CLINICAL PATHOLOGY IN GENERAL PRACTICE

THE LABORATORY DIAGNOSIS OF PULMONARY TUBERCULOSIS

BY

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In any case of pulmonary tuberculosis the laboratory has two important roles to fulfil. First, it plays an essential part in the diagnosis; for until *Mycobacterium tuberculosis* has been demonstrated in exudate or tissues of a patient the diagnosis remains incomplete. Secondly, the laboratory has an important function to fulfil in the control of treatment. This paper is concerned only with the diagnosis of pulmonary tuberculosis. Nevertheless, at the stage of diagnosis there are certain steps which should be taken if the laboratory is to exercise its function in the control of treatment. These steps are mentioned in the course of the paper. The essential step in diagnosis is the demonstration of tubercle bacilli, and consideration is given first to the various tests that may be employed for this purpose. Later, their application to specific excretions or tissues is discussed.

Microscopical Detection of Acid-fast Bacilli

This is the most widely employed laboratory investigation in tuberculosis and has the great merit of speed. A report on the presence or absence of acid-fast bacilli in a given specimen is available in a few hours.

Unfortunately, there is no method of differentiating between *Myco. tuberculosis* and non-pathogenic acid-fast saprophytes under the microscope. Mycobacteria are, by definition, a group of bacteria which, when stained, resist decolorization with acid and alcohol. Since 1894, the Ziehl-Neelsen technique has been the accepted standard procedure all over the world. In this method the bacilli on the film are stained with carbol-fuchsin, decolorized with acid and alcohol, and counter-stained with methylene blue. The acid-fast bacilli thus appear under the microscope as red rods against a blue background, as viewed by reflected light. Recently a new technique has been developed whereby the acid-fast bacilli are stained by fluorescent dyes, decolorized in the normal way by acid and alcohol, and counter-stained. The stained bacilli, when irradiated by ultra-violet light, have the property of emitting visual light. This makes them appear larger and more easily seen under the microscope. While this new technique is a big advance from the laboratory point of view, it is essential to remember that it does not constitute a new development and that the property of acid-fastness is still the property which is recognized by both procedures.

Cultural Methods

Myco. tuberculosis is a slow-growing organism. Even when seeded on a culture medium favourable to its growth an inoculum containing viable tubercle bacilli may take from two to eight or more weeks to produce a recognizable colony. The length of time taken depends upon the number of viable bacilli in the inoculum, the smaller the number the longer the time. Obviously, if other bacteria are present in the material to be examined, these will overgrow the whole surface of the culture medium and will swamp the *Myco. tuberculosis*. It is therefore necessary to kill off the other bacteria in the specimen before inoculating it on to culture medium. It is also desirable to concentrate as many as possible of the viable tubercle bacilli from the whole specimen into a small volume, for the larger the number of bacilli used the quicker the growth will become evident.

Fortunately, mycobacteria are far more resistant to the action of acids and alkalis than other bacteria, and the most satisfactory methods of concentration are based on this difference. It must be remembered that in a grossly contaminated specimen with much pus and secondary infection the amount of exposure to acid or alkali which is necessary to kill the secondary organisms in the specimen may also kill the majority of tubercle bacilli in that specimen. Therefore such specimens are apt to give false-negative results.

Animal Inoculation

Until the development of modern egg media, animal inoculation was a much more sensitive method of detecting *Myco. tuberculosis* than culture. From the large number of investigations which have been performed to compare the relative merits of the two methods, it is clear that cultures are now as sensitive as animal inoculation, and the high cost of the latter would prohibit a high proportion of the investigations now usefully performed by culture. In material in which contamination by acid-fast saprophytes is likely, as in specimens of urine, and in which typing of the strain is required, animal inoculation is still the method of choice. Indeed, the usefulness of animal inoculation is now very limited, and cultural methods on the more normally submitted material are every bit as accurate.

Pathological Material

These standard methods of investigation must now be considered in relation to the pathological material which is available for diagnosis in cases of pulmonary tuberculosis.

Sputum

The material most often examined in cases of suspected or proved pulmonary tuberculosis is undoubtedly sputum. The specimen should contain little saliva and be free from food particles. The early morning, before breakfast, is the best time to collect such a specimen and the patient should be provided with a clean, dry, wide-mouthed sterile container. If the disease has not previously been diagnosed the specimen should be examined by a direct film for microscopy, and whatever the result the specimen should be concentrated and cultured for *Myco. tuberculosis*. Unless the first specimen shows many acid-fast bacilli in the direct film, it should be followed by two further specimens, which will also be put up for culture if the diagnosis of pulmonary tuberculosis is regarded as likely on clinical grounds. If these three specimens prove negative on microscopy it will be necessary to repeat the test at frequent intervals until either a positive result is obtained or some diagnosis other than tuberculosis is established. In any event, the wisest course at this stage is to persist in cultures until a positive result is obtained, especially if there is any likelihood that chemotherapy will be instituted or any possibility that the patient has contracted the disease from a patient who has been treated with streptomycin.

Gastric Lavage

The stomach washings are useful material from which to cultivate *Myco. tuberculosis* in patients with pulmonary tuberculosis who fail to expectorate.

The specimen should be taken before breakfast and there should be no delay in submitting it to the laboratory. It must be remembered that the gastric juice is strongly acid and if the specimen is left unneutralized for more

than one hour it is probable that the tubercle bacilli will be killed. To the gastric washings 5 ml. of 10% trisodium-phosphate should be added to neutralize the acid. The presence of acid-fast bacilli in a smear made from the gastric contents is not conclusive evidence of the presence of *Myco. tuberculosis*. Many foodstuffs, notably butter, are often contaminated with saprophytic acid-fast bacilli. It is therefore essential to culture all specimens of gastric contents.

Laryngeal Swab

Gastric lavage is often difficult and usually impossible in general practice, and to meet this situation with patients who do not expectorate the technique of a laryngeal swab has been developed. A sterile swab, moistened with sterile water, is passed with the aid of a laryngeal mirror into the larynx. The patient then coughs, and any sputum in the upper part of the bronchial tree will adhere to the swab. This swab is treated with 10% sulphuric acid for five minutes, and then placed in 2% sodium hydroxide for a further five minutes. It is then inoculated on to Löwenstein-Jensen medium and incubated in the ordinary way. Nassau* obtained a positive swab from 44% of 273 sanatorium patients who did not expectorate, and Forbes and his colleagues† showed that the results of laryngeal swabs were as reliable as gastric lavage in out-patients.

Faeces

In children the faeces may be investigated for the presence of *Myco. tuberculosis* in cases of pulmonary tuberculosis in preference or in addition to the gastric lavage. This method is also sometimes used in adults, but here the presence of large numbers of tubercle bacilli are more likely to indicate tuberculosis of the intestinal tract than pulmonary tuberculosis.

Pleural Fluids

Simple non-turbid pleural effusions, although most commonly of tuberculous aetiology, often contain only few tubercle bacilli. The probability of demonstrating *Myco. tuberculosis* is increasingly likely the larger the volume of fluid used. As these fluids often contain fibrinogen, an anticoagulant should be added—for example, 1 ml. of 20% sodium citrate. If possible about 100 ml. of fluid should be examined microscopically. If no organisms or only acid-fast bacilli are seen, six slopes of Löwenstein-Jensen medium may be inoculated. If other organisms are seen the material is used first for culture of secondary organisms and then treated by Petroff's or the trisodium-phosphate techniques before cultivation for *Myco. tuberculosis*. A small volume of the fluid should be examined for cells, and in simple tuberculous effusion the lymphocytes will predominate.

Turbid and purulent fluids should be treated in the same way, and in these cases there will be greater probability of successful cultivation.

One of these procedures will result in a positive culture from a case of pulmonary tuberculosis. With the widespread use of chemotherapeutic agents in treating tuberculosis, it is inevitable that some cases of pulmonary tuberculosis will be found in which the organisms are resistant to one or other of the chemotherapeutic agents. It is therefore necessary that the first culture from a new case of pulmonary tuberculosis should be preserved and preferably have its sensitivity tested against streptomycin, P.A.S., and isoniazid, as, even if not immediately, chemotherapy is very likely to be used sooner or later in the course of the patient's treatment.

The definitive diagnosis of pulmonary tuberculosis depends upon the demonstration of *Myco. tuberculosis* in the tissues or excretions. Nevertheless, there are other ancillary tests which, while not actually diagnostic of pulmonary tuberculosis, are of great assistance in estimating its severity and the course of treatment. These tests may be divided into the serological tests and the haematological tests.

Ancillary Investigations

Middlebrook and Dubos‡ showed that sheep's erythrocytes suitably activated with an extract of the strain *Myco. tuberculosis* H37Rv were agglutinated by the blood serum of some cases of pulmonary tuberculosis. Various other workers have made investigations along these lines, each of them making minor modifications in the technique. Sohler, for instance, used purified tuberculin to sensitize the red cells and obtained good results with tuberculous sera but had a high percentage of false-positive results in normal controls. While this relatively recent development may eventually prove satisfactory, there is at present no serological diagnostic test of proved value available.

Haematology

The most constant alteration in the blood picture in all forms of pulmonary tuberculosis is a progressive hypochromic anaemia. This usually reacts well to adequate treatment.

There have been many investigations into the changes in the total and differential leucocyte counts which may be associated with pulmonary tuberculosis. The only fact which emerges with certainty is that there is no specific change in the leucocyte count of diagnostic value. On the other hand, the disease may be associated with either a leucopenia or a moderate leucocytosis. There have also been reports of a leukaemoid reaction in a few cases of tuberculosis. The monocyte count is often raised (over 10%), especially when fresh tuberculous tissue is being formed, while a raised lymphocyte count is often associated with healing tuberculous lesions.

Erythrocyte Sedimentation Rate

Although the erythrocyte sedimentation rate is not in any way specific for tuberculosis, it is nevertheless of great value. In all toxic conditions associated with tissue destruction the sedimentation rate is raised, and thus the test is no more specific than pyrexia or leucocytosis. The sedimentation rate is always raised in active tuberculosis and falls as healing takes place. Serial examinations are thus of great value in the control of patients suffering from pulmonary tuberculosis, and a spread of the disease may manifest itself by a rise in the sedimentation rate before there is any radiological or clinical evidence of it. The test is also often valuable in cases in which radiographs reveal a lesion which is of doubtful activity, and in these cases a raised sedimentation rate is strong evidence of activity.

Westergren's method is commonly employed in tuberculosis. In this method 0.4 ml. of 3.8% citrate solution is drawn into a syringe which is then introduced into a vein. Blood is withdrawn until there is 2 ml. of mixed blood and citrate in the syringe. The sedimentation tube, which has an internal diameter of 2.5 mm. and is calibrated in millimetres from 0 to 200, is then filled in the manner of an ordinary 1-ml. pipette. The column of blood is drawn up to the zero mark on the scale and the tube is placed to stand vertically in a rack. At the end of one hour the number of millimetres through which the erythrocytes have sedimented is recorded. The disadvantage of the method lies in the fact that it is impossible to correct for anaemia, but the ease of the technique more than compensates for this somewhat technical disadvantage. It has been found in practice that serial readings of the sedimentation rate by the Westergren method are of very great assistance in controlling individual cases, and it is therefore always desirable to take a reading as early as possible in every case of pulmonary tuberculosis.

Next article on Clinical Pathology.—"The Normal Blood Count," by Dr. George Discombe.

* *Proc. roy. Soc. Med.*, 1941, 34, 397.

† *Lancet*, 1948, 2, 141.

‡ *J. exp. Med.*, 1948, 88, 521.