warfarin was found in the plasma. This is difficult to explain since diazepam and paracetamol do not usually depress warfarin absorption or accelerate its metabolism (Breckenridge, 1972). From day 155 to day 164 when she was taking co-trimoxazole (two tablets twice daily) for a urinary tract infection the plasma warfarin levels increased, producing the expected effect on prothrombin activity. Interestingly the co-trimoxazole appeared to exert its effect by inhibition of warfarin metabolism rather than by displacement from protein binding.

Anticoagulant resistance has been reported by other workers. O'Reilly *et al.* (1964) gave the first report of a kindred of patients exhibiting inherited (probably autosomal dominant) resistance. In such patients the resistance is present at the start of treatment and is believed to occur as a result of altered enzyme or receptor site affinity for vitamin K or the drug. Other patients have been described in whom resistance was due to increased drug metabolism resulting in normal blood levels despite large oral doses (Lewis *et al.*, 1967). We have found only one reference to patients gradually developing tolerance to anticoagulant drugs though the actual doses attained were not exceptional (Soulier, 1961). Our patient gradually acquired resistance to several drugs but adequate control was not achieved even with high doses.

We feel that the anticoagulant resistance in our patient was

not related to reduced absorption (normal rate of appearance of warfarin in the plasma after ingestion) or to increased warfarin metabolism (high plasma levels and normal plasma half life). It was more likely to be due to a gradually developing altered affinity such as that discussed by O'Reilly *et al.* (1964).

The precise cause of our patient's recurrent embolic episodes was not elucidated though we are at present considering further cardiological investigations.

We are grateful to Dr. E. W. J. Gumpert, Professor R. Kilpatrick, and Mr. A. G. Norman for permission to report details of this patient, and to Dr. A. Breckenridge (Royal Postgraduate Medical School) for plasma warfarin measurements and helpful criticism.

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Endocarditis due to *Actinobacillus actinomycetemcomitans*

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There is only one report of a case of endocarditis due to *Actinobacillus actinomycetemcomitans* from the United Kingdom (Mitchell and Gillespie, 1964) though several have been described elsewhere. (Kayser and Bircher, 1967; Underhill, 1968; Vandepitte *et al.*, 1970; Block *et al.*, 1973). The rarity with which this organism is isolated, and thus recognized, may result from the fastidious nature of its growth requirements. We describe here these requirements and emphasize the importance of beginning treatment in clinically probable cases of bacterial endocarditis even if blood cultures are initially negative.

Case Report

A 55-year-old local authority officer was referred as an outpatient in March 1974 with a three-month history of general malaise and a weight loss of 28 lb (12.7 kg). For three weeks he had also noticed night sweats. His previous health had been good, but as a child he had not been allowed to play school games because of a heart murmur. He had been unfit for military service because, he had understood, of severe myopia. There was no history of rheumatic fever or chorea. He had not received any antibacterial chemotherapy before his referral.

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On examination he looked unwell, but not obviously anaemic, and was sweating with an axillary temperature of 37.8°C. His pulse was regular at 120 beats/min and there was a loud (grade 3/4) pansystolic murmur at the apex, which was not displaced. There were no signs of heart failure, the spleen and liver were not palpable, and no splinter haemorrhages, Osler nodes, or retinal lesions were observed. His teeth and gums were healthy. Haemoglobin was found in the urine (Labstix). He was immediately admitted to hospital with a presumptive diagnosis of infective endocarditis.

In the ward his temperature varied between 37°C and 38°C and rose to 39°C on two evenings out of three. Urine microscopy showed red cells with granular and red-cell casts. Initial investigations: appearances on chest radiography and screening were normal, and plasma urea was 7.6 mmol/l (46 mg/100 ml); creatinine 141 µmol/l (1.6 mg/100 ml); and haemoglobin 11.1 g/dl with normochromic and normocytic red cells. The W.B.C. was $10.3 \times 10^9/l$ ($10\,300/mm^3$) (71% neutrophils, 26% lymphocytes, 3% monocytes) and E.S.R. was 123 mm/h. Serum albumin was 27 g/l; globulin 42 g/l; bilirubin 6.8 µmol/l (0.4 mg/100 ml); SGOT 27 IU/l; alkaline phosphatase 100 K.A. units/l. The serum β_1A -globulin was 0.78 g/l (normal 1-1.5 g/l). No evidence of brucellosis or psittacosis was found. Fifteen blood samples were cultured for bacteria and fungi and all showed no growth at 48 hours.

On the third day in hospital a high-pitched diminuendo, early diastolic murmur was noted at the left sternal edge in the fourth intercostal space and the plasma urea was 9.5 mmol/l (57 mg/100 ml) with a creatinine of 168 µmol/l (1.9 mg/100 ml). Treatment was started with benzylpenicillin 18 MU daily by intermittent intravenous infusion, and he was digitalized. On the fifth day of treatment his temperature had fallen to a steady 37°C. Echocardiography at this stage showed mitral regurgitation.

By the 16th day of therapy 11 of the 15 blood samples taken before chemotherapy was started had grown a gram-negative coccobacillus when subcultured under carbon dioxide. After antibiotic sensitivity tests on this organism therapy was changed to ampicillin 4.5 g/day by intravenous injection.

On the 19th day of treatment the patient developed nocturnal dyspnoea with basal crepitations and raised jugular venous pressure; a chest radiograph showed small bilateral pleural effusions. These symptoms and signs responded rapidly to an increase in digoxin and a daily dose of frusemide 40 mg. The haemoglobin concentration had slowly fallen to 9.4 g/dl, and three units of packed cells were given.

The diastolic murmur gradually diminished in intensity and was inaudible after the 43rd day of therapy. Two weeks of intravenous penicillin had been followed by four weeks of ampicillin intravenously, and a 10-day course of streptomycin 0.5 g twice daily by intramuscular injection was added as a final sterilization procedure in case cell-wall-defective forms of the organism had survived the ampicillin. All chemotherapy was then stopped, and as the patient

remained well and afebrile he was discharged from hospital. Digoxin and frusemide were subsequently withdrawn and four months later the patient was feeling perfectly well. He declined further investigation of his cardiac lesion.

BACTERIOLOGY

Blood was cultured without additional carbon dioxide in brain-heart infusion medium (Oxoid) containing 0.03% sodium polyanethol sulphate (Liquoid). The blood cultures were incubated at 37°C, examined daily macroscopically, and subcultured on to plates after one, three, eight, and 15 days. Growth was obtained only from the 15-day subcultures. The organism was a small, slightly pleomorphic, gram-negative rod which produced visible growth on 5% horse-blood agar incubated at 37°C in an atmosphere of 5% carbon dioxide on the second day of incubation. It also grew in an atmosphere of 10% carbon dioxide and 90% hydrogen but not in pure hydrogen or aerobically. Growth occurred also on nutrient agar and chocolate agar and in a granular form along the sides of tubes containing Todd-Hewitt broth, but not in tryptone broth or Hartley's broth.

Because of the superficial similarity to *Haemophilus aphrophilus* tests were carried out on minimal agar according to the stringent criteria laid down by Khairat (1940) to determine whether the organism was dependent on X factor (haematin) for its growth. No growth occurred on minimal agar alone or in the presence of X factor, V factor (nicotinamide adenine dinucleotide), or X and V factor combined. The organism was catalase positive; when grown in serum water sugars it produced acid from glucose but not from lactose, sucrose, and mannitol.

The organism was thus identified as *A. actinomycetemcomitans*, which was confirmed by Dr. S. P. Lapage and Mr. M. R. Hill of the National Collection of Type Cultures, Colindale, London, who informed us that the characteristics shown in the table are the only ones of value in distinguishing *H. aphrophilus* from *A. actinomycetemcomitans*.

Cultural Characteristics of *A. actinomycetemcomitans* and *H. aphrophilus*

Test:	Catalase	CO ₂ Needed	CO ₂ Improves	Lactose	Sucrose	Mannitol
<i>A. actinomycetemcomitans</i> . . .	+	+	+	-	-	d
<i>H. aphrophilus</i> . . .	-	-	+	+	+	-

d = 79-21% strains positive.

When tested by the disc diffusion technique, using the Oxford staphylococcus N.C.T.C. 6571 as the control, the organism was less sensitive to penicillin (minimal inhibitory concentration (M.I.C.) 2 µg/ml) but equally sensitive to ampicillin, tetracycline, streptomycin, and chloramphenicol. Both ampicillin and streptomycin were bactericidal when tested by the cellophane transfer method (Garrod and Waterworth, 1962; Chabbert and Waterworth, 1965), neither synergy nor antagonism being demonstrable between these antibiotics.

Comment

Bacillus actinomycetemcomitans was first described by Klinger (1912), who isolated the organism from patients with actino-

mycosis in association with *Actinomyces* spp. The present generic name was given by Topley and Wilson (1929). It has subsequently been reported in association with *Actinomyces* spp. by many authors, including Holm (1950, 1951, 1954), Heinrich and Pulverer (1959), Klaber (1934), and Goldsworthy (1938). Vallée and Gaillard (1953) briefly reported the association of *A. actinomycetemcomitans* with endocarditis in two patients, and Page and King (1966) reported a further 23 patients in the U.S.A. The organism has been described as present in the normal mouth flora by Lentz (cited by Heinrich and Pulverer, 1959). There seems to be only one report of endocarditis due to *A. actinomycetemcomitans* in the United Kingdom (Mitchell and Gillespie 1964), but Khairat (1940) reported a case of endocarditis due to a superficially similar organism, *Haemophilus aphrophilus*. The literature suggests that endocarditis due to *A. actinomycetemcomitans* carries a high fatality rate of about 33% compared with 7% for *Haemophilus aphrophilus* (Page and King, 1966). This, together with its fastidious cultural requirements, justifies particular attention being paid to the possibility of *A. actinomycetemcomitans* infection in cases of endocarditis. The long delay before we isolated this organism was probably due to incubating the blood cultures aerobically. This procedure had been adopted in this laboratory for suspected endocarditis after experimental evidence had shown that our blood-culture technique of adding 10 ml of patient's blood to 100 ml of medium achieved adequate conditions for the isolation of other more frequently encountered pathogens which need carbon dioxide. In view of our results, however, it is obviously necessary to incubate all blood cultures under added carbon dioxide and this is now our practice.

This case report also emphasizes the importance of starting chemotherapy empirically when the diagnosis of endocarditis is made on clinical grounds and an adequate number of blood specimens have been collected for culture.

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