Haemophilus aphrophilus endocarditis in pregnancy

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SYNOPSIS The clinical and microbiological features of a case of *Haemophilus aphrophilus* endocarditis in pregnancy are described. The complicating effect of pregnancy on treatment and the difficulties in identifying the organism in the laboratory are discussed.

We have observed a patient with *Haemophilus aphrophilus* endocarditis in pregnancy. Endocarditis due to *H. aphrophilus* is rare and the organism has not been previously reported as causing endocarditis in pregnancy. Subacute infective endocarditis from any cause is rare in pregnancy, only five cases in 36 392 deliveries being found in one British series (Ward and Hickman, 1971).

Because of the infrequency of this type of endocarditis, the difficulties in identifying the organism in the laboratory, and the complicating effect of pregnancy on treatment, we report on the management of this patient.

Case report

A 36-year-old housewife presented, when 31 weeks pregnant, with a two-week history of intermittent rigors, generalized limb pains, and mild exertional dyspnoea. She was known to have mild mitral incompetence and had been under regular antenatal care. There was no history of dental or surgical intervention.

She had an intermittent pyrexia and was anaemic. Her pulse was regular and there was no evidence of heart failure. There was left ventricular hypertrophy and auscultation revealed mitral incompetence. An Osler's node appeared three days after admission. Her teeth were non-carious.

Investigations on admission: Haemoglobin 10.5 g/dl, WBC 12 000 with 90% polymorphs, ESR 100 mm in one hour. Urine microscopy showed 40 RBCs per high-power field. Chest x-ray showed left atrial prominence. An electrocardiogram confirmed sinus rhythm and was within physiological limits. *H. aphrophilus* was isolated from six separate blood cultures taken over three days. The identity

of the organism was confirmed by the National Collection of Type Cultures, Central Public Health Laboratory, London.

Bacterial sensitivities were determined and treatment was started with intravenous ampicillin, 2 g two-hourly. The patient became afebrile and had no further rigors nor 'embolic phenomena'.

At 39 weeks the patient went into spontaneous labour. Intravenous gentamicin, 80 mg 12-hourly, was added to therapy during the first stage of labour. A healthy child was delivered with forceps without maternal cardiac complications. Parenteral antibiotics at the above dosages were continued for two weeks after delivery. After a further week on oral ampicillin at normal dosage the patient was discharged home. Oral ampicillin was continued for a further two weeks after discharge.

One week after stopping ampicillin she developed arthralgia and a tender finger pulp. When she presented six days later she had already started to take ampicillin again. Examination showed both an Osler's node and a Janeway lesion. She was afebrile. Cardiovascular findings were unchanged. The ESR was 72 mm in one hour. After repeated blood cultures treatment was started with penicillin G, 5 mega units six hourly, probenecid, 500 mg three times daily, and streptomycin, 500 mg twice daily, and continued for six weeks. There were no further 'embolic phenomena' and the ESR fell to normal limits. Blood cultures with added penicillinase were sterile after 21 days' incubation. There is no evidence of relapse three months after cessation of therapy.

BACTERIOLOGY

The organism was isolated from the patient's blood by inoculating 5 ml of the blood into each of two bottles containing 100 ml of 1% glucose broth (Southern Group) and one bottle containing 100 ml of Brewer's thioglycollate broth. Six sets of blood

cultures were taken over 48 hours. The cultures were incubated at 37°C and subcultures were made after two, five, and nine days' incubation on to two horse blood agar plates, one incubated anaerobically (BBL Gas Pack) and one aerobically, and on to chocolate agar incubated in a candle jar. After 24 hours minute colonies of Gram-negative small pleomorphic coccobacilli had appeared on the anaerobic and chocolate agar plates. There was no growth on aerobic plates after 72 hours' incubation. Six bottles of broth cultures yielded growth after two days' incubation while the remaining 12 broths yielded growth after nine days' incubation. Growth was dependent upon added CO₂ and growth on chocolate agar was similar to that on blood agar.

On subculture on horse blood agar, colonies were pinpoint after 24 hours, developing to 0.1 mm-0.5 mm at 48 hours, when the colony was circular, smooth, domed, opaque, and grey in colour. In liquid medium the growth was granular, settling to the bottom with growth along the sides of the tube.

X and V factor dependence was tested by the methods of Boyce *et al* (1969) before a possible X-factor dependence could be lost as a result of repeated subculture. A freshly isolated strain of *H. influenzae* was used for control tests. The *H. aphrophilus* isolated was found to be independent of X or V factor on initial subculture.

Biochemical reactions were tested using the medium described by Zamiri (1973) for testing the fermentation reactions of Corynebacteria. Acid and gas were formed between 24 and 48 hours from glucose, lactose, maltose, sucrose, trehalose, dextrin, and raffinose. There was no fermentation of mannitol, salicin, sorbitol, xylose, starch, or glycogen. Nitrate (Cook, 1950) was reduced to nitrite. Hydrogen sulphide was produced. Negative results were obtained for catalase, oxidase, urease, indole, and gelatin liquefaction tests.

By the disc sensitivity method (Stokes and Waterworth, 1972) the organism was sensitive to penicillin, ampicillin, cotrimoxazole, tetracycline, erythromycin, streptomycin, and gentamicin. By the tube dilution method the minimum inhibitory concentration of penicillin was 0.12 µg/ml and that of ampicillin was 0.25 µg/ml. The minimum bactericidal concentration of penicillin was 0.25 µg/ml and that of ampicillin 0.25 µg/ml.

Discussion

Originally isolated by Khairat (1940) from blood culture, *H. aphrophilus* was so named because it required X factor and CO₂ for growth. It was found not to produce gas in fermentation reactions. Reports since then, while generally agreeing on

most cultural characteristics, have conflicted on two important features: the requirement of X-factor for growth and gas production in the fermentation of carbohydrates.

King and Tatum (1962) could not demonstrate dependence on X-factor by *H. aphrophilus* but, because the strains studied by them had been subcultured an unknown number of times, the requirement for X-factor could have been lost. Boyce *et al* (1969) tested original isolates and concluded that although most bacterial cells in each culture of *H. aphrophilus* were X dependent, a small proportion of X independent variants could be selected and isolated. Lack of dependence on X-factor was more conclusively demonstrated by Kraut *et al* (1972) by a quantitative culture procedure. This has also been reported by others (Farrand *et al*, 1969; Sutter and Finegold, 1970; Dorff and Kilian, 1974). Our strain was X independent.

Zinnemann (1970) has emphasized the need for an organism to be dependent on either X or V factor or both to be included in the genus *Haemophilus*. In numerous reports, including ours, the putative *H. aphrophilus* does not comply with this prerequisite and reinforces Cowan's (1974) contention that this organism, along with *Actinobacillus actinomyces-comitans*, is difficult to allocate to a particular genus. Further support for this view comes from the controversy over gas production in fermentation reactions. Zinnemann does not accept that any member of the genus *Haemophilus* produces gas when it forms acid from carbohydrates. Khairat (1971) has reiterated his original finding that *H. aphrophilus* does not form gas and this has been confirmed by Boyce *et al* (1969). King and Tatum (1962), however, using the unusual technique of Hormaeche and Munilla (1957) of plunging a red hot wire into the medium, and Sutter and Finegold (1970), using more conventional methods, were able to demonstrate gas production in many strains of *H. aphrophilus* that they studied.

Apart from these two features, the fermentation reactions listed for this organism are consistent. This forms the mainstay of identification and differentiation from other closely related bacteria, namely *Actinobacillus*, HB-I strains as well as *Pasteurella* and *Brucella*. Important positive reactions that distinguish *H. aphrophilus* are acid production from lactose, maltose, sucrose, and trehalose and failure to ferment xylose. The serum-free medium used supports good growth of *H. aphrophilus* to give results within 48 hours and lacks the tedium of King and Tatum's (1962) unconventional method.

The occurrence of this infection during pregnancy in our patient limited our choice of therapy. Ampicillin was used initially because the strain of

H. aphrophilus isolated showed greater *in vitro* sensitivity to ampicillin than to penicillin. An aminoglycoside was avoided during the pregnancy because of the risk of eighth cranial nerve toxicity to the fetus. The relapse after ampicillin alone and the satisfactory response to penicillin and streptomycin, after delivery, in our patient provide further support for the latter combination as the treatment of choice in *H. aphrophilus* endocarditis (Sutter and Finegold, 1970) in non-pregnant women.

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