Bacteriological Aspects of Mycobacterial Infections

D. A. MITCHISON

British Medical Journal, 1972, 1, 424-425

Pathogenesis

Most mycobacterial infections are caused by *Mycobacterium tuberculosis*. Infections with *M. bovis* are increasingly rare in Britain,⁸ even in non-pulmonary disease, and are even less often encountered in developing countries. Pulmonary disease, due to atypical mycobacteria, is of increasing importance in Britain.¹¹⁻¹³ About 1.5% of patients with the radiographic appearances of pulmonary tuberculosis yield cultures of atypical mycobacteria and this percentage has probably been increasing during the past two decades.^{8, 14} Disease due to these organisms is extremely rare in many developing countries presumably because the particularly susceptible patients likely to develop disease more often encounter *M. tuberculosis*.

The most important pathogen is *M. kansasii*, which is responsible for about two-thirds of these infections and is associated with previous exposure of the patients to dust. Infections with *M. avium* and *M. intracellulare* (the avian-Batley complex) and with *M. xenopi*, are less common. *M. scrofulaceum* usually causes glandular disease, while pulmonary or glandular disease due to *M. fortuitum* is most uncommon. *M. marinum* (*M. balnei*) and, rarely, *M. ulcerans* cause lesions confined to the skin and other superficial areas because they can only grow at temperatures slightly lower than 37°C. Close contacts of patients with atypical infections may show skin sensitivity to the specific tuberculin,¹⁵ but there is no evidence that they have a higher rate of disease due to the organisms.^{16, 17} These infections, particularly those due to *M. avium* and *M. intracellulare*, are very serious because they may not respond to antituberculosis chemotherapy.¹⁸

Diagnosis

Mycobacterial infections are most effectively diagnosed by bacteriological methods. The demonstration of acidfast bacilli in smears or the isolation of the organism in culture is indisputable evidence of the nature of the disease. Nevertheless, material for examination is not always so easily obtainable, particularly in non-pulmonary disease. Furthermore, cultures take 3-4 weeks (on average) to become positive and more rapid methods would be advantageous. Several attempts have been made to develop immunological methods which would distinguish between inactive and active tuberculosis, but most of these have failed to yield a satisfactory distinction between these two groups. Recently, Nassau and Merrick¹⁹ have described a new fluorescence antibody method with promising initial results. Should these results be confirmed and extended, an important diagnostic advance will have been made.

Direct smears are examined most economically and efficiently by fluorescence microscopy,^{20, 21} and therefore the Ziehl-Neelsen technique should now be abandoned, except for small laboratories in developing countries (Table I). The benefits to be gained by culture, as well as smear examination, depend on the severity of the disease in the patients presented for diagnosis. The proportion of culture-positive cases of pulmonary tuberculosis with negative smears is much higher in Britain with

TABLE I—Approximate Cost of Direct Smear Examination by Ziehl-Neelsen and Fluorescent Microscopy

No. of Smears Examined per Day	Method of Examination	Cost of microscope (£)			Technician's Salary (£1000 p.a.) (c)	Total Annual Costs (£) (a + b + c)
		Capital	Annual			
			Depreciation (a)	Maintenance (b)		
10 ..	Ziehl-Neelsen Fluorescence ¹	300 550	30 55	5 30 ²	120 24 ³	155 109
30 ..	Ziehl-Neelsen Fluorescence	300 550	30 55	5 50	360 72	395 177
100 ..	Ziehl-Neelsen Fluorescence	600 ⁴ 550	60 55	10 70	1200 240	1330 365

¹ For instance, a suitably equipped Leitz model SM.

² Mainly the cost of high pressure mercury vapour bulbs, whose life depends partly on number of switchings and partly on total operating time.

³ Bennedson and Larson²⁰ found that fluorescence microscopy was five times more rapid, for similar efficiency, than Ziehl-Neelsen microscopy.

⁴ For 100 smears daily, at least one additional microscope would be necessary for the Ziehl-Neelsen method, but about 500 smears per day can be examined with a single fluorescence microscopy.

frequent minimal disease than in most developing countries.²¹ Thus it is always advisable to examine specimens by both smear and culture in Britain, whereas it may be more economical to use smear examinations only in developing countries.

BACTERIOLOGICAL CULTURE

Culture for *M. tuberculosis* should be done by fairly efficient methods, since the time and money spent on medium preparation, adequate incubator space, and regular culture readings offset any small gains made by the use of relatively inefficient procedures. Efficient methods are modified Petroff procedures^{22, 23} and the sodium lauryl sulphate procedure.²⁴ The sputum swab method is not advisable.

In laboratories which inoculate each specimen on to two slopes of Löwenstein-Jensen medium, one should contain 0.5% sodium pyruvate, since this addition encourages the growth of *M. bovis*.^{25, 26} Nevertheless, retention of medium without the addition is necessary as pyruvate inhibits *M. kansasii*.

Cultures of mycobacteria should be routinely screened to distinguish between mammalian tubercle bacilli and other mycobacteria.

Though atypical mycobacteria are often human pathogens when isolated in British laboratories, it is often not appreciated that they are frequently obtained totally unrelated to the causation of disease in cultures set up in tropical laboratories. Presumably these organisms are common because mycobacteria, multiplying as saprophytes in a hot, humid climate, are better adapted to growth at 37°C on culture medium than in temperate climates. Useful screening procedures include the niacin test^{27, 28, 30} and ability to grow on egg medium containing *p*-nitrobenzoate³¹ or normal medium incubated at 25°C. One or more of these tests should be carried out routinely on every positive isolate.

The full identification of atypical mycobacteria should be done in a reference laboratory. Some of the most useful methods,³² such as serology, lipid analysis,³³ agar-gel diffusion against mycobacterial antigens^{34, 35} and bacteriophage lysis patterns, are specialized procedures and only a few people have the necessary skill to interpret the results. Nevertheless, preliminary identification can usually be achieved by using a small series of tests including those for speed of growth and formation of pigment of exposure to light,³⁶ catalase activity, sensitivity to thiocetazone 10 µg/ml, Tween-80 hydrolysis³⁷ and growth at 42°C. Catalase and Tween-80 hydrolysis activity are particularly useful in distinguishing potentially pathogenic strains from saprophytes (Table II).

TABLE II—Provisional Identification of *Mycobacteria* Growing at 37°C

Species	Speed of growth	Pigment	Catalase	Tween-80 hydrolysis	Growth	
					on thiocetate 10 µg/ml	at 42°C
<i>M. tuberculosis</i>	slow	buff	2+	—	(—)	—
<i>M. bovis</i> ¹	slow	buff	2+	—	(—)	—
<i>M. kansasii</i>	slow	orange in light yellow dysgonic	3+	+	—	—
<i>M. xenopi</i>	slow	yellow in dark	1+	—	+	+
<i>M. scrofulaceum</i>	slow	buff	3+	—	+	+
<i>M. avium</i> ²	slow	buff	1+	—	+	+
<i>M. intracellulare</i> ²	slow	buff	1+	—	+	+
<i>M. fortuitum</i> ³	rapid	buff	3+	+	+	—

¹ Distinguished from *M. tuberculosis* by a negative niacin test, no nitrate reduction and no growth on medium containing 1 µg/ml furan-2-carboxylic acid hydrazide.

² Best identified by serological methods.⁴⁶

³ Identified by special methods, particularly lipid analysis.⁴⁷

Sensitivity Tests

A description of the three main methods of doing sensitivity tests on cultures (indirect tests) and when they should be done have been given by Canetti *et al.*³⁸ In brief, tests done by the absolute concentration method are simple and accurate, but require individual calibration in each laboratory using them, especially for some antibacterial drugs such as streptomycin. The resistance ratio method is slightly less accurate and requires more medium, but there is less need for calibration in individual laboratories. The proportion method is the most time-consuming, though it gives the most accurate answer with some of the second-line drugs.

Sensitivity tests should always be done when the results of serial smear and culture examinations show that a patient is relapsing during or after the end of a period of chemotherapy. In a true relapse during chemotherapy the organisms are always resistant to at least one of the drugs used.³⁹ This is uncommon, provided that the regimen is efficient. Nevertheless, if chemotherapy has been terminated intentionally by the physician or, unintentionally by failure of the patient to take the drug, the organisms are often still drug-sensitive,⁴⁰⁻⁴³ and the originally prescribed chemotherapy may be given again.

The value of routine pre-treatment sensitivity tests is dubious.

A recent study in Hong Kong has investigated the benefits gained by such tests in patients treated with isoniazid, streptomycin and PAS for three or six months, followed by isoniazid and PAS.⁴⁴ The patients were treated in Hong Kong but standard sensitivity tests (and proportion sensitivity tests) were done at the M.R.C. Unit for Laboratory Studies of Tuberculosis. The patients were randomly allocated to three policies. In policy A, they were given the standard regimen, irrespective of the results of pre-treatment sensitivity tests. The drugs prescribed were changed only if serial smears and cultures taken during chemotherapy showed evidence of a relapse. In policy B treatment was modified along conventional lines by substitution of one or more reserve drugs (ethionamide, pyrazinamide, cycloserine) for those to which the organisms were initially resistant by the standard test. Particular care was taken to get the test results to the clinicians in Hong Kong as early as possible—usually 6-8 weeks after chemotherapy had begun. In policy C rapid slide-culture tests were done in Hong Kong before treatment was started and the drugs used depended on the results of this test.

Very little difference was found in the success rates obtained with these three policies despite the high prevalence of initial drug resistance in Hong Kong (Table III). Applying the results to Britain, the proportion of patients who could be prevented from having a relapse during treatment, by the use of initial sensitivity tests (policy B), was estimated as only 0.6%. In practice, even less benefit would result since the tests might well be done under less favourable conditions and less promptly reported. Furthermore, the few relapses which were probably due to initial resistance

TABLE III—Summary of Results of Hong Kong Study of Policies of Sensitivity Testing

Policy	Total patients	Patients with initial resistance ¹ to:		Favourable response ² at 12 months No. (%)
		One or More Drugs No. (%)	Isoniazid and streptomycin No. (%)	
A	187	62 (33)	25 (13)	167 (89)
B	192	48 (25)	17 (9)	176 (92)
C	187	62 (33)	23 (12)	175 (94)

¹ By standard indirect M.R.C. tests.

² Bacteriologically quiescent disease.

would be detected if serial examinations of sputum, by smear and culture, are done during treatment. Such examinations are necessary in any case, because as many relapses were found to occur as a result of patients failing to take their drugs regularly as were due to initial resistance. On the other hand, treatment without initial sensitivity tests (policy A) had the advantages of lower drug toxicity, lower costs of hospital care, lower drug costs, lower laboratory costs, and simplicity of operation.

Sensitivity tests for reserve drugs should not be done unless a patient has already received a course of treatment with such a drug.³⁸ In the absence of previous treatment, there is no evidence of the occurrence of primary resistance to rifampicin. Primary resistance to ethambutol occurs very rarely and, since sensitivity tests to this drug are both technically difficult and have a problematic interpretation, no real benefit is achieved by doing them. Other drugs, such as pyrazinamide or capreomycin, contribute so little to the chemotherapeutic potency of the reserve regimen that sensitivity tests are unwarranted.

Control of Chemotherapy by Smear and Culture

Provided that treatment starts with an effective triple-drug regimen, little benefit will be gained by examining sputum during the first four months of treatment. The most economical and effective rhythm of examination is to do smear and culture examinations at four months, six months, and at three-monthly intervals thereafter until at least six months after the course of chemotherapy has ended. If any of the results from six months onwards are positive, a further one or two specimens should be examined to assess the situation. Isolated positive results, usually with only one or two colonies on culture, may be obtained from patients making satisfactory progress for as long as two to three years at least after the start of chemotherapy. On the other hand, two or more positive cultures suggest a bacteriological relapse due either to initial chemotherapy or to failure in drug taking. Sensitivity tests should be done as soon as there is suggestive evidence of a true relapse, though even more bacteriological examinations may be necessary to establish whether it is necessary to retreat the patient.

Centralization of Laboratory Services

A strong case may be made for increasing the degree of centralization of laboratory services for the diagnosis and treatment of mycobacterial disease. Specimens of sputum can easily be sent by post without important loss of viability of the organisms.²¹ All aspects of the laboratory work, including smear and culture examination, are most efficiently done in large laboratories with specialized staff. It is particularly important that as few as possible laboratories should be doing sensitivity tests, and the decision of the Public Health Laboratory Service to restrict laboratories doing this work to a small number is to be commended.