

The Bacteriological Examination in Leprosy.

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TO a leprosy worker accustomed to the making of bacteriological examinations as a matter of simple routine, it comes as something of a shock to discover how many neglect this procedure, which is so essential in diagnosis, classification, and the evaluation of the results of treatment. Again, there is often cause for astonishment at the technique by which smears for the examination are prepared by those who do make it. Little has been said in the recent literature on this subject, perhaps because it is not realised how many of those who deal with patients are not well informed about it.

Exemplifying the condition spoken of, one correspondent tells of having had a bacteriological examination made in a case with lesions probably of a tuberculoid nature, as if it were an unusual procedure. Another who has recently begun to make such examinations, remarks, apparently puzzled, that he found cases that are obviously leprosy but that give negative smears. One has learned of an active clinic in which striking cases of the tuberculoid variety were classified as ordinary "nodular" simply because they had not been examined bacteriologically.

As regards technique, it seems that in some places to speak of the bacteriological examination brings to mind only the nasal smear; and at that, usually, smears made with a cotton swab used more or less blindly. Methods in use for obtaining material from skin lesions include blistering, and aspiration with a syringe. Of the "snip" method nothing will be said at this point. The "scraped-incision" method is often applied awkwardly and inefficiently. A radical departure from it that has been seen is to obtain serous and cellular material by scraping off the epidermis, and only the epidermis, from an area 0.5 cm. or more in diameter—with much discomfort for the patient and little chance of finding bacilli when they are few.* In the

* Two facts should be emphasised: *First*, that probably none of the active skin lesions is entirely bacillus-free, not even the leprides of neural leprosy, though in them it is often difficult or impossible to demonstrate bacilli by any available method. In many instances, as in new (reaction) lesions in cutaneous leprosy, or lesions almost cured, bacilli may be difficult to find by any standard method unless care is used in the examination, and with care they may sometimes be found in florid tuberculoid lesions that otherwise would be called negative. *Second*, that the leprotic infiltration is in the dermis, the epidermis and a thin layer immediately under it are uninvolved, as shown in Text-figure 1, so that when an infiltration is of slight degree the lesion-foci may be fairly deep, requiring considerably more than a superficial scratch to get to them.

examination of annular lesions material is sometimes taken only from the oldest part, the healed central area. The situation is such, one has come to feel, that attention should be drawn to it somewhat vigorously. Since the use of poor technique is due to lack of understanding of good technique, and since failure to make the examination freely, is probably due largely to an idea that the procedure is more or less difficult and disturbing, this paper is confined to a discussion of technique.

METHODS OF SKIN EXAMINATION.

For the routine, standard examination of skin lesions there are two widely known ways of obtaining smear material, namely, by excision and incision. The former involves removal of pieces of tissue, the latter requires only small simple cuts in the skin. Unusual methods and special procedures such as the aspiration of lymph nodes and the scraping of exposed nerves, need not be considered because none of them is suitable for a standard, routine examination.

1.—THE EXCISION METHOD.

Excision, done in one way or another, is the time-honoured method of obtaining material and the only one mentioned by older writers. The removed tissue has, of course, been dealt with in various ways, but evidently for many years the demonstration of bacilli was usually by histological sections, which made it more a research procedure than a routine measure. The first recorded effort to simplify the procedure of which the writer is aware was that of Alvarez, of Hawaii (1) who reported that he triturated the piece of excised tissue and smeared on slides the pulp so obtained. This method, though rather cumbersome, constituted a real advance because, as Alvarez pointed out, it permitted making a diagnosis in a comparatively short time, and it could be done by physicians who did not possess laboratory facilities.

The present-day survival of the excision procedure, essentially similar to that of Alvarez, is the snip method apparently devised by Muir, of Calcutta. This is described briefly in an appendix of the report of the Leonard Wood Memorial Conference (4) as follows :—

" . . . a small portion of the dermis, at least 2 millimeters thick, is nipped off with a sharp pair of scissors, curved on the flat. The raw surface of the tissue so obtained is applied to a slide and firm pressure is exerted so as to express as much as possible of the cellular elements."

To meet the objection that this method of smearing spreads the material diffusely over too large an area of the slide, Lowe (2) holds the snip with forceps, raw surface uppermost,

and scrapes it with a knife, thus obtaining pulp with which a more concentrated smear can be made.

2.—THE INCISION METHOD.

The beginnings of this method are not known, but it was of course only a natural development to meet the needs of those seeking a simple, practicable method of getting bacteriological confirmation of diagnosis. The blister method was also evolved for this purpose, but it was much simpler and quicker to obtain material from a small incision.

Such a method had long been in use in Manila when, in 1916, the writer was assigned to the official diagnostic committee there. The local term for the examination as then made, was "examiner el sangre," and that was actually what was done; the material examined was chiefly blood or bloody lymph that exuded or was expressed from a small cut. As a rule the cellular material in such smears was so diluted and so spread over the slides that when bacilli were few or absent the examination was greatly prolonged, and even at that it is probable that many erroneously negative reports were made. In the following year the writer was in a position to modify the technique to essentially that now used, the "scraped-incision" variety of the so-called "slit" method. The following is a revision of published descriptions of it (6, 7). It is really simple and rapid, but attention should be given to the points discussed under "comments."

TECHNIQUE.

1. Cleanse the area to be examined by rubbing briefly though vigorously with a small cotton-wool sponge, wet with alcohol; soap and water, or even ether or gasoline may be used. Wipe dry with cotton-wool.

2. Pinch up the skin in a fold, applying enough compression to stop or minimise bleeding. When it cannot be actually picked up, compress it laterally as much as possible.

3. With a properly cleansed scalpel of suitable style and size make a small but real cut, 5 mm. or so long and deep enough (about 2 mm.) to get well into the infiltrated layer.

4. If blood or lymph exudes in any quantity wipe it off. With the knife-blade turned transversely to the line of cut, scrape the side and bottom of the cut repeatedly and with sufficient vigour to obtain a little actual tissue pulp from below the epidermis.

5. With the knife transfer the small amount of material obtained to a microscopic slide and make a uniform, moderately thick smear over a small area. Multiple smears from the same patient are best put on to a single slide.

6. For after-treatment of the cut the patient is simply given a bit of cotton-wool to compress it until oozing stops. No dressing is necessary. Though a wisp of cotton with collodion may be applied if desired.

Comments.—(a) The sites for examination should be selected with some care, choosing lesions that appear to be most active. Routine procedure does not include the examination of apparently normal skin, but it is well to recall that slight infiltration of the ear is difficult to detect and that apparently normal earlobes are sometimes found positive (3).

(b) It is often desirable to make *multiple smears*, one from each of several sites, for bacilli may be fairly numerous in one lesion and very few in another. A single negative smear means little, and it is only after repeated examinations that one is justified in declaring a case that is clinically suspicious or positive to be bacteriologically negative.

(c) One of the objectives of the cleansing is to avoid *contamination of the smear* by surface material. There may be free-living acid-fast bacilli on the skin, or fragments of fungus spores which may retain the stain. When getting the tissue pulp the epidermis should not be scraped.

(d) A possible source of error is *instrumental transfer* of bacilli from the smear of one lesion or patient to that of another. After the knife has been used it should be wiped clean with an alcohol-soaked sponge and heated in the direct flame sufficiently to destroy the bacilli, though the blade edge should not be burned.

(e) Nearly any kind of *knife* can be used, but the most satisfactory is a rather small, fairly full-bellied scalpel (see Text-figure 2, A). Interchangeable blades (Text-figure 2, B) are useful and may be economical but being less rigid they are on the whole less satisfactory. In scraping the incision (Text-figure 1), the knife should be held as one would a pencil but more vertically, and the effort applied more to the side of the cut than to the bottom.

(f) Twists or balls of *cotton-wool*, small for convenience and economy, preferably sterile, should be available in abundance. Alcohol-soaked ones are used in cleansing the skin and the knife, and dry ones for drying the skin, for wiping incisions when there is an excess of blood, and by the patient to stop oozing. Something into which to throw used cotton should be handy.

(g) Excessive *dilution of the tissue pulp* with blood should be avoided. The bacilli are mostly in the tissue itself and, while escaping blood may carry some of them with it, they are necessarily dispersed, and if they are few they may escape detection.

(h) Excessive *dispersion on the slide* is also a source of difficulty. Only a little pulp is required from a given skin spot*, and this should be concentrated in a small area (Text-figure 2). However, should a smear be too thin it is helpful to outline the smeared area with a wax pencil or even an ordinary pen, before the immersion oil is applied, so that it will be seen when the lens reaches the edge of the area. On the other hand *excessively thick smears* are to be avoided, especially if there is much blood in the material.

(i) When multiple smears are made they are best put on a *single slide* (Text-figure 2). Record is to be made of the source of each smear, but the individual smears need not be numbered; the first from the labelled end is always No. 1, and so on.

NASAL EXAMINATION.

The foregoing has only to do with the examination of the skin as required for diagnosis, clinical classification of cases, and evaluation of progress under treatment. In connection with the last, and also with administrative classification (*i.e.*, distinction between "open" and "closed"

* From a thick lepromatous lesion it is easy to obtain an abundance of pulp, often quite free from blood. From a lesion with little or no gross infiltration only a little pulp can be obtained without excessive scraping. It is in such cases that bacilli are apt to be few, and that it is particularly important to concentrate the material so that all of it may be examined.

cases), the nasal examination is of much value, though it should be considered a supplementary measure.

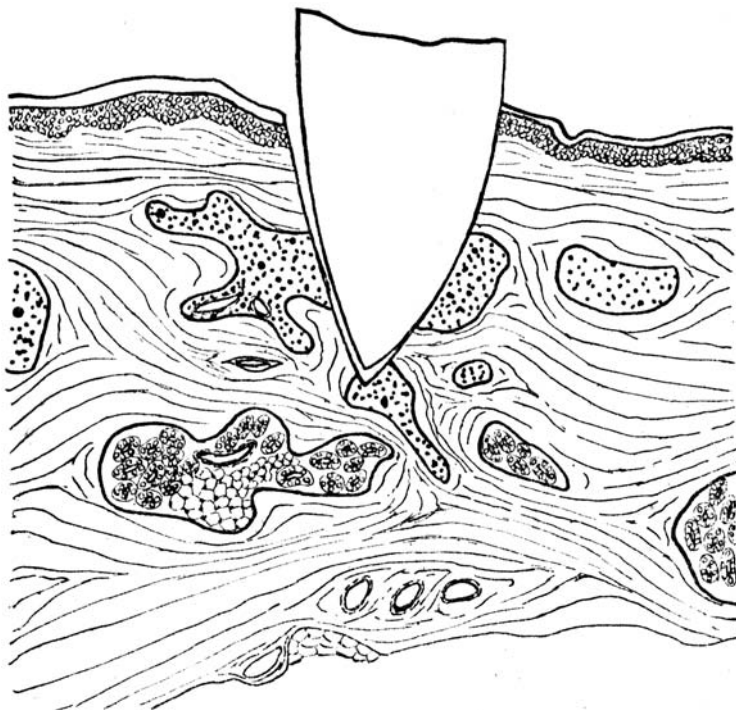


FIG. 1.—A semi-diagrammatic sketch showing the location of leprotic infiltration of slight degree, the proper depth of incision to reach it, and the position of the knife blade in obtaining tissue pulp.

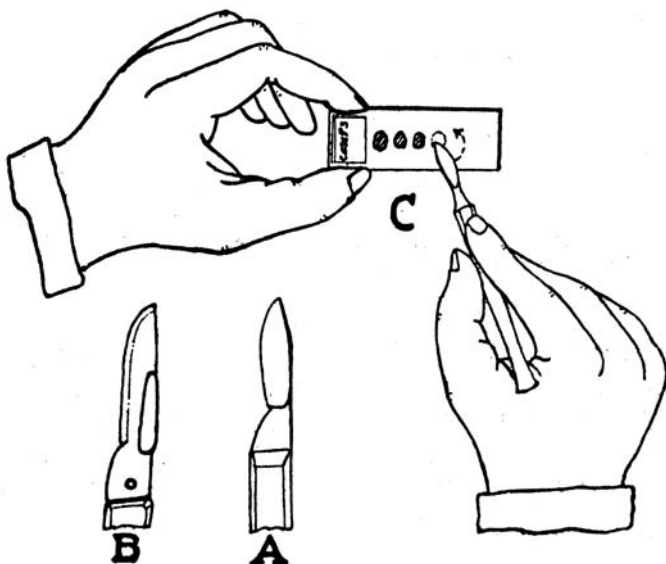


FIG. 2.—Showing A, a satisfactory scalpel for taking material (the size of blade indicated by the width of the handle), and, B, an interchangeable-blade knife that is also useful. In C is shown both the method of making the small, concentrated smear for examination, and of making multiple smears on a single slide.

With regard to the technique, if there is an unbroken mucous surface over a leprotic infiltration the bacilli may or may not be escaping at any given moment. Consequently, though a definitely positive finding in a smear of mucous swabbed from the surface is conclusive, * a negative finding is valueless. With the remark that both sides of the nose should be examined, the following is quoted from the appendix of the Memorial Conference report :—

“ With the use of a nasal speculum the interior of both nares is carefully examined for infiltrations, nodules, and ulcers. If any of these is found, material should be removed therefrom with a blunt narrow-bladed scalpel, or a similar instrument, by scraping deep enough to cause slight bleeding. Even when there is no visible lesion, a scraping should be taken from the septum. *Mycobacterium leprae* may be found on the septum, the inferior and middle turbinates, or the floor of the nose.”

It may be added that it is often advantageous to wipe away mucous or other surface material before taking material for the smear. Also, that in a good light much can often be seen in the nose without speculum and head-mirror. The locations and appearances of the lesions that may be found cannot be recounted here (5).

DISCUSSION.

The main question that arises is how the incision and snip methods compare as regards practicability and efficiency. The writer's opinion, more and more firm as time passes, is that when properly applied the former is decidedly superior in simplicity and convenience for both physician and patient, and not inferior in efficiency.

Simplicity.—It is much easier to obtain the smear material by the incision method, and much simpler to deal with the little cut, which closes and really needs no dressing, than to dress a gaping scissors wound. Furthermore, only a scalpel has to be cleansed before the next specimen is taken.

Discomfort.—Done with any skill at all the incision technique involves much less discomfort to the patient than the other.

After-effects.—The incision has practically no after-effect ; it heals promptly, without infection, and usually without any perceptible scar even after repeated examinations. The snip-wound heals with a scar, and after a few examinations an earlobe, for example, may be conspicuously mutilated.

Practicability.—Smears may be made with the utmost freedom, from practically any site, including the alae nasae, nipple, and glans penis—regions that with most patients could not be examined at all by the more painful, mutilating method.

* Such a finding would not, by the way, be made on discovering a single bacillus or two that might be of a free-living, air-borne saprophyte.

Multiple and Repeated Examinations.—The advantage of being able to make at a given time smears from as many places as desired, and of repeating the examination as often as desired, is an important one. This is frequently seen in diagnostic work, but more generally in observing cases that have become negative under treatment.

Multiple Smears on a Slide.—The reduction of labour and time in staining and examining and the economy of material that is gained by the multiple method of smearing—at Culion 6 or 8 smears are regularly made on one slide, and often more—cannot be had when smears are made by either variety of the snip method.

Efficiency.—In comparative tests made by Lowe and Christian (3) it was found that the snip method was “slightly more efficient than the slit method.” It is granted that, specimen by specimen, this may be so; in view of the amount of tissue taken in a snip it would be rather surprising if it were not so when bacilli are few. But considering the limitations of that method as regards multiple and repeated examinations one must strongly question any claim for superiority, or even equality, on that ground.

With regard to the general question of the use of the bacteriological examination, it cannot be insisted too strongly that it is a clinical procedure, and an essential and simple one. Many men working with lepers have no bacteriological laboratory at their disposal, but any one who can examine sputum for the tubercle bacillus can make this examination. It is quite as simple, and many intelligent, conscientious ward and clinic helpers, both patients and others, have been trained to make it. It is to be hoped that it will not be long before no one will attempt to diagnose, classify or treat leprosy without routine use of this procedure, for until that time comes cases cannot be properly classified or the results of treatment properly evaluated.

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