Haemophilus aphrophilus endocarditis

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SYNOPSIS   *Haemophilus aphrophilus* was isolated from the blood of a 31-year-old man with sub-acute bacterial endocarditis.   Subsequently the patient died with acute tubular necrosis of the kidney, probably secondary to cardiac failure. The characteristics of the species are described and pathogenicity to mice is reported for the first time.

Amongst the many infections caused by species of haemophilus, only rarely has endocarditis been reported. The incidence of Haemophilus in subacute bacterial endocarditis lies between 0·5 and 1 % (Keith and Lyon, 1963), and the species most often involved are *H. influenzae* and *H. parainfluenzae*. Endocarditis due to a third species, *H. aphrophilus*, was first described in London (Khairat, 1940), and recently interest in this organism has been shown in America although fewer than 30 cases of *H. aphrophilus* endocarditis have been reported. Since the original account, before the advent of antibiotics, no infection due to *H. aphrophilus* had been reported in Britain until microorganisms resembling this species were described in reports (Speller, Prout, and Saunders, 1968; Vickerstaff, Pease, and Rogers, 1968) which suggested the possibility that the presence of such organisms may remain undetected unless appropriate methods are used. Therefore we considered it of value to describe the features of *H. aphrophilus* isolated from a recent fatal case of endocarditis.

CASE REPORT

A 31-year-old man was admitted to hospital with a history of haematuria for two weeks and ankle swelling for two months. One year previously he had experienced a bout of fever, rigors, and flitting joint pains, and was thought to have had rheumatic fever, after which he had continued to feel unwell. On admission to hospital he had gross congestive cardiac failure, with haematuria and a petechial rash on the hands, torso, and legs. The teeth were carious and the gums septic. The haemoglobin was 4·4/100 ml and the white cell count 16,000/cmm with 91% neutrophils. The blood urea level was 52 mg/100 ml.

A diagnosis of subacute bacterial endocarditis with mitral incompetence was made, and two blood cultures were taken. Both yielded *Haemophilus aphrophilus*. Treatment was begun with ampicillin together with

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diuretics, digoxin, and blood transfusion. After four days on ampicillin, the temperature rose from 37°C to 37·8°C, and cephaloridine was given in addition. The temperature remained normal thereafter.

The blood urea level rose to 200 mg/100 ml after 10 days, and the oedema resisted all therapy. Three weeks after admission, the patient died of renal and cardiac failure.

Postmortem examination (by Dr J. M. Drennan) revealed old healed rheumatic valvulitis with mitral incompetence and hypertrophy of both ventricles. A small friable vegetation was present on the mitral valve; this was not submitted for culture, and on microscopical examination no microorganisms were recognized. The myocardium contained numerous granulomatous lesions, and one small coronary arteriole was plugged by amorphous material which may have been embolus from the vegetation. The coronary arteries were free from atheroma and of good calibre. In the spleen there was an abscess, possibly an infected infarct. The kidneys were grossly enlarged and the histological picture was that of acute tubular necrosis; glomeruli and blood vessels were normal.

The report comments that 'the renal changes are non-specific and, in the absence of any other aetiological factor, can be attributed to cardiac failure due in its turn to the multiple small focal lesions seen in the myocardium. Although it seems not unreasonable to attribute these to embolization from the mitral vegetation, it would be surprising for such a small vegetation to produce so many lesions in the myocardium with so few elsewhere. The granulomatous appearance and the extensive granular calcification are also very unusual features in embolic microinfarcts of the heart.'

BACTERIOLOGY

The organism was isolated from the patient's blood by inoculating 5 ml volumes of the blood into bottles containing 50 ml of nutrient broth with added glucose (1% w/v). The bottles were incubated at 37°C, but no visible signs of bacterial growth were noted. After seven days the blood culture bottles were subcultured on to horse blood agar plates, one

incubated aerobically and one anaerobically, and on to a chocolate agar plate incubated in 10% carbon dioxide. After 18 hours minute colonies had appeared on the chocolate and anaerobic plates, but no growth became visible on the aerobic culture until after 72 hours' incubation. At first the organism was a small, non-motile, Gram-negative coccobacillus; on subculture it became more rod shaped, often curved and with pointed ends. Both aerobically and anaerobically growth was enhanced by the addition of carbon dioxide. On chocolate agar, growth was no better than on blood agar, and on simple nutrient agar growth was poor. In all atmospheres, growth was independent of both X and V factors, as were all the strains tested by King and Tatum (1962). The optimum temperature was 37°C.

With added carbon dioxide the colonies on blood agar were about 0·1 mm in diameter after 24 hours, enlarging to about 2 mm after a week, when the colony was circular, smooth, domed, opaque, and grey in colour. Alphahaemolysis occurred both in carbon dioxide and aerobically, but no green colour was produced on anaerobic culture. Some cultures produced, in addition to colonies as described above, much smaller colonial variants; on subculture these reverted to the larger type. After 10 days the colonies tended to grow outwards around their periphery, become wrinkled, and grow into the agar, but without adherence or indentation. In liquid media growth was adherent to the sides of the container, appearing as discrete colonies on the glass, while the broth remained clear.

Nitrate was reduced to nitrite. Weak hydrogen sulphide production occurred. The following tests were negative: catalase, oxidase, urease, indole, gelatin liquefaction, and no growth occurred on MacConkey's agar. In Hugh and Leifson's medium, fermentation was demonstrated. Acid was produced from glucose, lactose, sucrose, maltose, trehalose, raffinose, and levulose. There was no fermentation of mannitol, dulcitol, xylose, sorbitol, salicin, arabinose, adonitol, or inositol. No gas production was observed.

The organism was sensitive to penicillin, ampicillin, cephaloridine, tetracycline, chloramphenicol, streptomycin, kanamycin, gentamicin, and colistin; it was resistant to cloxacillin, sodium fusidate, lincomycin, and erythromycin.

Pathogenicity to mice was demonstrated. Of 10 mice given an intraperitoneal inoculation of one hundred million organisms (10^8) in saline, seven died and three survived. Six died within 24 hours, and the seventh after eight days. At necropsy, in all seven, *H. aphrophilus* was obtained in pure growth from the heart blood and spleen. A further six mice were given the same dose of *H. aphrophilus* killed by heat, and all of these survived. Doses of 10^7 or fewer living organisms were not lethal, and mice given 10^9 or more killed organisms died, presumably of the toxic effect. Animal pathogenicity has not previously been attributed to *H. aphrophilus* (Khairat, 1940; Toshach and Bain, 1958).

This species received its name because of its preference for carbon dioxide, 'aphros' meaning the foam of fermenting wine. A comprehensive description of *H. aphrophilus* is given by King and Tatum (1962), and its differential characterization is discussed by Russell (1965). *H. aphrophilus* may produce no visible signs of growth in blood culture bottles and grows poorly on aerobic culture; the possibility of these organisms remaining undetected in clinical specimens may be reduced by routine culture in an atmosphere containing added carbon dioxide.

DISCUSSION

The disease most often caused by *H. aphrophilus* is subacute bacterial endocarditis, but the species has also been isolated from the following infections: brain abscess, sinusitis, wound infection, septic arthritis, meningitis, and in association with actinomycosis (Page and King, 1966). Microorganisms resembling *H. aphrophilus* have been found in many infections in children (Vickerstaff *et al*, 1968).

As in *Streptococcus viridans* endocarditis the usual portal of entry of *H. aphrophilus* is the mouth, often following a dental operation, and only previously damaged heart valves are infected (Witorsch and Gordon, 1964). All strains of *H. aphrophilus* have been found sensitive to streptomycin, chloramphenicol, and tetracycline, but sensitivity to the penicillins is variable. In the above case renal insufficiency restricted the antibiotics considered suitable; for uncomplicated infection with *H. aphrophilus* the treatment of choice seems to be penicillin plus streptomycin (Dung and Lin, 1966).

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