

# Bacteriological examination of pus from abscesses of the central nervous system

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**SUMMARY** The methods used successfully to examine pus from abscesses of the central nervous system are described. The association between direct inoculation of intracranial pus into a liquid anaerobic culture medium and the isolation of viable bacteria is emphasised. Cultural methods for the recognition of the streptococci associated with brain abscess and methods for the assay of antimicrobial drugs in pus are presented. The role of gas liquid chromatography is discussed.

Pus from brain abscesses and from subdural and extradural empyemata of the brain and spinal cord has been reported as sterile in up to 60% of cases in published series (Kao, 1973; see review of de Louvois (1978)). Bacteria have sometimes been seen in Gram-stained films but have failed to grow on culture (Shaw and Russell, 1975). If meticulous attention is paid to microbiological technique, bacteria can be cultured from all abscesses on primary investigation. Ingham *et al.* (1977) recorded positive cultures from nine consecutive abscesses and de Louvois *et al.* (1977a) from 46 studied.

This report details the microbiological procedures that afford the best chance of culturing bacteria from abscesses of the central nervous system.

## Material and methods

For satisfactory results the pus must be inoculated with a minimum of delay, and it is important that examining laboratories realise that brain abscess is no less an emergency for the laboratory than it is for the neurosurgeon. Ideally, the bacteriologist should be on hand when the abscess is tapped in the operating room. If this is not possible or feasible, precise instructions should be issued to the neurosurgical team so that the pus may be inoculated on to suitable media as soon as it has been aspirated and the specimens transported to the examining laboratory as rapidly as possible.

## EXAMINATION FOR BACTERIA

### *Transport of samples*

Should there be risk of delay, the specimen should be divided, one aliquot being placed into an anaerobic liquid medium (thioglycollate or thiol broth) containing  $\beta$ -lactamase (Whatman) and the other into a suitable sterile, screw-capped container. Samples should be sent to the examining laboratory so as to reach it in the shortest possible time and should ideally be transported at 4°C. This can be done most satisfactorily by means of a freezable sachet (Freezella-Raven Scientific) in a suitable container.

### *Direct inoculation of plates and liquid media for the cultivation of microorganisms*

The following media should be inoculated from the thioglycollate/ $\beta$ -lactamase broth:

- 1 6% horse blood agar incubated aerobically;
- 2 6% horse blood agar incubated anaerobically;
- 3 6% horse blood agar containing 0.01% neomycin (Upjohn) incubated anaerobically;
- 4 chocolate blood agar incubated in 10% carbon dioxide.

Direct antibiotic sensitivity tests should be set up on chocolate blood agar and incubated anaerobically and in 10% carbon dioxide. The anaerobic environment should contain 10% carbon dioxide.

Plates are incubated for up to five days at 35°C, during which time they are examined daily. Individual colonies are filmed and subcultured for identity and antibiotic sensitivity using standard microbiological procedures (see below). Primary sensitivity of the infecting organisms is determined to penicillin, ampicillin, chloramphenicol, co-trimoxazole, clin-

damycin, streptomycin, and metronidazole, for these are the antibiotics most frequently used in the treatment of brain abscess. Staphylococci are also tested against cloxacillin and fusidic acid.

The thioglycollate broth is incubated for 48 hours and subcultured to the range of media above, irrespective of the culture results already obtained.

#### BLOOD CULTURE

Blood for culture taken at the time of aspiration of pus should be inoculated into 0.1% dextrose digest broth containing added *p*-aminobenzoic acid and  $\beta$ -lactamase. Subcultures should be made after 1, 2, 3, 7, and 14 days' incubation at 35°C on to blood agar plates incubated aerobically and anaerobically and chocolate blood agar incubated in 10% carbon dioxide.

#### IDENTIFICATION OF BACTERIA

The bacteria commonly isolated from intracranial and intraspinal abscesses are listed in Table 1.

Table 1 *Bacteria commonly isolated from intracranial and intraspinal abscesses*

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|  |
|--|
| Streptococci                                   |
| <i>Strep. milleri</i> *                        |
| Other viridans and non-haemolytic streptococci |
| Enterococci                                    |
| $\beta$ -haemolytic streptococci               |
| Peptostreptococci                              |
| Bacteroides group                              |
| Enterobacteriaceae                             |
| <i>Proteus</i> sp.                             |
| <i>Escherichia coli</i>                        |
| <i>Klebsiella</i> sp.                          |
| <i>Staphylococcus aureus</i> †                 |

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\*Most frequent organism isolated from intracranial pus

†Associated with post-traumatic and spinal lesions

Routine bacteriological procedures based on the methods and nomenclature of Cowan and Steel (1965) can be used to identify aerobic isolates other than the viridans streptococci. Anaerobic Gram-negative rods can be identified initially by antibiotic sensitivity tests (Leigh, 1976) and subsequently by the API 20 A test kit, which is also of value in identifying anaerobic cocci. Organisms of the bacteroides group when present in intracranial pus often occur as a mixed population.

The identification methods used for the viridans and non-haemolytic streptococci are, in general, those described by Colman (1970), and organisms should be classified according to the scheme proposed by Colman (1970) and Colman and Williams (1972). In cases of doubt the organisms should not be allocated specific status.

#### CULTURAL METHODS FOR CHARACTERISING STREPTOCOCCI

Acid production from glucose mannitol arabinose, lactose, sucrose, trehalose, raffinose, salicin, inositol, and sorbitol is tested in phenol red peptone water with 20% horse serum. Cultures are incubated in 10% carbon dioxide (CO<sub>2</sub>) and examined after one, two, and seven days. Hydrolysis of aesculin is tested on 0.1% aesculin agar containing 5% horse serum. Acetylmethyl carbinol production is determined by the method described by Poole and Wilson (1976) and hydrolysis of arginine by the method of Niven *et al.* (1942). The liberation of free ammonia is detected by adding 1 ml of 1/10 dilution of a seven-day arginine broth culture to a mixture consisting of 1 ml 0.17 M sodium nitroprusside in 0.106 M phenol water and 1 ml 0.125 N sodium hydroxide in water containing 11 mM sodium hypochlorite. A positive reaction is demonstrated by production of a blue colour. Production of extracellular polysaccharide from sucrose is demonstrated after growth in the medium of Bailey and Oxford (1958) for five days by the method of Hehre and Neill (1946). Growth on media containing bile salts is demonstrated on a blood agar plate, one-half of which is removed and replaced with 10% or 40% bile salts in nutrient agar with 5% added horse serum. Strains are streak inoculated on to both halves of the plate and examined for growth after 48 hours' incubation at 37°C. The biochemical reactions of the viridans and non-haemolytic streptococci are shown in Table 2.

#### ANTIBIOTIC ASSAY

A number of factors affect the penetration and concentration of antimicrobial drugs in intracranial pus. It is therefore important, if antibiotics are to be used effectively in the management of brain abscess, that antibiotic concentration should be assayed whenever possible.

Samples of pus and serum can be assayed for the presence of antimicrobial drugs most satisfactorily by the plate diffusion technique. Thirty to forty millilitres of appropriate assay medium at 50°C is poured into a 6 in diameter petri dish (Sterilin) on a levelling tray. Once set the medium is dried and surface seeded with a dilution of a log phase broth culture of the indicator organism which results in semiconfluent growth after 18 hours' incubation. The inoculated medium is dried and stored at 4°C for use within three days. Before use wells are cut with a sterile cork borer. The wells are filled at random with test and standard solutions in duplicate. After prediffusion at 4°C for 1 hour, the plate is incubated at 37°C overnight (30°C for assay of cloxacillin). The log of the concentration of antibiotic standards is plotted against the inhibition

Table 2 *Biochemical characters of the viridans and non-haemolytic streptococci*

|                      | <i>Strep. milleri</i> | <i>Strep. mutans</i> | <i>Strep. mitior</i> | <i>Strep. sanguis</i> | <i>Strep. salivarius</i> |
|----------------------|-----------------------|----------------------|----------------------|-----------------------|--------------------------|
| Acid production in:  |                       |                      |                      |                       |                          |
| Glucose              | +                     | +                    | +                    | +                     | +                        |
| Mannitol             | -                     | +                    | -                    | -                     | -                        |
| Arabinose            | -                     | -                    | -                    | -                     | -                        |
| Lactose              | +                     | +                    | +                    | +                     | +                        |
| Sucrose              | +                     | +                    | +                    | +                     | +                        |
| Trehalose            | +                     | +                    | v                    | +                     | v                        |
| Raffinose            | -                     | +                    | v                    | v                     | v                        |
| Salicin              | -                     | +                    | v                    | +                     | +                        |
| Inositol             | -                     | +                    | -                    | +                     | v                        |
| Sorbitol             | -                     | +                    | -                    | -                     | -                        |
| Hydrolysis of:       |                       |                      |                      |                       |                          |
| Aesculin             | +                     | +                    | -                    | +                     | +                        |
| Arginine             | +                     | -                    | -                    | +                     | -                        |
| Voges Proskauer      | +                     | +                    | -                    | -                     | v                        |
| Levan from sucrose   | -                     | -                    | -                    | -                     | +                        |
| Dextran from sucrose | -                     | +                    | -                    | +                     | -                        |
| Growth on bile       |                       |                      |                      |                       |                          |
| 10%                  | v                     | v                    | -                    | -                     | v                        |
| 40%                  | -                     | -                    | -                    | -                     | v                        |

(Parker and Ball, 1976)

v = variable reaction

zone diameter in millimetres. The concentration in the test fluids is determined from the line of best fit drawn through the standard points. Table 3 gives essential details of assay systems that have been found satisfactory. The accuracy of plate diffusion assays is greatly increased if the zones are read on a zone reader.

The following factors should also be considered when undertaking antibiotic assay:

#### *Avoidance of falsely low readings due to decay of antibiotics in transit*

The laboratory should be notified during or immedi-

ately after operation that pus has been located. Serum standards for the anticipated assay should be prepared as soon as the sample is collected and stored under identical or comparable conditions. This is especially necessary for the  $\beta$ -lactam antibiotics, which tend to be degraded in pus.

#### *Separation and assay of serum*

Samples of serum for assay should be separated from the blood clot immediately on arrival in the laboratory and stored with the prepared standards at 4°C or -20°C, depending on the anticipated delay before the assay is performed.

Table 3 *Assay systems for the common antimicrobial drugs used in treatment*

| Agent           | Indicator organism                      | Medium                          | pH      | Well size (mm) | Sensitivity of assay in serum (mg/l) |
|-----------------|---|---------------------------------|---------|----------------|--------------------------------------|
| Penicillin      | <i>Sarcina lutea</i>                    | DST* or Penassay Agar No. 1†    | 6.8     | 6.0            | 0.02                                 |
|                 | <i>Staph. aureus</i> NCTC 6571          | " " " "                         | 6.8     | 6.0            | 0.2                                  |
| Cephalosporins  | <i>Staph. aureus</i> NCTC 6571          | " " " "                         | 6.8     | 6.0            | 0.5                                  |
|                 | Cloxacillin                             | Penicillin-resistant            | " " " " | 6.8            | 8.0                                  |
| Ampicillin      | <i>Staph. aureus</i>                    | " " " "                         | 6.8     | 6.0            | 1.0                                  |
|                 | <i>Escherichia coli</i> NCTC 10418      | " " " "                         | 6.8     | 6.0            | 0.5                                  |
| Chloramphenicol | <i>Staph. aureus</i> NCTC 6571          | " " " "                         | 6.8     | 6.0            | 0.5                                  |
|                 | <i>S. lutea</i> NCTC 8340               | 0.5% Yeast extract agar         | 6.2     | 8.0            | 2.5                                  |
| Sulphonamides   | <i>B. subtilis</i> (ATCC 6051)          | Anaerobic agar†                 | 6.8     | 8.0            | 6.0                                  |
|                 | <i>Alkaligenes</i> sp.                  | DST + 4% lysed blood            | 7.4     | 9.0            | 2.0                                  |
| Trimethoprim    | <i>Klebsiella</i> sp.                   | DST + 4% lysed blood            | 7.4     | 6.0            | 2.0                                  |
| Metronidazole   | <i>Clostridium sporogenes</i>           | Anaerobic agar† + 5 mg/l haemin | 7.2     | 7.5            | 0.25                                 |
|                 | <i>C. perfringens</i>                   | " " " "                         | 7.2     | 4.5            | 4.0                                  |
| Fusidic acid    | <i>Corynebacterium xerosis</i> FF VIII‡ | Fusidic acid agar               | 6.0     | 6.0            | 0.1                                  |
| Aminoglycosides | <i>Klebsiella</i> sp. NCTC 10896        | DST                             | 7.8     | 6.0            | 0.5                                  |

\*Oxoid †Difco ‡Leo Laboratories

### Liquefaction of pus

Mucoid samples may be liquefied by adding 1 ml pancreatin solution\* to approximately 1 ml pus and incubating the mixture at 37°C for 30 minutes. An accurate volumetric measurement of the pus can be made at the end of this time by the method of subtraction (de Louvois and Hurley, 1976).

Samples of pus that require pancreatin treatment are assayed in agar to which 1 ml pancreatin solution had been added before pouring.

Pus samples that require pancreatin treatment are assayed against standards that have been similarly treated.

### ASSAY OF ANTIBIOTIC MIXTURES

The procedures used to assay antimicrobial drugs in a mixture include the use of indicator organisms resistant to the unwanted drug, pH control of the assay system, use of variation in diffusion rates between different drugs, dilution of samples, and drug inactivation. The inactivators used are  $\beta$ -lactamase I,  $\beta$ -lactamase I and II, *p*-aminobenzoic acid, and thymidine. Aminoglycosides can be removed by treating samples with cellulose phosphate (Sigma) (Stevens and Young, 1977).

A further feature that complicates the assay of antimicrobial drugs in pus is that of inactivation. This most often affects  $\beta$ -lactamase sensitive penicillins in the presence of  $\beta$ -lactamase producing bacteria. However, the inactivation of some penicillins by purulent material alone and in the absence of  $\beta$ -lactamase or  $\beta$ -lactamase producing bacteria has been reported (de Louvois and Hurley, 1977).

### GAS LIQUID CHROMATOGRAPHY

The presence of short and long chain fatty acids in samples of pus, cerebrospinal fluid, and broth cultures is highly indicative of anaerobic bacteria and can be demonstrated by gas-liquid chromatography (GLC). A Pye series 104, model 64, gas chromatograph with a glass column 5 ft long and 4 mm internal diameter containing Chromosorb 101, mesh 80/100 (Field Instruments Ltd) which is not coated is a suitable system. Using this column, it is not necessary to extract or esterify the specimens. Samples of 1  $\mu$ l of crude material are injected directly on to a precolumn also packed with Chromosorb 101. The injection port is maintained at a temperature of 200°C while the flame ionisation detector should be at 240°C. The carrier gas is nitrogen, at a flow rate of 40 ml per minute, and the

machine is run at an attenuation of  $2 \times 10^{-2}$ . The results are traced on to a linear chart recorder and compared with those obtained from a standard solution of fatty acids in water (Sepulco). Eradication of anaerobic bacteria is closely followed by the disappearance of fatty acids from clinical samples, and this may be of value in assessing the effectiveness of treatment.

A suitable broth medium for growing bacteria before GLC is as follows:

|                                 |         |           |
|---------------------------------|---------|-----------|
| Sodium thiosulphate             |         | 0.05 g    |
| Sodium formaldehyde sulfoxylate |         | 0.03 g    |
| Peptone                         | (Oxoid) | 1.00 g    |
| Yeast extract                   | (Oxoid) | 1.00 g    |
| Sodium chloride                 |         | 0.25 g    |
| Glucose                         | (Oxoid) | 1.00 g    |
| L-cysteine hydrochloride        |         | 0.05 g    |
| Vitamin K/Hemin                 | (Sigma) | 1.00 g    |
| Distilled water                 |         | to 100 ml |

The pH of the medium is adjusted to 7.0 and it is sterilised at 115°C for 15 minutes.

### Discussion

Viable bacteria were isolated from all the samples of intracranial pus studied by de Louvois *et al.* (1977a) and Ingham *et al.* (1977). In direct contrast to all previous studies on unselected patients, a common feature of which have been reports of sterile pus ranging in incidence from 4% (McFarlan, 1943) to 62% (Kao, 1973), Liske and Weikers (1964), Morgan *et al.* (1973), and Samson and Clark (1973) suggested that sterile cultures are due to antibiotics administered before aspiration. This may well be so if no attempt is made to dilute out or to neutralise any antibacterial agents present as soon as the sample is aspirated. The finding that pus samples containing anaerobic bacteria may be rendered sterile if allowed to stand at room temperature overnight supports this hypothesis (de Louvois, 1978).

Samples should, therefore, be collected into a liquid anaerobic culture medium containing antibiotic inactivators. Recent work suggests that thiol broth may be superior to thioglycollate for this purpose (Griffiths and Shoosmith, 1977). If samples are transported to the laboratory in this way, then routine bacteriological procedures are satisfactory for the isolation of infecting bacteria.

Standard identification procedures are suitable for the majority of isolates. The viridans and non-haemolytic streptococci are best identified using the scheme of Colman and Williams (1972) and Colman (1976). Care needs to be taken to avoid confusion between carboxyphilic, microaerophilic, and anaerobic streptococci since many isolates of

\*Pancreatin solution: Dissolve a pancreatin tablet (Oxoid) in 25 ml sterile distilled water at 37°C. Seitz filter the solution and store at 4°C for use within seven days.

*Streptococcus milleri* only grow anaerobically on primary culture. Initial separation of the various groups of anaerobic Gram-negative rods may be achieved using selected high concentration antibiotic discs (Leigh, 1976).

The problem of establishing the role of anaerobic bacteria in intracranial sepsis is complicated by the fact that the organism described and recognised as *Strep. milleri* (Colman, 1970; Colman and Williams, 1972) is biochemically indistinguishable from that referred to as *Peptostreptococcus intermedius* in the earlier Virginia Polytechnic Handbook (Holdeman and Moore, 1972) and the API system for identification of anaerobic bacteria. Acetic acid and sometimes propionic acid are the only fatty acids found in the GLC profiles of *P. intermedius* (VPI Handbook) and of *Strep. milleri* (de Louvois, 1977). Neither of these organisms is a strict anaerobe, but both require additional carbon dioxide for growth.

GLC using Chromosorb 101 as the stationary phase has the advantage that samples do not have to be extracted. The demonstration of volatile fatty acids (butyric, caproic, valeric, etc) in 1  $\mu$ l crude pus can confirm the presence of anaerobic bacteria within 30 minutes of receiving the sample. The procedure, carried out on subsequent aspirates, may also provide useful information on the effectiveness of therapy on anaerobic bacteria.

Duffy (1969) pointed out the hazards of lumbar puncture in patients with intracranial abscess. Despite this, many patients do undergo lumbar puncture before referral and before an intracranial or intraspinal abscess has been recognised. Cerebrospinal fluid from such patients is usually bacteriologically sterile, and some will have normal cell counts, sugar, and protein (de Louvois, 1978). The isolation of an organism unlikely to cause uncomplicated meningitis or of a mixed bacterial population should alert the bacteriologist to the possibility of an intracranial abscess.

There is an association between the site of an abscess in the central nervous system, the proximate cause, and the types of microbes that are likely eventually to be isolated (de Louvois *et al.*, 1977a, 1977b). Thus an early report on the Gram film of the original sample and of the GLC profile of the pus may provide useful therapeutic information, and the neurosurgeon should be informed of these as soon as possible. Initial therapy may have to be modified after presentation of definitive reports.

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