Propionibacteria as a cause of shunt and postneurosurgical infections

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SUMMARY *Propionibacterium acnes* was isolated from postoperative specimens from nine neurosurgical patients. The role of *P. acnes* in postneurosurgical infection is discussed.

Propionibacterium species are Gram-positive, non-motile, non-spore-bearing bacilli, usually pleomorphic, diphtheroid, or club-shaped with one end rounded and the other end tapered or pointed and staining less intensely. Cells may be coccoid, elongate, or even branched (Buchanan and Gibbons, 1974). Growth is anaerobic or aerotolerant, no growth occurring at atmospheric levels of oxygen. Eight species of *Propionibacterium* are recognised with *P. freudenreichii* as the type species.

Propionibacterium (*Corynebacterium*) *acnes* is a major member of the skin flora and is probably the dominant bacterium of sebaceous areas (Ray and Kellum, 1970). Evans (1975) found propionibacteria on the skin of the forehead in 44 out of 48 subjects in numbers up to 1.1×10^6 per cm².

P. acnes has been incriminated in the pathogenesis of acne in man. Leyden *et al.* (1975) studied carriage in three groups of subjects and found propionibacteria to be present in the skin of a pubertal group only in those with acne. In contrast in patients over 21 years old, carriage did not vary between acne and non-acne groups. One theory of the role of *P. acnes* is that bacterial esterases release fatty acids by hydrolysis of triglycerides, and these acids produce a primary irritant dermatitis of the wall of sebaceous follicles (Kellum *et al.*, 1970).

Reports in the literature incriminating *P. acnes* as a potential human pathogen and the known carriage of the organism in the region of neurosurgical operation sites led to a search for *P. acnes* in clinical specimens of neurosurgical origin. This paper

presents nine cases of post-surgical and shunt infection from which *P. acnes* was isolated.

Material and methods

SPECIMEN COLLECTION

Specimens were obtained from patients undergoing neurosurgery. Preoperative treatment involved shaving of the head area and swabbing with 0.5% chlorhexidine in 95% ethanol/5% methanol. This was left in contact with the skin for three to five minutes before the operation. The specimens received included scalp flap fluids, wound aspirates, shunts, and ventricular and lumbar spinal fluids.

BACTERIOLOGICAL INVESTIGATIONS

On fluid specimens, other than blood, a cell count was done on the uncentrifuged specimen. From centrifuged deposits differential cell counts were made on smears stained by Leishman's stain. When fluids were viscous an assessment of cell number was made on films of uncentrifuged specimens and reported as + + +, + +, +, or $\pm$. Protein was estimated by the sulphosalicylic acid technique of Meulemans (1960) and sugar by the glucose oxidase technique (Trinder, 1969). The centrifuged deposit was cultured on 5% horse-blood agar (lab M) incubated aerobically and anaerobically (Gas Pak), chocolate agar incubated in 10% CO₂, and MacConkey agar (lab M). All plates were incubated for 72 hours at 35°C and subcultured aerobically and anaerobically at intervals of one, two, and three days, and one, two, and four weeks or whenever visible growth was apparent. Subcultures were incubated for five days at 35°C. Shunts were placed directly into thioglycollate broth and subcultured as previously described.

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IDENTIFICATION OF ISOLATES

Presumptive identification of all anaerobic bacterial isolates was based on Gram-stain appearance and biochemical reactions in the API 20A system (API Ltd, Rayleigh, Essex). Confirmation of identity was obtained by detection of the end products of glucose metabolism and by biochemical reactions as described by Holdeman and Moore (1972). Cultures were grown in 5 ml of glucose broth (1% glucose, 1% Oxoid peptone, 1% yeast extract, 0.5 µg/ml menadione, 50 µg/ml haemin) incubated for a minimum of five days at 35°C. The broth was acidified with 0.1 ml of 50% sulphuric acid and transferred to chromatography sample bottles for analysis in a Perkin Elmer F.40 multifract automatic gas chromatograph designed for head-space analysis. Sample bottles were placed in a waterbath for 10 minutes at 96°C to equilibrate. The machine automatically sampled and analysed the vapour phase of each vial.

The chromatograph was fitted with a flame-ionisation detector and a 2-metre stainless steel column packed with 15% carbowax 20 m and 2% phosphoric acid on chromosorb W, 80-100 mesh (prepared column No. BS 1386S part No. 498-1222 Perkin Elmer). The carrier gas was oxygen-free nitrogen (BOC) passing at 30 ml/min. The column-oven temperature was 135°C and the detector oven was at 200°C. A four-second injection period was used.

Results

Clinical data from nine patients from whom 15 isolates of *P. acnes* were made are listed in Table 1,

and the laboratory findings on the specimens are given in Table 2. The biochemical characteristics of the *P. acnes* isolates are shown in Table 3.

Detailed histories are presented from cases 7, 8, and 9. These cases were selected as being the most characteristic of suspected *P. acnes* infection in post-neurosurgical patients.

CASE 7

An 11-year-old boy had a right occipital lobectomy for an unusual epileptogenic lesion subsequently reported as chronic encephalitis. On the third post-operative day he developed a fever which persisted at about 39.5°C for the next 11 days, during which time he remained alert but irritable with occasional neck stiffness. Physical examination and repeated bacteriological studies of blood, urine, and stool failed to reveal a systemic cause for the pyrexia. A diagnosis of meningitis was made. His peripheral white cell count was 11 800/mm³ (11.8 × 10⁹/l) (85% polymorphs, 13% lymphocytes). The lumbar CSF white-cell count was 2000/mm³ (2.0 × 10⁶/l) (90% polymorphs, 8% lymphocytes) with a protein of 2750 mg/litre and a sugar of 3.9 mmol/litre. Micro-organisms were not seen in a Gram stained smear but culture yielded *P. acnes*. Gentamicin and chloramphenicol were added to the ampicillin that had been started at the time of operation, and by the 16th postoperative day he became afebrile.

CASE 8

A 46-year-old woman with aqueduct stenosis had had a ventriculoatrial shunt performed in another hospital one year before referral. *Staphylococcus epidermidis* was found in blood cultures, and the

Table 1 Clinical details of cases from whom propionibacterium was isolated

Case	Operation	Significant pyrexia*	Administration of		Other information
			Steroids	Antibiotics	
1	Temporal lobectomy	39°C on 4th day	No	Ampicillin Cloxacillin Chloramphenicol	<i>P. acnes</i> isolated from initial culture. Staphylococcal osteomyelitis of craniotomy flap
2	Revision of blocked ventriculoatrial shunt	No	No	No	No clinical evidence of infection
3	Frontal lobectomy	No	Yes	No	Subgaleal fluid collection
4	Stereotaxic leucotomy	No	No	No	Subgaleal fluid collection
5	Revision of blocked ventriculoatrial shunt	No	No	No	No clinical evidence of infection
6	Craniotomy for arteriovenous malformation	No	No	No	Subgaleal fluid collection
7	Occipital lobectomy	38.5°C on 3rd day	Yes	Ampicillin Gentamicin Chloramphenicol Erythromycin	Clinical features of meningitis
8	Removal of infected ventriculoatrial shunt	No	Yes	No	Nephrotic syndrome due to chronic shunt infection
9	Craniotomy for pituitary adenoma	38.5°C on 4th day	No	Chloramphenicol	No clinical features of meningitis but CSF findings indicative

*Low-grade pyrexia in first 24 hours postoperatively was not considered significant.

Table 2 Laboratory data from patients with propionibacterial infections

Case and date of specimen	Peripheral WBC count (total per mm ³)	Fluid findings (total cell count/mm ³)	Gram smear
1 26 Oct. '76 Lobectomy cavity fluid	Total 13 100 Polys 88%, lymphs 9%, monos 3% Left shift	Cells WBC + Polys predominated. Diff. not done	MNS
2 7 Mar. '77 Shunt tubing	Total 10 200 Polys 75%, lymphs 15%, monos 10%		Not done
3 31 Jan. '77 Subgaleal fluid	Total 1800 Polys 77%, lymphs 20%, monos 3% Left shift. Toxic granulation	Cells WBC +++ Polys 90% Lymphs 2% Monos 8%	MNS
1 Jan. '77 Subgaleal fluid	Not done	Cells WBC ++ Polys 98% Monos 2%	MNS
4 16 June '77 Wound aspirate	Not done	Cells WBC ++ Polys 97% Monos 3%	Gram +ve cocco- bacilli ±
5 27 May '77 Ventricular CSF	Total 8100 Polys 58%, lymphs 38%, monos 4%	RBC 100 WBC none Protein QNS Sugar 0.9 mmol/l	MNS
6 31 Dec. '76 Subgaleal fluid	Total 23 000 Polys 87%, lymphs 10%, monos 3% Left shift	Cells WBC + Polys 89% Lymphs 49% Monos 7%	MNS
13 Jan. '77 Flap fluid	Total 13 800 Polys 67%, lymphs 29%, monos 4%	Cells WBC +++ Polys 64% Lymphs 25% Monos 11%	Gram +ve bacilli +
7 28 May '77 CSF lumbar	Total 11 800 Polys 85%, lymphs 13%, monos 2%	WBC 2000 Polys 90% Lymphs 8% Monos 2% Protein 2750 mg/l Sugar 3.9 mmol/l	MNS
8 20 Dec. '76 CSF ventricular	Total 14 000 Polys 86%, lymphs 14%	RBC 3020 WBC 196 Polys 60% Lymphs 20% Monos 20% Protein 660 mg/l Sugar 4.2 mmol/l	Gram +ve bacilli +
21 Dec. '76 CSF ventricular	Total 8800 Polys 78%, lymphs 22%	RBC 800 WBC 95 Polys 82% Lymphs 10% Monos 8% Protein 330 mg/l Sugar 4.1 mmol/l	Gram +ve bacilli +
22 Dec. '76 CSF ventricular	Not done	RBC 4800 WBC 53 Polys 70% Lymphs 18% Monos 12% Protein 275 mg/l Sugar 4.1 mmol/l	MNS
9 21 Apr. '77 CSF xanthochromic	Total 13 700 Polys 77%, lymphs 17%, monos 5%, Eosin 1% Left shift	RBC 6000 WBC 800 Polys 93% Lymphs 6% Monos 1% Protein 2750 mg/l Sugar 1.6 mmol/l	MNS

MNS = microorganisms not seen; QNS = quantity not sufficient

atrial shunt was changed. She subsequently developed a nephrotic syndrome presumed to be due to the persistence of the *Staph. epidermidis* in the shunt, and the microorganism was again isolated from the blood. Preoperative erythromycin (appropriate to sensitivity) was given, and the entire shunt system was removed with insertion of external ventricular drainage. The removed shunt grew *Staph. epidermidis*.

The ventricular fluid showed RBC 3020/mm³ ($3.0 \times 10^9/l$), WBC 196/mm³ ($0.2 \times 10^9/l$) (60% polymorphs, 20% lymphs, 20% monocytes), sugar 4.2 mmol/l, and protein 660 mg/l. Gram-positive bacilli were seen and cultures yielded *P. acnes*. Two more ventricular fluid samples obtained during drainage yielded *P. acnes* on culture, and in the first of these samples the microorganisms were again seen on Gram

Table 3 Biochemical characteristics of *P. acnes* isolates

Catalase	Positive
Gelatin liquefaction	Positive
Aesculin hydrolysis	Negative
Indole	Positive
Nitrate reduction	Positive
Haemolysis	Variable
Carbohydrate fermentations (acids only)	
Glucose	Positive
Fructose	Variable
Mannose	Variable
Xylose	Negative
Mannitol	Negative
Lactose	Negative
Sucrose	Negative
Rhamnose	Negative
End products of glucose fermentation	
Propionic acid	
Acetic acid	
Isovaleric acid (minor amounts)	

stain. A new ventriculoperitoneal shunt was subsequently put in.

CASE 9

A 44-year-old man became febrile (up to 38.5°C) on the fourth postoperative day after craniotomy for pituitary adenoma. There was no clinical evidence of chest or wound infection, and blood and urine cultures were sterile. Meningitis was therefore suspected. On the sixth day lumbar CSF was examined and found to contain 6000 WBC/mm³ ($6.0 \times 10^9/l$) (93% polymorphs, 6% lymphs, 1% monocytes), protein 2750 mg/l, and sugar 1.6 mmol/l. *P. acnes* was isolated from the CSF. The peripheral blood at this time was 13 700 WBC/mm³ ($13.7 \times 10^9/l$) (77% polymorphs showing a left shift). Intramuscular chloramphenicol was started and the next day his fever subsided. There had been no neurological deterioration throughout this period.

Discussion

Propionibacteria have been described as being of little significance and are often disregarded as contaminants when isolated in the microbiology laboratory (Kaplan and Weinstein, 1969). There have, however, been reports incriminating *P. acnes* in a range of infections in man other than acne vulgaris, for example, meningitis (Kimbrell, 1964; Graber *et al.*, 1965), infected cerebrospinal fluid shunts (Everett *et al.*, 1976; Beeler *et al.*, 1976), and wound infections, osteomyelitis, and bacterial endocarditis (Kaplan and Weinstein, 1969).

The isolation of *P. acnes* from these nine cases may be explained in one of three ways. The isolates may be laboratory contaminants or skin commensals, or may genuinely be playing a pathogenic role in some or all of these patients. Culture of *P. acnes*

in pure culture from 15 specimens submitted from nine patients over a period of six months makes laboratory contamination unlikely because most of the similar specimens from asymptomatic patients remained sterile. Simple culture of skin bacteria is also unlikely because *P. acnes*, although a dominant member of the skin flora, particularly on the head, is rarely the only species present, and many other common skin bacteria are far easier to grow in the laboratory than are propionibacteria. The case for *P. acnes* being a significant isolate rests upon the data in Tables 1 and 2.

Pyrexia was noted in three of the nine cases. There was a correlation between pyrexia and peripheral blood leucocytosis and neutrophilia in all three cases. Leucocytosis with neutrophilia is a common finding postoperatively, with tissue damage and general trauma, resulting in the redistribution of the neutrophils between the marginal and circulating neutrophil pools. The normal duration of this response is 12 to 30 hours (Wintrobe, 1975). Of all the cases with neutrophilia, only case 9 returned to within normal limits in less than four days. Pyrexia may not be a relevant feature of *P. acnes* infection; Schlesinger and Ross (1977) noted that in *de novo* diphtheroid meningitis focal signs of cerebral dysfunction preceded the afebrile course of the disease, microorganisms being occasionally seen in Gram-stained films. The picture in CSF was one of an aseptic meningitis with a mononuclear leucocytosis and normal levels of glucose. Pyrexia may not, therefore, be a relevant criterion for infection. The CSF glucose levels in our series of patients varied from case to case, and the predominant cellular response was neutrophilic. In the neurosurgical context the features noted by Schlesinger and Ross (1977) may not apply. In all cases except case 6, the CSF showed a leucocytosis; this may represent a leptomeningeal reaction to infiltration of blood into the fluid. Phagocytic activity provoked by the red blood cells is manifested by the macrophages, and these phagocytes may be seen with ingested red blood cells either as intact cells or as vacuoles in the cytoplasm. The macrophages may be present for several months after the initial bleed (Kölmel, 1977). However, none of these cellular elements was seen in the specimens examined.

Cases of propionibacterial infection previously reported have involved mainly meningitis or infected CSF shunts. In nearly all cases the patients had some predisposing illness or had undergone operation which had increased the likelihood of infection. In all nine cases described in this study the patients had, for a variety of causes, had a neurological operation that involved incision through areas of skin known to be heavily

colonised with this type of microorganism (Evans, 1975). As a skin disinfectant 0.5% chlorhexidine has been shown to be effective (Nielsen *et al.*, 1975). Repeated Sellotape swabbing of the foreheads of volunteers known to carry *P. acnes* failed to yield that organism after standard treatment with 0.5% chlorhexidine. The skin surface appears to be cleared of organisms but results of a survey now in progress, which involves freshly cut scalp tissue, suggest that *P. acnes* living in the sebaceous glands (Woodroffe and Shaw, 1974) may survive the skin preparation and be released on incision.

The spectrum of *P. acnes* involvement includes its presence in subgaleal effusions (cases 3, 4, 6). These arise from mechanical complications and were resolved without antibiotics. The presence of *P. acnes* in such effusions may be relatively insignificant, but a survey of a large series of scalp flap collections has been instituted.

P. acnes was isolated from shunt cases 2, 5, and 8. In two of these cases (2 and 5) operation was for blockage of shunt without clinical evidence of infection and with negative blood cultures. *P. acnes* was cultured from the tubing of both cases and from the ventricular CSF of one (case 6). These patients represent the occult shunt colonisation of the type described by Fokes (1970). The third case was diagnosed as chronic *Staph. epidermidis* septicaemia by pre-operative blood cultures at another hospital. However, the presence of *P. acnes* in Gram-stained ventricular CSF at the time of shunt removal suggests that this microorganism was well established. *P. acnes* has been reported previously as the sole cause of immune-complex glomerular nephritis in chronic shunt infection (Beeler *et al.*, 1976).

In case 1 *P. acnes* was cultured from the first needle aspirate of the lobectomy cavity on the fourth day postoperatively when the patient had fever, headache, and haematological indications of infection. This was followed on the seventh post-operative day by a *Staph. aureus* osteomyelitis, which necessitated bone flap removal and vigorous antibiotic therapy. The meningeal symptoms were associated with the *P. acnes* isolation and *Staph. aureus* followed, causing necrosis of the bone flap.

The range of possible infection is completed by the two postoperative meningitis cases 7 and 9. Both cases developed fever, with CSF polymorph leucocytosis and raised protein. Case 7 had mild meningitic symptoms while case 9 was neurologically unchanged yet had significant depression of CSF sugar (1.6 mmol/l). *P. acnes* cannot be implicated with certainty as the primary aetiological agent, but this possibility is highly likely since no other microorganisms were isolated and there was a response to antibiotics.

All the propionibacteria isolated in this study were sensitive by disc diffusion testing to tetracycline, carbenicillin, penicillin, clindamycin, chloramphenicol, and erythromycin but were resistant to metronidazole with MICs, determined by the agar dilution method, of > 100 mg/l. This correlates with the findings of Wüst (1977) and Henderson *et al.* (1977) that propionibacteria and *Actinomyces* sp are the only clinically important groups of anaerobes resistant to the nitroimidazole group of antimicrobial agents.

The isolation of *P. acnes* in pure culture correlated with the haematological and CSF findings, and the benign indolent nature of the cases of postoperative meningitis suggests *P. acnes* as the aetiological agent (Schlesinger and Ross, 1977). Postoperative meningitis associated with *P. acnes* has not been reported before, and it is clearly important to study prospectively a larger series of neurosurgical cases in which a 'sterile' meningitis occurs. Demonstration of circulating antibodies against the patient's organism (French *et al.*, 1974; Beeler *et al.*, 1976) and CSF LDH (Beaty and Oppenheimer, 1968) and IgA (Sheth, 1971) levels would provide further evidence of a genuine role for this group of microorganisms as a significant pathogen in some types of patient.

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