Mycobacteria Other than M. tuberculosis
in Sputum of Tuberculous Patients in South Africa

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Introduction

The three main factors contributing to the more frequent isolation of atypical
mycobacteria in recent years are probably the intensification of culturing in the
era of chemotherapy, the decline of human tuberculosis in certain countries, and
the ability of atypical mycobacteria to survive anti-tuberculosis therapy. It is
conceivable that persons who never come into contact with the classical patho-
genic mycobacteria or BCG are more prone to develop disease with other potentially
pathogenic mycobacteria. If this assumption is correct atypical mycobacteria
would occur less frequently in regions in which tuberculosis is prevalent.

In particular M. kansasii and M. intracellularis (RUNYON, 1965), also called Battey
bacilli, Group III or M. avium-like are important pathogens in some parts of the world,
especially the southern states of U.S.A. and Western Australia (RUNYON, 1960; KOVACS,
1962). From publications by MARKS (1960), MEISSNER (1965), EDWARDS and others (1957),
WONG (1964), ENGEZ and others (1965), YAMAMOTO and others (1965) and THOMAS and others
(1961), it appears that the incidence of pulmonary mycobacterioses, due to atypical myco-
bacteria is much lower in Europe and Asia. Reports on mycobacteria other than M. tuberculosis,
also come from Africa. In the Congo, VAN DEN ABBEELE (1961) isolated bovine and atypical
mycobacteria from pulmonary and extrapulmonary material of man. In various countries
of Central Africa, acid fast organisms have been seen and sometimes isolated from skin ulcers
by OYE and BALLION (1950), JANSSENS (1950), HENNEBERT and others (1961), VANDEPITTE
and others (1961), CLANCY and others (1961), CLANCY (1964) and ANDERSEN (1965). More
recently investigations on atypical mycobacteria in lung material of man were reported by
BEER and DAVIES (1965) from Nigeria, CAVANAGH (1965) from Sudan, and BOURDARDOT and
others (1965) from Senegal. The laboratory at the King George V Hospital in Durban collected
36 strains of atypical mycobacteria during the last 10 years. WORTHINGTON (1965) cultured
sputum of 600 natives in Ovamboland, S.W.A., and found 4 strains of atypicals which, in his
opinion, were of no pathogenic significance. Reviews by DORMER (1957) and NEITZ (1965)
from South Africa, and reports by VAN DEN ABBEELE (1961), CAVANAGH (1965) and BEER and
DAVIES (1965) from other parts of Africa, indicate that infection of man by M. bovis plays a
very minor part.

This report deals with the results of the examination of 4,000 sputa from
patients in South Africa. The aim of the study was to establish the incidence of
mycobacteria other than *M. tuberculosis* in cases provisionally diagnosed as pulmonary tuberculosis.

**Material and Methods**

The sputa of 4,000 Bantu patients, in whom a diagnosis of pulmonary tuberculosis had been made by X-ray, smear and/or clinical examination, were cultured. Sputum, accompanied by a questionnaire, was sent to this laboratory from hospitals in the Transvaal and Transkei. Those which were in transit (100--600 miles) for more than one day were mixed at the hospital with equal parts of Pancreatin-Desogen solution (STOTTMEIER and KLEEBERG, 1966), and the others treated on arrival with the same solution in the laboratory and shaken (SAXHOLM, 1958; MEISSNER and TUNÇMAN, 1962). Two drops of the treated sputum (0.1 ml) were inoculated on to 2--4 screw cap bottles with Löwenstein-Jensen medium with and without glycerine.

They were kept in a horizontal position until the next morning and then incubated at 37 °C for 4--8 weeks. Drug sensitivity tests were done in all positive cases by harvesting the primary culture with a loop and suspending in a 0.1% solution of Tween 80 in water, to give a suspension of 0.1 mg/ml of bacteria. One loop of the suspension (10^-3 mg) was used as inoculum. The absolute concentration method was used for testing the drug sensitivity. The following concentrations in Löwenstein-Jensen medium were used:

- **Isoniazid**: 0.2--1.0--10.0 mcg/ml
- **D-hydro-streptomycin**: 0.0--50.0 mcg/ml
- **PAS**: 1.0--10.0--100.0 mcg/ml
- **Thiacetazone**: 1.0--10.0 mcg/ml
- **Ethionamide**: 20.0--30.0 mcg/ml
- **D-cycloserine**: 20.0--30.0 mcg/ml
- **Viomycin**: 20.0--30.0 mcg/ml
- **Pyrazinamide**: 20.0--100.0 mcg/ml

(Control tube for testing pH 5.0 tolerance).

Isolations were first suspected to be atypical, when they were chromogenic, the drug sensitivity results were indicative of atypicals or the speed of growth was faster than seen with *M. tuberculosis*. Drug sensitivity tests were read after four weeks, but strains thought to be atypical were read weekly for four weeks. Only strains were included which grew as pure culture on the primary isolation and were not mixed with *M. tuberculosis* or other mycobacteria.

The following biochemical tests were employed for classification and identification — Konno’s niacin test as modified by RUNYON (1959), catalase peroxidase reaction (ANDREJEW and others, 1960), sensitivity to pyromucio acid hydrazide for the differentiation of INH sensitive *M. bovis* (BÖNICE, 1958), and Bönicke’s (1961) amidase tests. Further bacteriological differentiation was done by studying colony morphology of single cell colonies; temperature tolerance at 22°, 31°, 37°, 45° and 52 °C; and exposure to continuous light inside an incubator during the growth period.

Animal inoculations were done using rabbits, fowls, guinea pigs and mice. All atypical strains were inoculated subcutaneously into guinea pigs in a dose of 1 mg, the rapid growers were also injected intramuscularly and intracardially. Each rapid growing strain was also injected intravenously into eight mice, at a dose of 0.3 mg. All slow growing non-chromogenic strains were injected intravenously into rabbits at a dose of 1 mg, as was the strain thought to be a Nocardia. Rapid growth was defined as ability to grow at 37 °C as well developed colonies from single cells within five days on Löwenstein-Jensen medium. Non-chromogenic and chromogenic slow growers were injected into fowls intravenously at a dose of 0.1 and 1.0 mg. The sensitin specificity pattern was established in guinea pigs using six different sensitins.

**Results**

From the sputa of 4,000 African patients judged to be tuberculous by clinical symptoms and X-ray, about 2,000 strains of *M. tuberculosis* and 14 other strains