Basic studies on the biology of staphylococci are indispensable, but they tend to focus attention to an undue extent on the micro-organismal aspects of the host-parasite relation.

**SUMMARY**

When tested by intracerebral injection in mice, a strain of *Staphylococcus aureus* containing antigen 17 was found to be much more virulent than a variant that lost this antigen and contained antigen 1 in its place. Since 85.7 per cent. of the mice challenged died between 7½ and 11½ hr after inoculation it is suggested that a toxin was the cause of death.

A standardised test by intracerebral inoculation is considered to be a useful test of virulence in mice. Out of 100 recently isolated coagulase-positive strains assessed by this method, only 18 were considered to be avirulent. The degree of virulence was correlated with the clinical source of the strains.

**REFERENCES**


**ENDOCARDITIS DUE TO *HAEMOPHILUS PARAINFLUENZAE***

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Although a variety of micro-organisms may be implicated in infective endocarditis, viridans streptococci remain responsible for more than 50 per cent. of all cases and are the aetiological agent in 70–80 per cent. of cases of subacute bacterial endocarditis (Cates and Christie, 1951; Wedgewood, 1955; Morgan and Bland, 1959; Friedberg, Goldman and Field, 1961; Wilson, 1963; Lerner and Weinstein, 1966). Bacterial endocarditis due to Gram-negative bacilli of the *Haemophilus* group occurs relatively infrequently. In an extensive review covering the period from 1935 to 1948, Jones (1950) found only 25 cases caused by *Haemophilus* species, and these organisms were responsible for only nine of 1324 cases recorded by Keith and Lyon (1963).

Insufficient cases of haemophilus endocarditis have been reported in the antibiotic era to allow the proper formulation of optimum treatment of this type of infection (Garrod and O'Grady, 1968). We describe in this paper a case of bacterial endocarditis due to *Haemophilus parainfluenzae*.

**CASE REPORT**

A young man of 17 yr was rejected for service in the Royal Air Force because he had a heart murmur. He had no undue breathlessness, and remained in excellent health for 3 yr when he developed fever, sweating, and pain in his limbs. He received penicillin with temporary improvement, but later obtained no benefit from tetracycline or salicylate. After 3 wk he was referred for hospital treatment.

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On examination, he showed marked mental clouding and was pyrexial. The heart was of normal size and had a normal impulse, but a pansystolic murmur was heard at the apex and was well conducted to the left axilla diminishing towards the left sternal border. No haemorrhages were seen in the skin or nail beds, and there was no finger clubbing or splenomegaly. The other systems were normal.

The results of the special investigations were as follows. Chest X-radiograph showed no abnormality; ECG was within normal limits; haemoglobin 10.8 g per 100 ml; mean corpuscular haemoglobin concentration 30 per cent.; WBC 14,200 per µl (67 per cent. polymorphs); ESR (Wintrobe) 33 mm in 1st hr; urine microscopy showed occasional leucocytes and red cells with no casts; total serum proteins 6.3 g per 100 ml (albumin 3.0 g, globulin 3.3 g); blood urea 27 mg per 100 ml; Coxiella burnetii CF titre, 8.

Four blood cultures taken over the first 48 hr (when fever reached 104°F at times) were sterile, as were cultures taken on the 4th and 6th days, when he was again febrile. A culture taken on the 8th day, and five subsequent cultures, yielded growths of *H. parainfluenzae*. Meanwhile, skin petechiae and small haemorrhagic palmar lesions had appeared and treatment was started on an empirical basis with benzyl penicillin (2 M.units 6-hourly) and streptomycin (1 g 12-hourly), both intramuscularly. Probenecid (0.5 g 6-hourly) was given by mouth. The clinical response was not satisfactory, and in the light of the results of tests of the antibiotic sensitivity of the infecting organism, treatment was changed to ampicillin (0.5 g 4-hourly by mouth) and streptomycin (0.5 g 12-hourly, intramuscularly) with probenecid as before.

The pyrexia promptly subsided and there was gradual clinical improvement, punctuated by embolic episodes involving the lung, brachial artery and mesenteric vessels, all of which resolved uneventfully. The antibiotics were discontinued after 8 wk, by which time the patient had recovered clinically, haemoglobin concentration, white cell count, and sedimentation rate were normal, and blood cultures were sterile.

The patient was subsequently investigated at the Medical Cardiology Unit of Glasgow Royal Infirmary. The clinical findings were thought to indicate either mitral incompetence or a ventricular septal defect. On right heart catheterisation there was no evidence of any shunt, and normal pulmonary pressures and normal right-sided pressures throughout were observed. Normal results in ascorbate dilution tests were thought to exclude an intracardiac shunt and a diagnosis of mitral incompetence was made. It was thought undesirable to carry out left heart catheterisation within months of a valve infection.

**MICROBIOLOGICAL FINDINGS**

The organism isolated from the blood of the patient was first apparent in aerobic 0.1 per cent. glucose broth cultures after incubation for 72 hr at 37°C, as pleomorphic non-motile Gram-negative bacilli. On subculture to horse blood agar plates incubated aerobically at 37°C, the organism produced smooth, greyish, semi-transparent colonies reaching a size of 10-15 mm at 48 hr. No haemolysis was produced on horse blood agar. Growth was enhanced by incubation in air with increased CO₂, but no growth was obtained on unenriched nutrient agar or on MacConkey agar. The biochemical properties of the organism were investigated. Nitrate reduction was tested by the method of Cook (1950). The fermentation of sugars was tested with 2.0 per cent. of the test sugar in peptone water containing 25 per cent. freshly prepared yeast extract (Stokes, 1968), the tests being incubated for 10 days.

The biochemical properties of the organism were as follows. Acid, without gas, was produced from glucose and sucrose; no acid was produced from lactose, maltose, mannitol, salicin, trehalose, raffinose, or xylose; indole was produced; nitrate was not reduced to nitrite; the catalase and urease tests were negative; and H₂S was not produced.

A requirement for V factor was demonstrated in tests with commercially available disks (Mast Laboratories) on nutrient agar, and confirmed in peptone waters supplemented with X factor (haemin) and V factor (co-enzyme I) freshly prepared by the method of Stokes.

In antibiotic sensitivity tests performed by the controlled disk diffusion method (Stokes),
the organism was sensitive to ampicillin (2 µg), cephaloridine (5 µg), streptomycin (10 µg),
tetracycline (10 µg), chloramphenicol (10 µg), and was partially sensitive to benzyl penicillin
(1 unit).

The minimal inhibitory (MIC) and bactericidal (MBC) concentrations of ampicillin
and of streptomycin active against the isolate, determined by the tube dilution method
(Stokes) in 50 per cent. serum broth supplemented with 25 per cent. yeast extract, were:
ampicillin MIC 0.25 µg, MBC 1.5 µg; streptomycin MIC 2.4 µg, MBC 9.6 µg.

The Cellophane transfer technique of Chabbert and Waterworth (1969), for which we
employed blotting-paper strips impregnated with ampicillin (50 µg per ml) and streptomycin
(500 µg per ml), confirmed that these antibiotics constituted a synergistic bactericidal
combination.

Assays of the bactericidal activity of the patient's serum against the homologous isolate
were undertaken during the 6 wk of combined antibiotic therapy. The tube dilution method
(Stokes) was used with an inoculum of 10^6 organisms per ml in serum yeast extract broth.
The patient's serum was bactericidal in dilutions up to 1 in 6.

The presence of serum antibodies against the organism was investigated by agglutination
tests. The haemophilus strain was grown in serum yeast extract broth for 72 hr at 37°C.
The centrifuged bacterial deposit was treated with 70 per cent. alcohol for 30 min. and adjusted
in saline to an opacity corresponding to that of Brown's tube standard no. 1. Agglutination
was tested in Dreyer tubes and the results were read after incubation for 18 hr at 37°C.
Sera collected 12 days and 90 days after hospitalisation agglutinated the organism in dilutions
of serum up to 1 in 10 and 1 in 80 respectively. Ten randomly chosen sera submitted to the
laboratory did not agglutinate the prepared antigen.

**DISCUSSION**

Bacterial endocarditis caused by species of *Haemophilus* is uncommon. Craven, Poston
and Orgain (1939) collected only 36 cases in a review of haemophilus endocarditis covering
the half-century since the discovery of *H. influenzae* by Pfeiffer (1893).

The relative infrequency of this form of infective endocarditis has been reported by
several authors. In a survey of non-streptococcal bacterial endocarditis, Jones (1950)
found 25 recorded cases due to *Haemophilus* spp. between 1936 and 1949. A review of 442
patients with subacute bacterial endocarditis by Cates and Christie (1951) included only
three cases due to haemophilus, whilst Wedgewood (1955) reported the isolation of *H. influ-
enzae* in two of 65 cases of endocarditis. Geraci (1958), who documented the Mayo Clinic
experience of infective endocarditis, reported only one case due to a *Haemophilus* sp. in a
series of 172 cases. Morgan and Bland (1959) found only one example of haemophilus
endocarditis in a review of the records of 228 patients with bacterial endocarditis treated
at the Massachusetts General Hospital, and no cases of bacterial endocarditis attributable
to haemophilus were reported in a similar number of patients seen at the University of
Minnesota hospitals over the period 1939–59 (Pankey, 1961). More recently, Lerner and
Weinstein (1966) have published an extensive review of infective endocarditis based on
admissions to the New England Medical Center Hospitals between 1956 and 1964. A
*Haemophilus* species was the aetiological agent in only 1 per cent. of the diagnosed cases
during that period.

A number of earlier reports of haemophilus endocarditis failed to distinguish between
*H. influenzae*, *H. parainfluenzae*, and other species, though the elucidation of the nutritional
requirements of the various members of the group (Rivers, 1922; Fildes, 1924) permitted
a more detailed characterisation of the species in the later papers.

*H. influenzae* appears to be a very rare cause of endocarditis, *H. parainfluenzae* being
relatively much commoner (Miles and Gray, 1938; Craven *et al.*, 1939; Rose, 1941;
Garrod and O’Grady, 1968). Double infection of the heart valves by *H. parainfluenzae*
and viridans streptococci has been recorded by Goudie and Lowther (1951). The other
member of the *Haemophilus* group that has occasionally been encountered in subacute
bacterial endocarditis is *H. aphrophilus*, 25 cases having been reported in the literature since
the original description of this form of valvular infection by Khairat in 1940 (Speller, Prout and Saunders, 1968).

Before the advent of antibiotics, bacterial endocarditis was almost invariably fatal. The infection can now be cured in the majority of patients by rational antibacterial therapy conducted under proper laboratory control. In contrast, however, with streptococcal or staphylococcal endocarditis for which standardised treatment regimens have been formulated and widely applied, insufficient cumulative experience of haemophilus infections exists to define the optimum management of this form of infective endocarditis. As in other forms of bacterial endocarditis, cure depends on the complete sterilisation of vegetations by the administration of a bactericidal antibiotic or combination of antibiotics chosen on the basis of comprehensive laboratory tests.

Various antibiotic combinations have been used in the management of haemophilus endocarditis. Goudie and Lowther successfully treated a double infection by viridans streptococci and *H. parainfluenzae* with penicillin and streptomycin, and this bactericidal combination has also achieved cure of *H. aphrophilus* endocarditis (Keith and Lyon, 1963; Witorsch and Gorden, 1964). Boughton (1965) employed oral ampicillin (with probenecid) and tetracycline in a case of subacute bacterial endocarditis due to *H. parainfluenzae*, a combination which many would not regard as ideal in view of possible antagonism between a bactericidal and a predominantly bacteriostatic drug. Goldberg and Cecil (1966) also used ampicillin and streptomycin, though supplemented by kanamycin, in the treatment of *H. aphrophilus* endocarditis. Ampicillin, combined with streptomycin if necessary, has been recommended by Garrod and O'Grady as a logical first choice of treatment in subacute bacterial endocarditis due to *Haemophilus* species. This combination proved to be highly satisfactory in eradicating the infection in the present case, levels well in excess of the minimum bactericidal concentration being easily attained in the serum by the addition of probenecid to the chosen regimen.

The importance of strict laboratory control of antibiotic therapy in infective endocarditis cannot be overemphasised. It is insufficient merely to determine the minimum bacteriostatic concentrations of antibiotics active against the isolated pathogen. Since the aim of treatment must be a totally bactericidal effect, sensitivity tests must demonstrate whether the organism has been killed or merely inhibited. The determination of the maximum dilution of the patient's serum that exerts a bactericidal effect on the causative organism is of great value in the control of antibacterial therapy in such cases. This test is widely favoured by American workers (Geraci; Pankey; Jawetz, 1962), the clinician's aim being to achieve a bacteriostatic effect at a dilution of 1 in 16 or greater, and a fully bactericidal effect at a dilution of 1 in 4. Other tests should be done to determine whether there is a synergistic combination of drugs that may be exploited, especially in cases of endocarditis due to organisms relatively resistant to penicillin; suitable tests for this purpose are the "half chess-board" bactericidal sensitivity test (Garrod and O'Grady) or the Cellophane transfer technique (Garrod and Waterworth, 1962; Chabbert and Waterworth, 1965).

**SUMMARY**

The clinical and microbiological features of a case of subacute bacterial endocarditis due to *Haemophilus parainfluenzae* are described. The incidence of haemophilus endocarditis is reviewed and the treatment is discussed with special reference to the laboratory control of antibiotic therapy.

**REFERENCES**

Pamela
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The value of a combination of trimethoprim and sulphonmethoxazole in the treatment of a variety of infections is now well established. Little has yet been published, however, on the possible effectiveness of the combination in bacillary dysentery. As a first step it was thought that it would be of interest to ascertain the in-vitro sensitivity of recently isolated strains of *Shigella sonnei* to the two drugs. A collection of 209 strains of *Sh. sonnei* of various colicine types and antibiotic-resistance patterns were tested; these were isolated mainly in the Greater London Area.

Materials and methods

Most of the strains of *Sh. sonnei* tested were isolated from specimens submitted to the Public Health Laboratory, St George’s Hospital, London, and the Public Health Laboratory,