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CHANGES AND ADVANCES IN CURRENT TECHNOLOGY FOR THE BACTERIOLOGICAL
DIAGNOSIS OF TUBERCULOSIS⁽¹⁾

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1. INTRODUCTION

"We are henceforth confronted by a visible and tangible parasite...; this parasite can exist only in the body of human beings and animals: a very reassuring fact as far as tuberculosis control is concerned. It means that we must concentrate above all on eliminating the sources of infection. One of these sources, undoubtedly the chief one, is the sputum of consumptives which we must endeavour to disinfect and render harmless." In these few sentences, taken from the paper describing the discovery of the bacillus that bears his name, Robert Koch¹ a century ago implicitly defined the functions of bacteriology in tuberculosis control: to identify the sources of infection and make sure that they are sterilized. The functions of bacteriology remain the same today. Nevertheless, as a result of scientific progress, the bacteriological methods available are more numerous and more specific than in Koch's day. In order of priority they are: direct smear examination, culture, identification of positive cultures as belonging to species responsible for tuberculosis, and sensitivity testing.

2. AVAILABLE BACTERIOLOGICAL METHODS

2.1 Microscopic examination

Since pulmonary tuberculosis is the most widespread form of the disease and the one responsible for transmission of the bacilli, it is sputum specimens that are most commonly subjected to microscopic examination. Accordingly the microscopic examination of sputum is described here in detail.

(1) A french text may be obtained from the Tuberculosis and Respiratory Infections Unit, WHO, Geneva, Switzerland.

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The first stage in microscopic examination is to smear a purulent or haemorrhagic portion of sputum on a new* slide carefully freed from grease. After drying at room temperature the smear is fixed by heat (by passing it three times through a flame). It is then stained either by the conventional Ziehl-Neelsen method or by the auramine method. Both methods are based on the fundamental property of the mycobacteria, acid-fastness and alcohol-fastness. Slides stained by the Ziehl-Neelsen method are examined under an ordinary microscope with an oil immersion objective (x 100), those stained by the auramine method are examined under the fluorescence microscope with a low power objective (x 25). The bacilli stain red in the former case, fluorescent yellowish-green in the latter.

The advantage of fluorescence microscopy is that the microscopic field examined is 16 times as large as that examined after Ziehl-Neelsen staining. Reading is speeded up accordingly. The drawback is the much higher cost of fluorescence microscopy, not just for purchase (at least double) but also for maintenance (especially the replacement of the mercury vapour lamp).

Whether an ordinary optical microscope or a fluorescence microscope is used, the slides must be read in a uniform manner and the visible acid and alcohol-fast bacilli must be expressed quantitatively.²

Two points of detail need to be clarified. In the Ziehl-Neelsen method it is possible to decolorize the smears with hydrochloric acid (HCl) and avoid the use of alcohol. In fluorescence microscopy it is important to use two objectives, x 20 or 25 and x 40, the former to detect the bacilli and the latter to determine their morphology. They should both be uncorrected objectives, as the smears are examined without a cover-slip.

2.2 Culture

For routine use the best culture medium for isolating Mycobacterium tuberculosis is coagulated egg medium. The most widely used variant is the Löwenstein-Jensen medium without potato starch (IUAT medium). The culture medium should be placed in screw-capped bottles and inspissated at 85°C for 50 minutes.

The sputa and other specimens contain a mixture of mycobacteria and innocuous microorganisms, and the latter must be removed prior to seeding. This is done by various decontamination - homogenization techniques. One of the oldest, and certainly one of the simplest and best, is Petroff's sodium hydroxide method. The sputum is shaken up in twice its own volume of 4% sterile sodium hydroxide. After a contact time of 30-45 minutes the sodium hydroxide is neutralized in the presence of a pH indicator and the final product, with or without centrifuging, is seeded on several tubes of Löwenstein-Jensen medium. If the contact time with the sodium hydroxide is reduced, not all the innocuous micro-organisms are killed and the culture will be contaminated. If the contact time is too long, all the innocuous micro-organisms will be killed but so will a large proportion of the mycobacteria. Whatever method of decontamination is used, therefore, the decontamination time must be strictly complied with.

The seeded culture tubes are incubated at 37°C and examined every week for at least six weeks and a maximum of 12 weeks. The culture is declared positive as soon as typical colonies of acid-and alcohol-fast bacilli have developed, having biochemical characteristics whereby they can be identified as among the mycobacteria responsible for human tuberculosis (M. tuberculosis, M. africanum, M. bovis). Finally, as with the results of microscopic examination, the culture results should include a quantitative assessment of the number of colonies.

* Since used slides may carry residual micro-organisms or display streaks that respond to Ziehl-Neelsen stain, they should be thrown away.

2.3 Identification of cultures

In view of the frequency with which atypical commensal mycobacteria are isolated, particularly in the tropics, it is necessary to identify the colonies growing on the tubes of culture medium. Identification should include the following series of tests:

1. Ziehl-Neelsen staining on a smear from the colonies. This will verify that the bacilli are acid- and alcohol-fast.
2. Careful examination of the morphology and pigmentation of the colonies, bearing in mind that the pigmentation of some atypical mycobacteria sometimes appears only after exposure to light (photochromogenic organisms).
3. A niacin test and if possible a nitrate reduction test (by Virtanen's method) and a catalase test. A positive result to the first test is a virtually pathognomic sign of M. tuberculosis, provided the test is performed on a plentiful culture at least four weeks old and at most six weeks old. The second test is positive with M. tuberculosis but negative with M. bovis and with most M. africanum strains. The third test is pathognomic for the mycobacteria of tuberculosis, since virtually all the atypical mycobacteria have a heatstable catalase.^{3,4}

2.4 Tests for sensitivity to antibiotics

Many methods are available for testing the sensitivity of mycobacteria to antibiotics (Canetti et al., 1969). To produce valid results all these tests must be performed according to a strictly standardized procedure by qualified technicians, and great skill is needed to interpret their results. The easiest sensitivity tests to carry out and interpret are the isoniazid and rifampicin tests. Since they are also the most useful for clinical purposes, it is these tests which the investigator will endeavour to perform first. It must be emphasized, however, that sensitivity tests are usually indirect tests, performed on colonies from primary culture. It takes one month on average to obtain a positive primary culture and a further month to perform the sensitivity test, so the results of sensitivity tests do not become available until two months after tuberculosis is diagnosed and the patient is placed under treatment. In view of this time-lag the prescription of antibiotics is not routinely based on the results of the sensitivity tests.

3. CHANGES AND ADVANCES NEEDED

In 1974 the ninth report of the WHO Expert Committee on Tuberculosis⁶ pointed out that "from the point of view of their value in a tuberculosis control programme, bacteriological techniques may be ranked in the following order: examination of direct smears, culture and, lastly, sensitivity testing". There has been no recent discovery justifying a change in this order of priority. Nevertheless, it is worth while to consider what changes and advances might be forthcoming in the three areas of technology, organization and training.

3.1 Technological advances

There have been no recent modifications to the techniques for microscopic examination, culture, identification, and measurement of sensitivity to antibiotics, and it is unlikely that any decisive advances applicable in the field will be made in these areas in the near future. The most important consideration, therefore, is to select and use the available techniques and equipment in such a way that they are adapted as well as possible to the conditions of work and the climate.

3.2 Organizational advances

It is now evident that the progress still to be made is mainly of an organizational nature. Such progress will be made within a national tuberculosis control programme and will be based primarily on the standardization of methods, supervision by a national reference laboratory, and quality control. Standardization should cover technical aspects often ignored by the expert

biologists. For example, for the microscopic examination of sputum it is important to standardize the collection of specimens, their transport to the laboratory, the smear preparation technique, staining, reading under the microscope, and interpretation. Supervision by a national reference laboratory makes it possible to maintain a high technical quality in the examinations and at the same time preserves staff motivation. Lastly, quality control makes it possible to correct any shortcomings.

It is also necessary to ensure a regular supply of slides and stains and the maintenance of equipment, particularly the microscopes. This task could also be assigned to the national reference laboratory.

Finally, the technical staff must be given adequate protection against the risks of contamination during handling procedures, specifically by using safety cabinets.

3.3 Advances in staff training

In many countries there is a serious shortage of skilled technical staff. Considerable progress needs to be made in the training of skilled staff and in keeping up the skills of the staff already trained. Supervision, quality control and modern methods of continuous training should be used to maintain the skills and motivation of staff already trained.

An important final point concerns the implications of training technical staff. All training should lead up to an examination, and whenever a diploma is issued the recipient should be given increased responsibilities and a corresponding increase in remuneration. If these two requirements are ignored, failure is bound to result.

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