# DECOLORIZING OF *MYCOBACTERIUM LEPRAE*

By

A. R. DAVISON

*Medical Superintendent, Pretoria Leper Institution*

The diagnosis of neural leprosy may hinge on the discovery of a skin lesion accompanied by anaesthesia either of the lesion or in the distribution of a peripheral nerve. In other cases the diagnosis can be made on the discovery of a thickened nerve or nerves usually, but not necessarily, accompanied by disturbances of sensation and contractures or muscular atrophy. When the perineurium is incised and scraped, such thickened nerves almost invariably reveal bacilli. A scraping of the nasal mucosa may reveal bacilli but an incision into a macule seldom does. Approximately 80 per cent of our neural cases never show any bacilli. We, therefore, agree with Soule and McKinley (1): "a definite positive finding in a smear of mucous swabbed from the surfaces (of the nasal mucosa) is conclusive, a negative finding is valueless."

In lepromatous leprosy, at least in the early stages, no neural disturbances may be discovered, and the diagnosis is made on the discovery of infiltrated areas of skin and the presence of bacilli. We have made it an axiom that no case should be classed as lepromatous unless bacilli can be found.

Certain cases have been admitted to the institutions in this country without external manifestations of leprosy but with acid-fast bacilli in their noses. Some of these cases have been discovered by accident, e.g., during routine examinations such as for diphtheria bacilli. There is, also, at present one child under care whose mother died here as a patient four years ago; the routine follow-up of the contacts revealed acid-fast bacilli in his mucosa prior to admission, but in the institution this finding has not been confirmed. As laboratory technique is not standardized in the various laboratories, we cannot be sure this child has leprosy.

About six years ago three similar cases passed through the institution in the Transkei. Unfortunately the notes are not now at hand, but I recollect that we were unable to find bacilli during their stay in the institution and they never developed any leprotic lesions.

The following two cases are reported, as I believe they point to pitfalls in the diagnosis of leprosy.

CASE No. 8223: Admitted August, 1941. Immediately on seeing the case I remarked, "We don't admit impetigo cases here." The patient had eight impetigo-like crusts on his scalp and nares which exuded a most foul-smelling pus. On referring to his admission papers I was surprised to read: "Nasal smears sent to S.A. Institute, Port Elizabeth, positive for *B. leprae*." We took smears from his nose and from his scalp and these were stained by our laboratory technician.

The nose was negative but the pus from the scalp revealed the greatest number of acid-fast bacilli that I have ever seen. (Incidentally no bundles were present.) I asked that the slide be saved for demonstration to students. It was washed in xylol and then in absolute alcohol. When examined a month later only a few acid-fast bacilli could be found—the rest were bleached. After examination the case was admitted to hospital and a starch poultice applied to the scalp. On removal of the crusts, a fungating mass of granulation tissue was exposed, and this completely resolved in ten days under ammoniated mercury ointment. No attempts were made at culturing material from the crusts as at this stage I was satisfied that the slide had revealed *B. leprae*. When the case responded so rapidly to treatment I again questioned my diagnosis. Cultures from the nose at this late stage were sterile and smears were negative. No nerve involvement was ever found. We concluded the case was not leprosy, and the patient was discharged.

CASE No. 8354: Admitted December, 1941. This patient was admitted with induration of his face resembling acne pustules, which led to a diagnosis of acne indurata. Nasal smears were negative both prior to admission and on our examination, but pus from the pustule shown on the bridge of the nose showed acid-fast bacilli. These were plentiful, but no bundles were found. The staining in this instance was also done by my laboratory technician. Dr. Pyper of Pretoria cultured some of the pus and reported: ". . . I grew several colonies of diphtheroids, staining these for acid-fastness I find that some, if not too severely treated with acid, retain some of the fuchsin." I again expressed some pus and inoculated a blood agar slope. This was examined at the South African Institute for Medical Research, Johannesburg, under the impression that it had been incubated, and the Institute reported: "Report on a specimen of cultures:—Acid-fast bacilli morphologically indistinguishable from *B. leprae* have been detected on these cultures. These bacilli are scanty and do not appear to be growing on the medium. The appearance suggests that part of the original inoculum was removed and that the acid-fast bacilli were detected in this." I again inoculated and sent a blood agar slope and received the following report: "Cultivation yielded a mixed growth of diphtheroid bacilli and scanty *Staphylococcus albus*. No acid-fast organisms were seen on direct microscopic examination." A biopsy specimen of tissue from the face was sectioned and the report read: "Section of this specimen shows a small epidermoid cyst, generalized thickening of the squamous epithelium associated with chronic inflammation in the corium. Hair follicles and sebaceous glands are very numerous. There is no sign of leprosy." It was evident that the patient did not have leprosy and that the acid-fast bacilli were only partially acid-fast diphtheroids. The patient was discharged as non-leprous.

The Government pathologist at Cape Town, reported that in our nasal smears and in smears from other institutions he was constantly finding bacilli "morphologically indistinguishable from *B. leprae*" except that they were non-acid-fast. A culture was made from one such case and yielded a profuse growth in 24 hours. Smears from this were stained with steaming carbol-fuchsin for 5 minutes. A 5 per cent aqueous solution of sulphuric acid was then applied to one slide for one minute, to a second slide for 3 minutes, and to a third slide for 6 minutes. In the one-minute slide acid-fast bacilli were found. In the three- and the six-minute slides the bacilli were completely decolorized. The diphtheroids in this case were evidently only slightly acid-fast.

Our technician had been taught the "two, two, two", method of staining; i.e., two minutes each in fuchsin, acid, and methylene blue.

The decolorization here is hopelessly inadequate. We now stain for five minutes in warm fuchsin, decolorize for 20 minutes in 5 per cent sulphuric acid, and counterstain for 2 minutes with methylene blue. Leprosy bacilli are still acid-fast under this treatment.

As the danger of mistaking diphtheroids for *B. leprae* is so real, I recommend that laboratory technique be standardized and that no slide be regarded as showing acid-fast bacilli unless it stands up to such rigorous decolorization.

## Summary

1) Attention is drawn to the fact that cases are diagnosed as leprosy on the finding of acid-fast bacilli in nasal secretions or in pus.

2) Two cases are reported:—in one the acid-fast bacilli on the slide became decolorized after treatment with alcohol and it is inferred that these were diphtheroids. In the second case it was shown that bacilli which had been reported as "indistinguishable from *B. leprae*" were diphtheroids.

3) A plea is made for the standardization of laboratory technique.

## Reference

(1) Soule, M. H., and McKinley, E. B. Tuberculosis & Leprosy. Symposium Series Vol. I, p. 94. The American Association for Advancement of Science (1938)