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Rapid diagnosis of anaerobic infections by gas-liquid chromatography of clinical material

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SYNOPSIS Gas-liquid chromatographic analysis of samples of pus provides a rapid and reliable means for the presumptive differentiation of anaerobic from aerobic infections.

Infections due to non-sporing anaerobic bacteria are common. In recent years these organisms have been implicated in a wide range of infective processes of which intra-abdominal and pelvic infections are notable examples (Balows *et al*, 1974; Phillips and Sussman, 1974; Study Group, 1974, 1975, 1976). Characteristically, infections due to non-sporing anaerobes are associated with the formation of copious, foul-smelling pus from which these organisms may be isolated in abundance in 24 to 48 hours.

The production of volatile fatty acids is a primary metabolic activity of anaerobic bacteria and is used *in vitro* as a taxonomic tool (Moore, 1970; Holdeman and Moore, 1972). In a recent small study, Gorbach *et al* (1974) successfully correlated the isolation of *Bacteroides fragilis* from samples of pus with the detection by gas-liquid chromatography of significant amounts of isobutyric, butyric, and succinic acids in the specimens. This enabled a presumptive diagnosis of *B. fragilis* infection to be made by chromatographic analysis of the specimen within one hour of its collection.

In the present study, specimens of pus were analysed by gas-liquid chromatography for the presence of volatile fatty acids, and the results are compared with the organisms isolated by cultural methods.

Material and Methods

SPECIMENS

Forty-four consecutive samples of pus submitted to this laboratory for routine bacteriological examination were included in the study. The specimens were obtained from a variety of superficial and deep infections (tables I and II).

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BACTERIOLOGICAL STUDIES

Specimens were examined by direct microscopy of Gram-stained smears and were cultured on freshly prepared horse blood agar plates for aerobic and anaerobic incubation. Anaerobic cultures were incubated in jars in an anaerobic atmosphere containing approximately 7% carbon dioxide.

Two blood agar plates were incubated anaerobically for each specimen, one of which contained neomycin sulphate (100 μg/ml) to supress the growth of facultative organisms. Assessment of significant isolates was made after 24 hours' incubation. The plates were examined for the identity of the isolates and compared with one another for the relative proportions of aerobic, facultative, and anaerobic growth. Anaerobic cultures were reincubated for up to five days to allow full development of the flora present. Quantitative assessment of growth was recorded by the +/− notational system (+ = scanty growth . . . ++++ = very heavy growth).

GAS-LIQUID CHROMATOGRAPHY

A Pye Unicam 104 Series Chromatograph equipped with a flame ionization detector was used for analyses of volatile fatty acids. The column contained 10% polyethylene glycol adipate on Celite, and the carrier gas was nitrogen. The instrument was operated at 130°C. A chart recorder was used to produce a trace of the acid peaks detected.

Method

One millilitre of each sample of pus was acidified with a few drops of 50% sulphuric acid and the volatile acids were extracted in 1 ml of diethyl ether. One microlitre of the ether layer was withdrawn for injection into the chromatograph. A volatile acid control solution was chromatographed to allow

Specimen	*Source*	*Acids*	*Organisms*
1	Peritonitis	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic	++++ *Bacteroides fragilis* +++ *Bacteroides melaninogenicus* +++ *Clostridium* sp +++ Coliform sp
2	Peritonitis	Acetic, propionic, isobutyric, butyric, isovaleric, valeric	++++ *Bacteroides fragilis* ++++ Enterococcus ± *E. coli*
3	Peritonitis	Acetic, propionic, isobutyric, isovaleric	+++ *Bacteroides* sp +++ Coliform sp + *Proteus* sp
4	Peritonitis	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic	++++ *Bacteroides fragilis* ++++ Coliform sp +++ Enterococcus + *Clostridium* sp
5	Peritonitis	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic	+++ *Bacteroides fragilis* +++ *Bacteroides melaninogenicus* ++ *E. coli*
6	Peritonitis	Acetic, propionic, butyric, isovaleric	+++ *Bacteroides fragilis* ++ *E. coli*
7	Peritonitis	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic	++++ *Bacteroides fragilis* ++++ *Bacteroides melaninogenicus* ++++ Coliform sp
8	Abdominal wound	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic	+++ *Bacteroides* sp +++ *Clostridium* sp +++ Coliform sp
9	Abdominal wound	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic	++++ *Bacteroides fragilis* ++++ *Bacteroides melaninogenicus* +++ Coliform sp
10	Abdominal wound	Acetic, isovaleric	++++ *Bacteroides fragilis*
11	Abdominal wound	Acetic, propionic, butyric	++++ Anaerobic coccus
12	Pyothorax	Acetic, propionic, isovaleric	++++ *Bacteroides fragilis*
13	Pyothorax	Acetic, butyric	++++ Anaerobic coccus ++++ Enterococcus
14	Pelvic abscess	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic	++++ Anaerobic coccus
15	Pelvic abscess	Acetic, butyric	+++ *Bacteroides* sp +++ Coliform sp
16	Pelvic abscess	Acetic, propionic, isobutyric, butyric, isovaleric, valeric	++++ *Bacteroides fragilis* +++ *Bacteroides melaninogenicus* +++ *Bacteroides corrodens*
17	Appendiceal pus	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic	++++ *Bacteroides* sp ++++ Coliform sp
18	Caesarean section wound	Acetic, butyric	++++ Anaerobic coccus
19	Perianal abscess	Acetic, butyric	+++ *Clostridium perfringens* +++ Coliform sp
20	Pilonidal sinus	Acetic, butyric	++++ *Bacteroides fragilis*
21	Pilonidal sinus	Acetic, isobutyric, butyric, isovaleric	++++ *Bacteroides melaninogenicus* ++++ Anaerobic coccus ++++ *Bacteroides corrodens*
22	Axillary abscess	Acetic, propionic, isobutyric, butyric, isovaleric, valeric	++++ *Bacteroides melaninogenicus* +++ Anaerobic coccus
23	Axillary abscess	Acetic, butyric	++++ Anaerobic coccus ++++ *Bacteroides corrodens*
24	Carbuncle	Acetic, butyric	++++ Anaerobic coccus

Table I *Volatile fatty acids and organisms detected in 24 samples of pus from anaerobic infections*

precise identification of the acids detected in the specimens. This control solution contained approximately 1 mEq/100 ml of each of the following acids: acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and heptanoic (Holdeman and Moore, 1972).

Examination of Appendices

Appendices from 31 consecutive acute appendicectomies (9 normal, 15 acutely inflamed, 7 gangrenous) were examined for the presence of volatile fatty acids. After cultures had been made, the

Specimen	Source	Acids	Organisms
1	Peritonitis	No acids	+ + + Coliform sp
2	Abdominal wound	No acids	+ + Coliform sp
3	Abdominal wound	Acetic acid only	+ + + *Staphylococcus aureus*
4	Pyonephrosis	No acids	+ *Escherichia coli*
5	Pyocele	No acids	+ + + Coliform sp
6	Axillary abscess	Acetic acid only	+ + + + *Staphylococcus aureus*
7	Axillary abscess	No acids	+ + + + *Staphylococcus aureus*
8	Axillary abscess	Acetic acid only	+ + + + *Staphylococcus aureus*
9	Buttock abscess	Acetic acid only	+ + + + *Staphylococcus aureus*
10	Buttock abscess	No acids	+ + + *Staphylococcus aureus*
11	Abscess of neck	Acetic acid only	+ + + *Staphylococcus aureus*
12	Abscess of external ear	No acids	+ + *Staphylococcus epidermidis*
13	Maxillary pus	No acids	+ *Staphylococcus epidermidis*
14	Abscess of back	No acids	+ + *Staphylococcus epidermidis*
15	Palmar infection	No acids	+ + + *Staphylococcus aureus*
16	Infected ankle	No acids	+ + + *Staphylococcus aureus*

Table II *Volatile acids and organisms detected in 16 samples of pus from facultative infections*

proximal half of the organ was homogenized and the homogenate was extracted and chromatographed as outlined above for pus.

IN VITRO STUDIES

Pure fluid cultures of a variety of commonly encountered anaerobic and facultative organisms were incubated anaerobically in 1% glucose cooked meat broth. The culture fluids were extracted and analysed for the presence of volatile fatty acids. Fresh clinical isolates of the following organisms were used: *Bacteroides fragilis*, *Bacteroides melaninogenicus*, *Bacteroides corrodens*, *Bacteroides praeacutus*, *Fusobacterium nucleatum*, *Fusobacterium necrophorum*, *Fusobacterium mortiferum*, *Escherichia coli*, *Klebsiella* sp, *Streptococcus faecalis*, and *Staphylococcus aureus*. Aerobic glucose meat broth cultures of *Pseudomonas aeruginosa* were also analysed. In addition, culture fluids of various combinations of these organisms were examined. All cultures were analysed by gas-liquid chromatography after 2, 5, and 14 days' incubation.

Results

The volatile fatty acids detected in 40 of the 44 specimens of pus, and the significant bacterial isolates from each, are summarized in tables I and II. Four samples not included in the tables were sterile and contained no volatile acids.

As is clear from table I, anaerobes were always isolated in significant numbers (+ + + or + + + +) from samples of pus in which volatile fatty acids other than acetic acid were detected (figs 1a and b). The flora of these specimens was either exclusively anaerobic or anaerobic with an associated facultative flora. Obligate anaerobes were never isolated from specimens of pus which contained either no volatile acids or acetic acid only (table II; figs 2a and b).

Chromatographic analysis of both the pure and mixed cultures of facultative organisms showed that acetic acid was the only volatile fatty acid produced.

Pure and mixed cultures of obligate anaerobes and mixed cultures of anaerobes and facultative organisms produced multiple volatile fatty acids always including acetic acid.

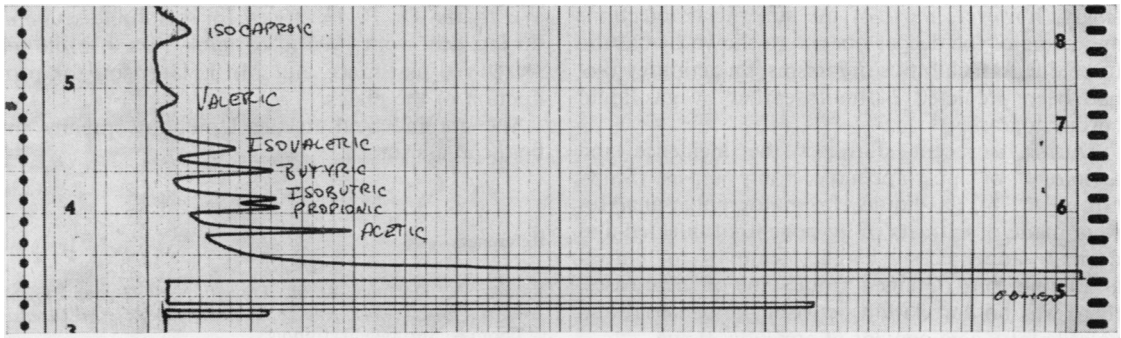
Chromatographic analysis of extracts of appendices was not helpful. There was no significant relationship between the chromatograms of volatile fatty acids present, the clinical state of the appendix, and its bacterial flora.

Discussion

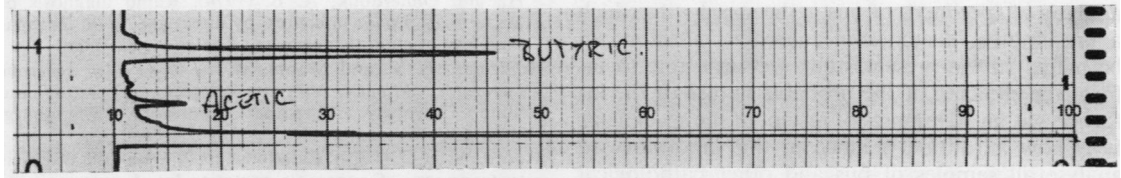
Laboratory recognition of infection due to non-sporing anaerobes currently depends on conventional methods of direct plating and at least 24 hours are required to establish a diagnosis. For those patients who need antimicrobial chemotherapy it is clearly important to establish the nature of the infection as soon as possible so that appropriate antibiotics may be chosen.

The results of the present study show that gas-liquid chromatographic analysis of samples of pus provides a rapid and reliable means for the presumptive differentiation of anaerobic from aerobic infection. As is clear from table I, specific identification of anaerobes present in the material is not possible.

Although non-clostridial anaerobic infections are characteristically associated with deep-seated, foul-smelling abscesses, these organisms may also be the cause of superficial infections not related to the gastrointestinal and female genital tracts. In the present study, pus from two axillary abscesses and a carbuncle (specimens 22, 23, and 24, table I) yielded obligate anaerobes only, an event that is by no means uncommon in our experience. Not surpri-

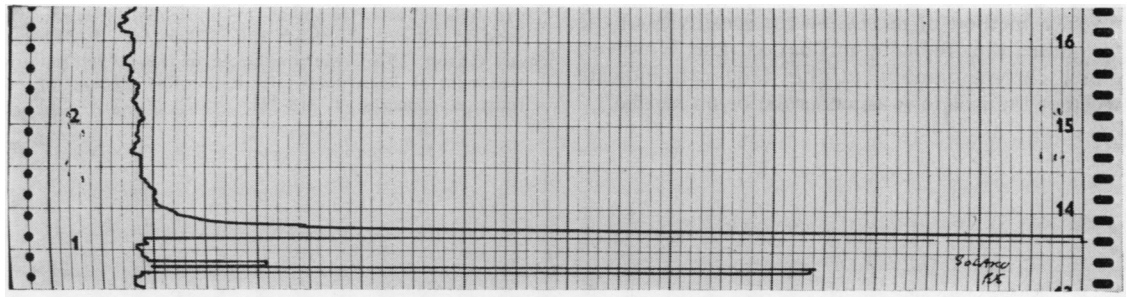


1a

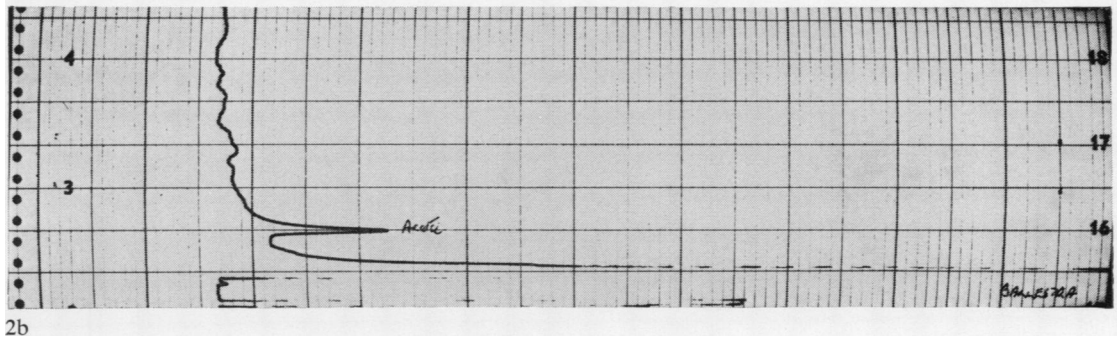


1b

Fig 1(a and b) *Chart records of chromatographic analysis of two typical specimens of pus from anaerobic infections (specimens 7 and 20, respectively, table I).*



2a



2b

Fig 2 (a and b) *Chart records of chromatographic analysis of two typical specimens of pus from facultative infections (specimens 1 and 6, respectively, table II).*

singly, however, most of the superficial infections and some of the deeper ones were shown by culture to be due to facultative organisms, an aetiology that had been predicted 24 hours earlier by gas-liquid chromatography.

During a recent study of the incidence and aetiology of post-appendicectomy sepsis (Study Group, 1976), gas-liquid chromatographic analyses were made of extracts of homogenized infected and normal appendices. Unlike samples of intra-abdominal pus obtained from some of these cases, there was no correlation between the bacterial and volatile fatty acid content of appendix tissue.

The great value of gas-liquid chromatographic analysis of specimens of pus is the speed with which a presumptive diagnosis of anaerobic infection can be made. In the present study we were able to inform the clinician of the probable nature of the infection within 30 minutes of receipt of the specimens. It is now a routine practice in this laboratory to analyse all samples of pus and other pathological exudates by gas-liquid chromatography.

Gas-liquid chromatography of blood cultures for rapid differentiation of anaerobic from aerobic bacteraemia may also be of value. However, it is our experience to date that volatile metabolic end products are not detectable in the blood culture medium before growth has been noted. Further study may reveal a method of increasing the sensitivity of the technique, thus reducing incubation time. However, direct chromatography at this time can indicate whether the isolate is an anaerobe or an aerobe.

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