A FLAVOBACTERIUM CULTURED FROM THE BLOOD OF A PATIENT WITH MENINGITIS

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Introduction

Meningitis may be caused by several distinct species of bacteria, viruses and protozoa. The initial clinical diagnosis is usually confirmed by chemical and cytological examination of the C.S.F. and the causal pathogen identified by bacteriological and virological techniques. Bacterial meningitis is often caused by *Neisseria meningitidis, Haemophilus influenzae* and various streptococcal species. Less common causative organisms belong to the Flavobacterium group. These are Gram-negative, slender, non-motile bacilli having restricted biochemical activities and are often resistant to several antibiotics, including penicillin. Some strains produce a pronounced yellow pigment.

The earliest description of this organism was given by Franklands (1889) who isolated it from deep wells in Kent. However, owing to incompleteness in the original description, one cannot be certain of its exact identity. Thereafter, several authors recorded the isolation of bacteria with characteristics similar to those described above and in circumstances suggesting that they were pathogenic for man. More recently, outbreaks of meningitis in new born infants caused by Flavobacteria were recorded by Vandepitte, Beeckmans and Buttiaux (1958) in the Congo and by Brody, Moore and King (1958) and George, Cochran and Wheeler (1961) in the United States. Cabrera and Davis (1961) put forward evidence to suggest that Flavobacteria usually lead a saprophytic existence but may also colonize the human nose.

The Flavobacterium genus contains 2 groups designated group IIa, named *Flavobacterium meningosepticum* and group IIb tentatively classified as *Flavobacterium* sp. (King, 1959). Strains in group IIa are often associated with infant meningitis and, unlike members of group IIb which produce a pronounced yellow pigment, show little or no pigmentation. These 2 groups although closely related show some differences in other biochemical reactions which enable laboratory workers to distinguish them (Tatum, 1970).

Clinical features and laboratory investigations

A 5-year old boy was admitted to St. Luke's hospital on the 5th November, 1973 with a history of mild frontal headaches for the previous 2 days. The pain later extended to the occipital region and to the neck. He vomited once, a few hours prior to admission to hospital.

On examination, his general condition was fair. The temperature was 101°F and the pulse rate was 140/min. Neck rigidity was present and Kernig's sign was positive. No other abnormalities were detected.

A diagnosis of meningitis was made and a lumbar puncture performed. The fluid was clear and the C.S.F. pressure was found to be within normal limits. The chemical and cytological examination gave the following result:-

Physical findings: fluid clear and co-

lourless. Globulin test: positive. Proteins: 40 mg/100 ml. Glucose: 44 mg/100 ml. Cells: 30/cu. mm. the great majority of which were polymorphs. Bacteriological examination of the sample was negative both on direct examination and on subsequent culture.

Other investigations included Hb estimation, WBC counts and blood urea levels. All were found to be within normal limits.

The patient was put on Penicillin 2 mega units 8 hourly, Chloramphenicol 125 mg 6 hourly and Sulphadiazine 125 mg 6 hourly. He recovered fairly rapidly and became afebrile on the 3rd day after admission. The CNS signs subsided about this time. He continued to make good progress and was discharged from hospital on the 8th day after admission.

Blood culture was also performed on admission. This consisted in taking 5 ml of blood under aseptic conditions from a suitable vein and inoculating it into a Castaneda bottle. This is simply a medical flat bottle of 100 ml. capacity in which is prepared a double (solid/liquid) medium. Tryptose agar constitutes the solid phase and covers one broad side of the bottle to a depth of about 5 mm. The liquid phase is made up of 35 cc tryptose phosphate broth with an anticoagulant which is added to each bottle after the solid phase has set firmly. The metal cap which has a rubber lining on the inside is perforated by a central hole of about 4 mm in diameter. Blood taken from a patient with a syringe is inoculated directly into the liquid phase of the medium without opening the bottle.

The inoculated blood culture bottle was incubated in an upright position at 37°C and examined daily. The solid medium was subcultured from the liquid phase on alternate days by gently shaking and tilting the bottle so that the liquid medium just covered the solid phase. On the third day after incubation, a yellowish confluent growth was detected. A Gram stain showed a small rod about 1.5μ x 0.4μ which was Gram-negative and non-acid-fast. Subcultures from the original blood culture bottle were made onto appropriate media and further tests to enable identification of this bacillus were carried out.

The bacillus was found to be nonmotile. It grew well on tryptose agar after 24 hours at 37°C forming smooth circular colonies which showed pronounced yellow pigmentation with a faintly greenish iridescence. The pigment was insoluble in water. No haemolysis was observed on blood agar. Growth on MacConkey's agar was not present at first but occurred later after some subcultures had been done. No growth at all was seen on SS agar. The catalase and oxidase tests were positive. Citrate utilization (Koser's), M.R. and V.P. tests were negative. Urea (Christensen's agar) was not hydrolysed. Indole production was very weak. Gelatin was hydrolysed by the third day. Growth was maximal at 37°C with no growth occurring at all at 5°C. Acid production was negative in liquid carbohydrate nutrient media employing several sugars. To test whether the attack on sugars was of the oxidative or fermentative type, the oxidation-fermentation (O - F) test of Hugh and Leifson (1953) was employed using glucose and sucrose. Acid production from glucose was observed in the open tube by the 3rd day; the result was doubtful in the sealed tube. The test was negative for sucrose in both tubes. On testing the organism for antibiotic sensitivity it was found that it was sensitive to chloramphenicol, moderately sensitive to trimethoprim-sulphamethoxazole and gentamycin and resistant to penicillin, streptomycin, amoxil, tetracycline, polymyxin, and penbritin. The cultural characters and biochemical reactions are summarised in Table 1 and compared with King's groups IIa and IIb.

With these findings we tentatively placed the isolate in the Flavobacterium genus and possibly belonging to King's group IIb.

Slide and tube agglutination tests with the patient's serum against a suspension of the cultured organism were negative. Nasal and throat swabs taken later from the patient and his 10-year old sister were negative for Flavobacterium.

Discussion

In a blood culture examination we always presume that a growth obtained after incubation is a potential pathogen which is

Characters	*Flavobacterium meningosepticum* Group IIa	*Flavobacterium* sp. Group IIb	Isolate referred to in text
Gram stain	negative	negative	negative
Motility	non-motile	non-motile	non-motile
Pigment (yellow)	none or very slight	pronounced	pronounced
Growth on MacConkey's agar	usually positive after several subcultures	usually postive after several subcultures	positive after several subcultures
Growth on SS agar	none	none	none
Catalase	positive	positive	positive
Oxidase	positive	positive	positive
O-F medium unsealed tubes (glucose)	acid some delayed	acid some delayed	acid by the 3rd day
Indole	weakly positive	weakly positive	v. weak positive
Urea hydrolysis	negative	negative	negative
Citrate utilization	negative	negative	negative
Gelatin liquefaction	positive	positive	positive
Pathogenicity	associated with infant meningitis	recovered mainly from adults	recovered from 5-yr old child

Table 1. Comparison of some characters of isolate referred to in the text with those of King's groups IIa and IIb.

causing a bacteraemia or septicaemia in the case concerned. Although blood is taken under strict aseptic precautions, contaminations do at times occur. This was shown by Cameron, Rae and Murphy (1931) who made blood cultures from 100 healthy individuals and obtained positive results in 18 cases. Of the positive blood cultures, 12 were shown to be due to large spore-bearing bacilli and were regarded as contaminants. Some, at least, of the remaining 6 positive blood cultures which included isolations of *Staph. aureus* and of diphtheroids were also dismissed as contaminants. Some cases are clear cut in the sense that it can be safely stated that the organism cultivated from the blood is causing the underlying disease in the patient. Thus, the isolation of *Salmonella typhi* or *Brucella melitensis* from the blood, especially if relevant clinical signs and symptoms are present, is irrefutable evidence that the patient is suf-

fering from the particular disease. At other times it is difficult to state whether an isolate is the cause of a bacteraemia or septicaemia or whether it is a contaminant picked up, say, from the patient's skin during the blood taking procedure. Points to help decide the issue include correlation of a particular positive blood culture with the clinical picture, repeated blood culture examinations and the performance of other supporting bacteriological tests. In the case of culturing *Staph. albus* from the blood, to mention one example, a diagnosis of an infection with this organism should never be made from the result of only one test. A bacteraemia due to this organism can be safely assumed following repeated isolation.

In the case under discussion, one can argue that the cause of the meningitis may have been the Flavobacterium which was cultured from the blood but for some reason not from the C.S.F. In favour of this statement is the fact that this organism is now recognised as being one of the causes of meningitis and culturing it from the blood is at least evidence of its presence in the tissues. It is not inconceivable that it may have found its way also into the C.S.F. Other possibilities include the proposition that the cause of the meningitis may have been some other type of organism and the positive blood culture was a separate, unrelated event. This however seems unlikely as it is difficult, although not impossible, to imagine a double pathology of ths nature. Lastly, the Flavobacterium may have been picked up as a contaminant from the patient's skin or from some other source during the blood culture procedure. In favour of this argument is the mild nature of the disease in this case (although this may have been due to early diagnosis and prompt successful treatment), the absence of leucocytosis and the fact that the patient's serum did not contain antibodies against the organism supposedly cultured from his blood.

As an additional note it may be mentioned that this child comes from a family of 4 children including himself. One brother died soon after birth. The other 2 siblings were both admitted to hospital in May 1973 with meningeal signs. One, a brother of 11 years was diagnosed as 'Fever' accompanied by meningeal irritation but no C.S.F. or blood culture examinations were carried out. The illness was mild and the patient was discharged from hospital after a few days stay. The other, a 10-year old sister was admitted to hospital with severe meningitis having a total of 13,300 cells/cu. mm in the C.S.F., the majority being polymorphs. A coagulase negative staphylococcus was cultured from the spinal fluid but this was thought to be a contaminant. The patient eventually recovered. Although a definite correlation between these three cases of 'meningeal involvement' cannot be proved, it is peculiar that 3 siblings in the same family should be affected by a common disease within a span of 6 months.

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References

BRODY, J.A., MOORE, H. and KING, E.O. (1958). Amer. J. Dis. Child., *96*, 1.

CABRERA, H.A. and DAVIS, G.H. (1961). Amer. J. Dis. Child., *101*, 289.

CAMERON, G.C., RAE, C.A. and MURPHY, G.N. (1931). Canad. M.A.J., *25*, 131.

FRANKLAND, G.C. and FRANKLAND, P.F. (1889). Z. Hyg. Infekt Kr., *6*, 373.

GEORGE, R.M., COCHRAN, C.P. and WHEELER, W.E. (1961). Amer. J. Dis. Child., *101*, 296.

HUGH, R. and LEIFSON, E. (1953). J. Bact., *66*, 24.

KING, E.O. (1959). Amer. J. Clin. Path., *31*, 241.

TATUM, H.W. (1970). Manual of Clinical Microbiology p. 197. American Society for Microbiology Bethesda, Md.

VANDEPITTE, J., BEECKMANS, C. and BUTTIAUX, R. (1958). Ann. Soc. belge Med. trop., *38*, 563.