depth of the section, and should be made systematically. As a rule the bacilli are found lying separately, and not in bundles or groups as they generally are in other lesions of leprosy. In their appearance they often resemble tubercle bacilli more than leprosy bacilli. There is no normal zone of tissue just below the epithelium, as in nodular leprosy. The bacilli may lie just below the basal cells, and may even be found occasionally in the lower layers of the epidermis. The number is very small as a rule, and one must not give up looking for them until the sections have been examined several times, and possibly also after longer staining in carbol-fuchsin, with perhaps more cautious decolorization with Gabbet's fluid. If one proceeds correctly in this manner one will nearly always be successful in finding bacilli in leprosy macules, and as far as my experience goes this includes those of tuberculoid nature.

² Potassium bichromate 2.5 gm. and sodium sulphate 1.0 gm. in 100 cc. of distilled water, plus 4 per cent formalin.

³ Sulphuric acid, 25 per cent solution, 100 cc., with 1 gm. methylene blue.

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