New Characteristics of *Mycobacterium haemophilum*

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The first isolation of *Mycobacterium haemophilum* in Victoria was from 8-month-old leg lesions in an immunosuppressed renal transplant patient. Characteristics of the isolate that had not been described previously for *M. haemophilum* included growth enhancement by carbon dioxide, a temperature optimum that was lower than previously reported, and the surprisingly slow growth of the primary isolate.

There have been only seven previous reports of the isolation of *Mycobacterium haemophilum*: one was from Israel (14), one from Ohio (2), four from Sydney (4; all six were from skin lesions of middle-aged immunosuppressed patients), and one from Rockhampton, Queensland (3) from the submandibular lymph node of an infant. This case report describes the unusual bacteriological features of our isolate.

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In January 1981, a 47-year-old Melbourne woman who had received a renal transplant 7 months previously and was under stable immunosuppresion on 25 mg of prednisolone per day presented with a discharging sinus from an interphalangeal joint on her left second toe. In the ensuing months, a purplish, infiltrated plaque appeared on her right ankle, progressing to several lesions. In June 1981, the toe was treated by debridement and arthroplasty, and a biopsy of one of the pigmented lesions on the right leg was subsequently taken. Acid-fast bacilli (AFB) were seen in the tissue from both the left toe and the right ankle. The toe tissue showed a granulomatous reaction including numerous Langhans' giant cells. No AFB were grown from any of the specimens on media for the cultivation of *M. tuberculosis*.

The patient was commenced on daily doses of 450 mg of rifampin, 300 mg of isoniazid, and 400 mg of ethambutol, and her prednisolone dose was reduced to a maintenance level of 20 mg/day: the lesions, however, failed to regress. A further skin biopsy from the right leg taken in September 1981 revealed AFB, and cultures from this specimen on Löwenstein-Jensen medium supplemented with 2% ferric ammonium citrate yielded colonies of AFB after 3 months of incubation at 20°C. Later in the year, all the lesions began to regress and had fully resolved by January 1982.

The isolate was a weakly gram-positive AFB. In subcultures it grew, in decreasing order of abundance, on chocolate agar, lysed horse-blood agar, plain horse-blood agar, and Löwenstein-Jensen medium supplemented with ferric ammonium citrate but failed to grow on plain Löwenstein-Jensen medium, nutrient agar, and MacConkey agar lacking crystal violet. In 12 days or less on chocolate agar at 20°C, the colonies were 1 mm in diameter but tending to become confluent, buff colored, rough, and nonphotochromogenic when tested by standard methods (12). At 28 and 33°C colonies were not so well developed as at 20°C in 12 days or an even longer period; at 37°C, there was either no growth or at most only a few pinpoint colonies after 7 weeks of incubation. However, 5 to 10% carbon dioxide in air greatly stimulated growth on chocolate agar at 37°C and yielded numerous 1-mm colonies after incubation for 7 weeks. There was no growth at any temperature in an anaerobic atmosphere containing 5% carbon dioxide.

The biochemical reactions of the isolate were typical of *M. haemophilum*, being negative for sodium chloride tolerance, niacin production, Tween hydrolysis, tellurite reduction (after 3 days), iron uptake, catalase (68°C), urease (3 days), and arylsulfatase (3 and 14 days), equivocal (negative or weakly positive) for nitrate reduction, and positive for pyrazinamidase (4 days) when tested by standard methods (12).

The identity of the isolate was confirmed by agglutination with an antiserum to *M. haemophilum* (ATCC 29548), and all agglutinins for the type culture were removed by absorption of the antiserum with the isolate.

The isolate was resistant to streptomycin, isoniazid, and ethambutol and sensitive to rifampin when tested by the resistance ratio method.

Mycobacterial disease in immunosuppressed patients is well documented. Infections are usually caused by *M. tuberculosis* (1, 5, 9, 16), but occasionally other species are responsible (1, 7, 8, 15). *M. haemophilum* has been described as a human pathogen only rarely (2–4, 14). In recent years, there have been reports of noncultivable AFB (6, 10, 11) causing skin infections, even in normal hosts, and some of these infections may have been due to *M. haemophilum*. Microbiologists and clinicians should be aware of this organism as a potential pathogen.

With the addition of ferric ammonium citrate to Löwenstein-Jensen medium containing glycerol but not pyruvate, recovery of *M. Haemophilum* can be expected if the incubation temperatures are kept at 20 to 33°C. At the Alfred Hospital, culture on Löwenstein-Jensen medium supplemented with ferric ammonium citrate is now part of the standard mycobacterial analysis on all specimens of skin biopsies and lymph nodes. Carbon dioxide is known to enhance the growth of *M. tuberculosis* (13), and we have now shown this enhancement to extend to *M. haemophilum*. Possibly, the growth of other mycobacterial species is improved by carbon dioxide; we suggest that an atmosphere containing 5 to 10% carbon dioxide be tried when microscopically apparent AFB prove difficult to cultivate. When attempting to culture *M. haemophilum*, apart from ensuring that the appropriate cultural conditions are provided, great patience must be exercised in view of the prolonged time that may be required to make a preliminary isolation. Previous workers have suggested (3, 11) that prior decontamination extends the apparent incubation time required, and it may be that this factor contributed to the extraordinary primary incubation time of our isolate, as our skin biopsies were treated for 30 min with 1 M sodium hydroxide solution before being cultured.

At first sight, our isolate might appear to be resistant to an unusually large number of antimicrobial agents, but it should be noted that the second skin biopsy was not even taken until 3 months after the commencement of antimicrobial therapy. The isolate was sensitive to rifampin but this agent may not have had a substantial effect on the course of the disease, as the prednisolone dosage was reduced when the patient was commenced on antimycobacterial chemotherapy. Furthermore, published case reports do not allow one to be certain that infections resolved owing to antimicrobial agents or natural defense mechanisms and the lessening of immunosuppression. Finally, the lower temperature optimum of *M. haemophilum* may explain why the majority of isolations have been from the skin of the extremities.

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