JCI The Journal of Clinical Investigation

Rapid diagnosis of anaerobic infections by direct gas-liquid chromatography of clinical speciments.

S L Gorbach, … , H Thadepalli, A B Onderdonk

J Clin Invest. 1976;57(2):478-484. https://doi.org/10.1172/JCI108300.

Research Article

Current methods to isolate and identify anaerobic bacteria are laborious and time consuming. It was postulated that the short-chain fatty acids (SCFA) produced by these organisms might serve as microbial markers in clinical material. 98 specimens of pus or serous fluid were analyzed by gas-liquid chromatography, and findings were compared with culture results. Good correlations were found for the recovery of anaerobic Gram-negative bacilli and the presence of isobutyric, butyric, and succinic acids. 19 of 20 specimens with significant amounts of these acids (greater than 0.01 mumol/ml) yielded bacteroides or fusobacteria. Culture of the single "false-positive" specimen failed to grow anaerobic Gram-negative bacilli, although clinical data and Gram-stain suggested their presence. 77 of 78 specimens which has insignificant concentrations of the marker acids failed to yield anaerobic, Gram-negative bacilli in culture. The single "false-negative" specimen yielded Bacteroides pneumosintes, an organism which does not ferment carbohydrates. It is concluded that direct gas-liquid chromatographic analysis of clinical specimens provides a rapid presumptive test for the presence of anaerobic, Gram-negative bacilli.

Rapid Diagnosis of Anaerobic Infections by Direct Gas-Liquid Chromatography of Clinical Specimens

SHERWOOD L. GORBACH, JAMES W. MAYHEW, JOHN G. BARTLETT, HARAGOPAL THADEPALLI, and ANDREW B. ONDERDONK

From the Infectious Disease Section, Veterans Administration Hospital, Sepulveda, California 91343, and The Department of Medicine, University of California Medical Center, Los Angeles, California 90024

ABSTRACT Current methods to isolate and identify anaerobic bacteria are laborious and time consuming. It was postulated that the short-chain fatty acids (SCFA) produced by these organisms might serve as microbial markers in clinical material. 98 specimens of pus or serous fluid were analyzed by gas-liquid chromatography, and findings were compared with culture results. Good correlations were found for the recovery of anaerobic Gram-negative bacilli and the presence of isobutyric, butyric, and succinic acids. 19 of 20 specimens with significant amounts of these acids (> 0.1 μmol/ml) yielded bacteroides or fusobacteria. Culture of the single "false-positive" specimen failed to grow anaerobic Gram-negative bacilli, although clinical data and Gram-stain suggested their presence. 77 of 78 specimens which had insignificant concentrations of the marker acids failed to yield anaerobic, Gram-negative bacilli in culture. The single "false-negative" specimen yielded *Bacteroides pneumosintes,* an organism which does not ferment carbohydrates. It is concluded that direct gas-liquid chromatographic analysis of clinical specimens provides a rapid presumptive test for the presence of anaerobic, Gram-negative bacilli.

INTRODUCTION

Comprehensive anaerobic bacteriology of clinical specimens is an expensive and time-consuming operation. Laudable pus from an intra-abdominal abscess, for example, may harbor multiple aerobic and anaerobic microorganisms, and it may require 1–2 wk for complete bacteriologic identification of these strains (1, 2). *Bacterioides fragilis* is particularly important to recognize since this organism appears to be more virulent than other anaerobes. In addition, *B. fragilis* has unusual antimicrobial susceptibility patterns since it is relatively resistent to penicillins, cephalosporins, and tetracyclines. Hence, an early indication of *B. fragilis* in infected material would be a useful guide to the choice of appropriate antibiotics.

We have attempted to develop a rapid test for the presence of anaerobic bacteria in clinical specimens based on their metabolic activities. These microorganisms, particularly bacteroides, clostridia, and fusobacteria, produce short-chain fatty acids (SCFA) [1] from metabolism of carbohydrate and protein. Such end products are so regularly produced that they can be used for taxonomic purposes (3). It was hoped that these acids could be detected in purulent material and thereby serve as fingerprints for the presence of putrefactive anaerobes.

METHODS

Specimens of pus and body fluids were rapidly delivered to our laboratory after collection. Care was exercised to collect fluid samples from the infected site and to avoid contamination with sources of normal flora such as skin, vaginal mucosa, or intestinal effluent. The specimens were collected in tubes rendered oxygen-free by gas exchange with CO_2 or in syringes that had been sealed to prevent entrance of air. Samples were divided for aerobic and anaerobic bacteriology and for gas-liquid chromatography (GLC) analysis.

Anaerobic bacteriology was performed with prereduced media in suitable anaerobic conditions, as previously described (3, 4). A Gram-stained slide was prepared of the initial specimen for interpretation by three experienced microbiologists. Culture results, Gram-stain findings, and GLC determinations were recorded for each specimen with-

Reprint requests should be addressed to Dr. Sherwood Gorbach, Infectious Disease Section, Tufts-New England Medical Center, Boston, Mass. 02111.

Received for publication 11 September 1974 and in revised form 8 October 1975.

[1] *Abbreviations used in this paper:* GLC, gas-liquid chromatography; PYG, peptone-yeast glucose; SCFA, short-chain fatty acids; VFA, volatile fatty acid.

out knowledge of results of the other studies. The code was broken at the conclusion of the study.

GLC. SCFA are resolved into two broad classes on the basis of susceptibility to steam distillation. The volatile compounds can be chromatographed directly i.e., acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids. Nonvolatile compounds such as lactic and succinic acids must be derivatized to encourage chromatographic volatility.

Specimens were prepared for GLC by dilution of 1 vol of purulent material with 1 vol of 5% aqueous H_2SO_4. After chilling in an ice bath, a small amount of NaCl and 1 vol of diethylether were added. The complete specimen was agitated, centrifuged at 5°C, and 2.0 μl of the organic phase was injected onto the chromatographic column for volatile fatty acid (VFA) determination. Relatively poor ethereal extraction of acetic (< 10%) and propionic (< 50%) acids are obtained with these procedures. Longer chain acids, including butyric acid, isobutyric acid, and succinic acid, those of primary interest to this study, are extracted well (> 90%).

For methyl esters, an additional acid-diluted sample was prepared. A volume of boron trifluoride methanol equivalent to the pus, and an acid diluent was added. The final product was incubated at 55°C in a water bath for 30 min, and extracted with 1 vol of chloroform. 2 μl was injected into the GLC for methyl ester analysis. If the volume of the specimen was very small, the ether phase was evaporated after VFA analysis at 35°C with a gentle nitrogen stream. The subsequent steps of methylation and injection were the same as described above. Parallel analyses with known acid concentrations revealed uniform lactic and succinic recoveries from identical portions whether methylated directly or shaken with ether, subjected to gentle organic-phase evaporation, and then methylated.

Packard 419 (Packard Instruments Co., Inc., Downers Grove, Ill.) and Shimadzu GC4BMPF dual-column gas chromatographs (Shimadzu, Seisakusho, Ltd., Kyoto, Japan) equipped with flame ionization detectors were employed. Chromatographic deflections were visualized on Honeywell (Honeywell Inc., Minneapolis, Minn.) and Shimadzu 1.0-mV span recorders, and peak areas were interrupted as μV·s on a Shimadzu ITG-2A electronic digital integrator. 2.0-μl samples were injected with a 5-μl Hamilton syringe (Hamilton Company Inc., Whittier, Calif.) directly onto coiled, silylated Pyrex columns, 6 feet long × 2 mm internal diameter. The analytical columns contained 6% Carbowax 20M-terephthalic acid on 80/100 mesh Gas-Chrom Q (Applied Science Labs, Inc., State College, Pa.). Peak identity was verified by chromatography on an alternate phase (10% Carbowax 20M, unconjugated at elevated column temperature, and carrier flow (150°C and 30 ml/min). The specimens were supplemented with a *n*-hexanoate (volatile analysis) and malonyl dimethyl ester (derivatized analysis) as relative retention times standards for both phases. Routine analysis conditions included: injection and detector blocks, 200°C: oven isothermal at 125°C: nitrogen, hydrogen, and air flows, respectively, at 30, 30, and 300 ml/min. Detector sensitivity was customarily set at 2.5×10^{-10} A while 2.5×10^{-11} A was utilized to detect and confirm trace amounts of metabolites.

Integration values compiled at the lower sensitivity were related to standard curves for conversion to micromoles per milliliter. Titrated aqueous solutions of SCFA were diluted in sterile human ascitic fluid, extracted, and chromatographed to obtain the standard values. The chromatogram development times were 9 min for valeric acid and 7 min for succinyl dimethyl ester. Thus, total time for submission of the pus specimen to the conclusion of chromatographic analysis was approximately 30 min.

To ascertain the accuracy of the method, ascitic fluid was supplemented with known organic acid concentrations at levels likely to be encountered in purulent specimens. No acids were detected in the ascitic fluid at 2.5×10^{-10} A before supplementation. Mean recoveries for triplicate injections of all acids had an average relative error of 9% from the true concentrations. The relative SD (precision) encountered was 10.2% based on replicate analyses of isovaleric acid. Acetic and propionic acids could be reliably detected to the 1 μmol/ml level, and all other acids to 0.1 μmol/ml.

SCFA production in vitro. All of the anaerobes recovered in this study were isolated in pure culture, and were grown for 48 h under anaerobic conditions in prereduced peptone-yeast glucose (PYG) broth, according to the method of the Virginia Polytechnic Institute, Blacksburg, Va. (3). SCFA were analyzed by GLC, and the results formed the basis of the taxonomic designation of the particular strain.

In an effort to more closely simulate the actual metabolic events in vivo, we incubated bacteria in human ascites fluid for prolonged periods of time. The chromatographic patterns of bacterial end products under these conditions were compared with those obtained in PYG after 48 h of growth. Five strains of *B. fragilis* and one strain of *Escherichia coli* were employed in these analyses. In each instance the isolate was recovered from a clinical specimen used in the study. For the anaerobes, approximately 0.1 ml of an 18-h PYG broth culture was inoculated into 10 ml of sterile, human ascitic fluid. This was incubated at 37°C in the anaerobic chamber with an atmosphere of 5% CO_2, 10% H_2, and 85% N_2. 0.5-ml aliquots were removed for GLC analysis at intervals of 0, 24, 48, 72, 168, 336, and 672 h. The *E. coli* strain was incubated in atmospheric air and was processed in a similar manner.

RESULTS

Comparison of bacteriological data and GLC determinations revealed that the acids that most reliably indicated the presence of putrefactive anaerobes were isobutyric or butyric acid in the volatile series, and succinic acid in the methylated group. Concentrations of 0.1 μmol/ml or greater of these acids were considered a significant concentration by the techniques of our analysis.

Purulent material with significant concentrations of isobutyric, butyric, or succinic acid. 20 clinical specimens of pus or body fluids had significant quantities of either isobutyric, butyric, or succinic acid (Table I). The sources of the infections were: abdominal abscesses or wounds (nine cases), perirectal abscess ($n = 2$), empyema ($n = 3$), decubitus abscess ($n = 2$), submandibular abscess ($n = 2$), prostatic abscess ($n = 1$), and brain abscess ($n = 1$). Isobutyric acid was detected in 12 samples, butyric acid in 14, and succinic acid in all 20. Small concentrations of acetic and lactic acid were present in many of these specimens, but these acids appeared in the noninfected body fluids as well (see below).

TABLE I

Purulent Material with Isobutyric, Butyric, or Succinic Acids

Case no.	Site of infection	SCFA			Cultures	
		iB	B	S	Aerobes	Anaerobes
			μmol/ml			
1	Intra-abdominal abscess	—	2.6	14.4	*E. coli* *Pr. mirabilis* *Enterococcus*	*B. fragilis, Cl. ramosum* *F. nucleatum, Ps. intermedius* *Pc. prevotii, V. parvula*
2	Intra-abdominal abscess	—	—	1.4	*E. coli* *Pr. mirabilis* *Enterococcus*	*F. nucleatum*
3	Intra-abdominal abscess	0.1	0.7	0.2	*E. coli*	*B. fragilis, F. nucleatum*
4	Intra-abdominal abscess	—	0.1	0.3	*Enterococcus*	*B. fragilis, B. melaninogenicus* *E. lentum, F. symbiosum*
5	Intra-abdominal abscess	—	—	0.1	*E. coli* *Klebsiella* *Enterococcus*	*B. fragilis, Cl. sporogenes,* *Cl. ramosum*, clostridium species
6	Abdominal wound	—	0.1	0.3		*B. fragilis*
7	Perirectal abscess	0.1	0.1	1.8	*E. coli* *Pr. mirabilis*	*B. fragilis, B. melaninogenicus* *E. aerofaciens, F. gonidiaformans* *Ps. anaerobius*
8	Perirectal abscess	—	0.4	24.0	*Pr. mirabilis*	*B. fragilis, Cl. paraputrificum* *Pc. prevotii*
9	Subphrenic abscess	0.1	—	0.1	*E. coli* *Enterobacter* *Klebsiella*	*B. fragilis*, clostridium species
10	Appendiceal abscess	—	—	0.1	*Enterococcus* *P. aeruginosa*	*B. fragilis, F. symbiosum*
11	Pelvic abscess	0.2	4.0	5.1		*B. fragilis, Pc. prevotii* *Ps. micros*
12	Prostatic abscess	0.7	8.2	31.0	*Pr. mirabilis* *Pr. rettgeri*	*B. fragilis, Pc. prevotii* *Ps. micros*
13	Decubitis abscess	6.5	—	30.0	*Pr. mirabilis*	*B. fragilis, Pc. micros* *Pc. asaccharolyticus*
14	Decubitis abscess	6.1	—	27.0	*Klebsiella* *Pr. mirabilis*	*B. fragilis, Ps. micros*
15	Submandibular abscess	0.5	0.4	2.5		*B. fragilis, B. melaninogenicus* *E. lentum, Ps. intermedius*
16	Submandibular abscess	0.5	2.1	2.8	*N. catarrhalis*	*F. nucleatum*
17	Empyema	1.1	0.9	13.6	*E. coli*	*B. fragilis, E. lentum*
18	Empyema	1.1	4.3	26.0	*N. catarrhalis*	*B. melaninogenicus*
19	Empyema	0.2	0.8	15.2	*E. coli* *Enterococcus*	*Ps. intermedius, V. parvula*
20	Brain abscess	—	0.1	2.2	*S. viridans*	bacteroides species

E, Escherichia; B, Bacteroides; F, Fusobacterium; P, Pseudomonas, Cl, Clostridium; E, Eubacterium; Pr, Proteus; Ps, Peptostreptococcus; Pc, Peptococcus; N, Neisseria; V, Veillonella; S, Streptococcus.

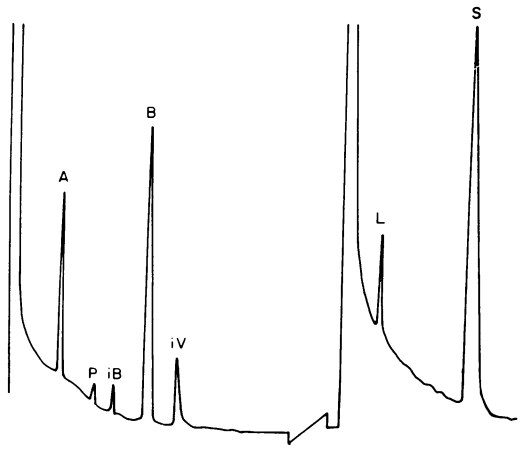

FIGURE 1 Chromatographic pattern of exudate from a pelvic abscess. VFA (pattern on left) were acetic (A), propionic (P), isobutyric (iB), butyric (B), and isovaleric (iV); methyl esters (pattern on right) were lactic (L) and succinic (S) acids. Culture yielded *B. fragilis, Ps. intermedius,* and *Peptococcus prevotii.*

Examination of Gram-stained slides of these 20 purulent specimens revealed Gram-negative bacilli in 14, as determined by three observers in double-blind readings. However, the morphologic features in only five slides were sufficiently distinctive to suggest anaerobes.

Cultures generally yielded a complex miscellany of aerobic and anaerobic forms. *B. fragilis* was present in 15 specimens, usually associated with other anaerobic bacteria. In only one specimen, a suppurative abdominal wound (case 6) was *B. fragilis* recovered in pure culture. *Bacteroides melaninogenicus* was isolated in one case of putrid empyema, associated with a neisseria species. An unclassified bacteroides species was recovered from a case of brain abscess. Fusobacteria were found in 7 cases; in 5 instances a bacteroides was coexisting, but in 2 patients (cases 2 and 15), *Fusobacterium nucleatum* was the only anaerobic isolate. Other anaerobes commonly found in combination with either bacteroides or fusobacteria were Gram-positive cocci (peptostreptococci or peptococci) in nine cases, clostridia in four, and eubacteria in four.

There was one false-positive specimen that contained isobutyric, butyric, and succinic acids (case 19). This was a sample of foul-smelling pleural fluid from a case of empyema associated with a subphrenic abscess. Culture of the empyema fluid yielded *E. coli, enterococcus, Peptostreptococcus intermedius,* and *Veillonella parvula,* but no bacteroides or fusobacteria. The Gram stain of the pus, however, showed thin pleomorphic Gram-negative bacilli. Furthermore, culture of the adjacent subphrenic abscess yielded *B. fragilis* in addition to the organisms recovered in the pleural fluid specimen. In this instance, there may have been a failure of culture techniques with the empyema specimen since other parameters suggested the presence of Gram-negative anaerobic bacilli.

Fig. 1 shows the chromatographic pattern of pus obtained from a pelvic abscess (case 11). This specimen yielded acetic, propionic, isobutyric, butyric, and isovaleric acids in the volatile fraction, and lactic and succinic acids in the methylated series. Our cultures revealed *B. fragilis,* peptococcus, and peptostreptococcus from the same specimen.

Another example is purulent discharge from a perirectal abscess (case 8) which showed large concentrations of butyric and succinic acids by GLC analysis (Fig. 2). The culture of this specimen produced *B. fragilis,* anaerobic cocci, a clostridia species, and coliforms.

Purulent material without significant concentrations of isobutyric, butyric, or succinic acids. There were 18 specimens of infected material that yielded microorganisms on culture but failed to demonstrate significant concentrations of isobutyric, butyric, or succinic acid (Table II). All samples were grossly purulent, and were obtained by direct aspiration from empyema (six cases), intra-abdominal abscess ($n = 4$), perirectal abscess ($n = 2$), liver abscess ($n = 2$), septic joint ($n = 2$), breast abscess, ($n = 1$), and stump abscess ($n = 1$). A variety of enteric Gram-negative rods, pseudomonas, staphylococci, and streptococci were recovered. In addition, there was one fungal infection with *Coccidioides immitis,* and one case of an amebic liver abscess. Anaerobic bacteria were recovered in six specimens including four with peptostreptococci. An-

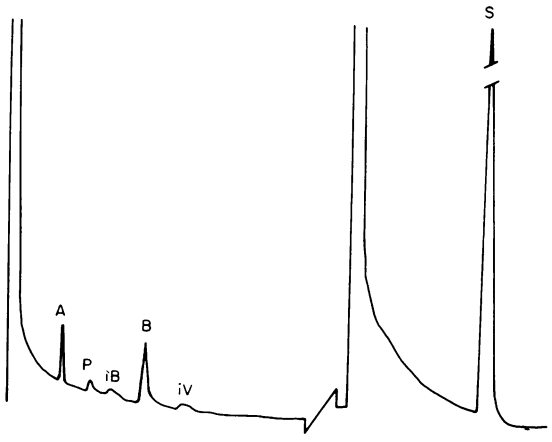

FIGURE 2 Chromatographic pattern of purulent drainage from a perirectal abscess showing a butyric acid (B) peak, and in the methylated series, succinic acid (S), Other acid peaks are acetic (A), propionic (P), isobutyric (iB), and isovaleric (iV). Culture of this specimen yielded *B. fragilis, Clostridium paraputrificum, Peptococcus prevotii,* and *Proteus mirabilis.*

TABLE II

Purulent Material without Detectable Isobutyric, Butyric, or Succinic Acids

Clinical conditions		No. of cases
Empyema		6
Intra-abdominal abscess		4
Perirectal abscess		2
Liver abscess		2
Septic joint		2
Breast abscess		1
Stump abscess		1
Total		18
Types of microorganisms		
Aerobes		
E. coli	(4)*	
S. aureus	(4)	
Proteus	(3)	
Pseudomonas	(3)	
S. faecalis	(2)	
Enterobacter	(1)	
Coccidioides	(1)	
Entamoeba histolytica	(1)	
Anaerobes		
Peptostreptococcus	(4)	
Cl. perfringens	(1)	
B. pneumosintes	(1)	

* Number of specimens.

other patient had clinical gas gangrene of the thoracic wall associated with empyema. The specimen submitted for GLC analysis had no detectable SCFA, although typical Gram-positive rods were noted on Gram stain and *Clostridium perfringens* was grown in heavy culture. Another specimen of empyema fluid grew *B. pneumosintes* and *Enterobacter aerogenes;* GLC determination revealed trace concentrations of isobutyric and lactic acids (< 0.1 μmol/ml), but no other SCFA. It should be noted, parenthetically, that this unusual bacteroides species does not ferment carbohydrates and fails to produce SCFA, in vitro.

Sterile body fluids. There were 60 specimens of sterile body fluids including pleural effusion (30 cases), ascites ($n = 9$), bile ($n = 7$), joint aspirate ($n = 7$), urine ($n = 6$), and pericardial fluid ($n = 1$). Significant succinic, isobutyric, or butyric acids were not detected in any of these samples. Some samples showed trace deflections in the isobutyrate-butyrate region on routine analysis. However, this tentative identification was not verified by cochromatography on unconjugated Carbowax 20M. In addition, trace concentrations of acetic, propionic, and lactic acids were found in some samples.

Specimens cultured in outside laboratories that failed to grow anaerobes. During the period of our study, six specimens of pus that were cultured in other laboratories not possessing optimal anaerobic capabilities were submitted to us for GLC analysis. These were samples collected from cases that clinically suggested anaerobes i.e., three cases of abdominal abscess, one pelvic abscess, one rectal abscess, and one gluteal abscess. The outside laboratory was unable to isolate anaerobes from these specimens, although coliforms and enterococci were present. A considerable period of time elapsed (1–3 days) before the specimens reached our laboratory, and we were also unable to recover anaerobes. All six specimens yielded significant concentrations of succinic acid, and three also showed isobutyric or butyric acids. It appears that these metabolic markers may have persisted in clinical specimens longer than the anaerobes themselves. Thus, these specimens technically represented false-positives, although it could reasonably be argued that the delay in culture precluded the isolation of fastidious anaerobic bacteria from such specimens.

Effect of growth media on SCFA production. The presence of C4 volatile acids, particularly in cases 6, 14, and 17, led us to examine in vitro organic acid production by *B. fragilis.* This organism was the only likely source of the isobutyrate and butyrate in these cases. However, C4 acids are seldom recovered with the conventional chromatographic methods employed for laboratory identification of *B. fragilis.* Using human ascitic fluid for culture medium and extended periods of observation, all five strains of *B. fragilis* produced isobutyrate and butyrate (Table III). Both compounds were detected in limited quantities, but isobutyrate production was clearly favored. In a similar study with *E. coli,* abundant succinic acid was produced in vitro. The patient with an abdominal wound abscess involving only this strain of *E. coli* paradoxically had no detectable succinic acid in the purulent exudate. These results indicate that there is not necessarily a direct correlation between in vitro and in vivo acidic end products of bacterial metabolism.

DISCUSSION

Anaerobic bacteria are now considered significant pathogens in human infections, particularly those related to contamination by the normal flora of the gastrointestinal tract, oropharynx, female genital tract, and skin (2). Reports of increased isolation of anaerobes probably reflect greater awareness and improved laboratory techniques, rather than a shift in infectious agents. At the present time it is reckoned that anaerobes are associated with 80–90% of intra-abdominal sepsis, 70–80% of female pelvic infections, 90% of pulmonary

TABLE III

Concentrations of Isobutyric and Butyric Acids in Ascitic Fluid over 672 h of Anaerobic Incubation

Acids	Hours						
	0	24	48	72	168	336	672
iB	0	<0.1	0.1±0.10*	0.1±0.10	0.4±0.18	0.5±0.09	0.6±0.13
B	0	0	0	0	0.1±0.04	0.1±0.02	0.1±0.02

* Mean concentration from five strains of *B. fragilis* expressed as micromoles per milliliter ± SD.

infections caused by aspiration, and 90% of brain abscesses, to mention the major forms of anaerobic infections.

Rapid bacteriologic diagnosis would be desirable since *B. fragilis,* the pre-eminent pathogen, is relatively resistant to many antibiotics used in clinical practice. Unfortunately, clinical laboratories are unable to produce results in an expeditious manner for several reasons: (*a*) anaerobes are slow growing, particularly after oxygen exposure during transport and processing of the clinical specimen; (*b*) these organisms are often present in a complex culture, on the average involving five different bacterial species in each specimen of purulent material; (*c*) isolating and identifying each microorganism is time-consuming since the taxonomy of anaerobes is more complex than that of facultative species, and several procedures, including GLC analysis may be required for identification; and (*d*) culture plates of these organisms are incubated in anaerobic jars that must be opened even to look at the plates. As a rule, the jars are exposed to room air only after 48–72 h of growth, rendering quick bacteriologic determinations rather impractical.

Shortcuts to the diagnosis of anaerobic infection are useful in certain settings, but do not have widespread application. A Gram-stained slide of the clinical specimen should always be examined. Distinctive anaerobes such as clostridia and fusobacteria can be recognized. Anaerobic streptococci, on the other hand, are indistinguishable from facultative strains; coliforms may be pleomorphic in purulent exudate making it difficult to differentiate these organisms from *B. fragilis.* Thus, three experienced bacteriologists in our laboratory could only identify 5 of 20 positive specimens as having Gram-negative anaerobes when read in a blind experiment.

The distinctive odor of anaerobes is apparent to all laboratory workers, although many clinicians fail to appreciate this smell. Although it is true that all infections exhibiting this odor of excrement are infected with anaerobes, we have found that even experienced anaerobists with a highly developed olfactory sense can only detect the stench in approximately one-half of clinical cases. Furthermore, at the bedside, our noses have been unable to differentiate infections caused by anaerobic cocci and clostridia, strains sensitive to penicillin, from those caused by resistant *B. fragilis.*

Our studies suggest that direct gas chromatography of clinical specimens offers a rapid and reliable method of identifying putrefactive anaerobes in clinical specimens. All purulent samples containing *B. fragilis* had significant quantities of succinic acid, and many also had isobutyric and butyric acids, as measured by these techniques. It should be noted that these acids were not specific for *B. fragilis* since there were specimens with fusobacteria and *B. melaninogenicus* that showed these products. However, this pattern was not seen in sterile specimens or in infections caused by aerobic organisms or anaerobic organisms such as Gram-positive cocci and Gram-positive rods. Although GLC is not absolutely diagnostic of *B. fragilis,* in that other Gram-negative anaerobes may be responsible, there is enough evidence, in our view, from this rapid diagnostic technique to initiate specific antibiotic treatment for this virulent pathogen. Therapy should be modified by results of culture which will eventually become available.

In the 20 yr since GLC has been developed, many workers have used this sensitive technique for microbial taxonomy and the diagnosis of infectious diseases (5). The classification of microorganisms have been based either on analysis of metabolites in spent culture media (6–10) or on the structural components of the bacterial cell itself (11–15). Variations of these basic themes have been used to classify clostridia, neisseria, coliforms, bacillus, mycobacteria, and pseudomonas.

Several investigators have attempted to identify specific infections in laboratory animals and in patients by GLC analysis of blood, stool, or urine. Mitruka and co-workers (16–20) have analyzed serum samples with GLC to diagnose experimental viral and bacterial infections. A recent report by Brooks et al. (21) has suggested that GLC might be useful in the differential diagnosis of arthritis.

The use of GLC for detecting bacterial infections represents a departure from classic bacteriology in that we are not as concerned in the first instance with growth of the microorganisms in artificial culture media as we are in detecting metabolic fingerprints of its presence in pathologic tissue. Our demonstration of SCFA is only one approach that may be useful with anaerobes. Microorganisms have other metabolic pathways that are distinct from their host, and these may prove susceptible to analysis with the gas-liquid chromatograph for rapid diagnostic purposes.

REFERENCES

1. Gorbach, S. L., H. Thadepalli, and J. Norsen. 1974. Anaerobic microorganisms in intraabdominal infections. *In* Anaerobic Bacteria; Role in Disease. A. Balows, R. M. DeHaan, V. R. Dowell, Jr., and L. B. Guze, editors. Charles C. Thomas, Publisher, Springfield, Ill. 399–407.
2. Gorbach, S. L., and J. G. Bartlett. 1974. Anaerobic infections. *N. Engl. J. Med.* **290**: 1177–1184, 1237–1245, 1289–1294.
3. Holdeman, L. V., and W. E. C. Moore. 1972. Anaerobe Laboratory Manual. Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg, Va. 132 pp.
4. Thadepalli, H., S. L. Gorbach, P. W. Broido, J. Norsen, and L. Nyhus. 1973. Abdominal trauma, anaerobes, and antibiotics. *Surg. Gynecol. Obst.* **137**: 270–276.
5. Cherry, W. B., and C. W. Moss. 1969. The role of gas chromatography in the clinical microbiology laboratory. *J. Infect. Dis.* **119**: 658–662.
6. O'Brien, R. T. 1967. Differentiation of bacteria by gas chromatographic analysis of products of glucose catabolism. *Food Technol.* **21**: 1130–1132.
7. Mitruka, B. M., and M. Alexander. 1968. Rapid and sensitive detection of bacteria by gas chromatography. *Appl. Microbiol.* **16**: 636–640.
8. Carlsson, J. 1973. Simplified gas chromatographic procedure for identification of bacterial metabolic products. *Appl. Microbiol.* **25**: 287–289.
9. Brooks, J. B., C. C. Alley, J. W. Weaver, V. E. Green, and A. M. Harkness. 1973. Practical methods for derivatizing and analyzing bacterial metabolites with a modified automatic injector and gas chromatograph. *Anal. Chem.* **45**: 2083–2087.
10. Moss, C. W., and S. B. Samuels. 1974. Short-chain acids of *Pseudomonas* species encountered in clinical specimens. *Appl. Microbiol.* **27**: 570–574.
11. Moss, C. W., and V. J. Lewis. 1967. Characterization of clostridia by gas chromatography. I. Differentiation of species by cellular fatty acids. *Appl. Microbiol.* **15**: 390–397.
12. Thoen, C. O., A. G. Karlson, and R. D. Ellefson. 1971. Fatty acids of *Mycobacterium kansasii*. *Appl. Microbiol.* **21**: 628–632.
13. Amstein, C. F., and P. A. Hartman. 1973. Differentiation of some enterococci by gas chromatography. *J. Bacteriol.* **113**: 38–41.
14. Wade, T. J., and R. J. Mandle. 1974. New gas chromatographic characterization procedure: preliminary studies on some *Pseudomonas* species. *Appl. Microbiol.* **27**: 303–311.
15. MacGee, J. 1968. Characterization of mammalian tissues and microorganisms by gas-liquid chromatography. *J. Gas Chromatogr.* **6**: 48–52.
16. Mitruka, B. M., M. Alexander, and L. E. Carmichael. 1968. Gas chromatography for detection of viral infections. *Science (Wash. D. C.)*. **160**: 309–311.
17. Mitruka, B. M., L. E. Carmichael, and M. Alexander. 1969. Gas chromatographic detection of *in vitro* and *in vivo* activities of certain canine viruses. *J. Infect. Dis.* **119**: 625–634.
18. Mitruka, B. M., A. M. Jonas, and M. Alexander. 1970. Rapid detection of bacteremia in mice by gas chromatography. *Infect. Immun.* **2**: 474–478.
19. Mitruka, B. M., A. M. Jonas, M. Alexander, and R. S. Kundargi. 1973. Rapid differentiation of certain bacteria in mixed populations by gas-liquid chromatography. *Yale J. Biol. Med.* **46**: 104–112.
20. Mitruka, B. M., R. S. Kundargi, and A. M. Jonas. 1972. Gas chromatography for rapid differentiation of bacterial infections in man. *Med. Res. Eng.* **11**: 7–11.
21. Brooks, J. B., D. S. Kellog, C. C. Alley, H. B. Short, H. H. Handsfield, and B. Huff. 1974. Gas chromatography as a potential means of diagnosing arthritis. I. Differentiation between Staphylococcal, Streptococcal, Gonococcal, and Traumatic arthritis. *J. Infect. Dis.* **129**: 660–668.