Electron Capture Gas-Liquid Chromatographic Study of Metabolites Produced by Some Arthritic Transudate-Associated Organisms In Vitro and In Vivo in Rabbit Models

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Computerized, frequency-pulsed, modulated electron capture gas-liquid chromatography was used to study the acid metabolites produced in vitro in fetal calf serum and in vivo in an animal chamber model. Several strains of *Diplostreptococcus agalactiae*, *Propionibacterium acnes*, *Staphylococcus aureus*, and *Streptococcus* serogroups A, B, and G were studied. All of these organisms have been reported to be associated with arthritic transudates in humans. Metabolites were detected by this method from derivatized extracts of both spent fetal calf serum and chamber fluids. Since there was little host response to the organisms cultured in the chambers, it is highly probable that the products detected represent metabolites produced in an in vivo type of environment. The metabolic patterns were reproducible and exhibited many similarities in vitro and in vivo. Production of the acids detected was reproducible, and these acids were useful identification markers. The data support published reports (J. B. Brooks, C. C. Alley, and J. A. Liddle, *Anal. Chem.* **46**:1930-1934, 1974; J. B. Brooks, G. Choudhary, R. B. Craven, D. Edman, C. C. Alley, and J. A. Liddle, *J. Clin. Microbiol.* **5**:625-628, 1977; J. B. Brooks, R. B. Craven, A. R. Melton, and C. C. Alley, in H. H. Johnson and W. B. Newson, ed., *Second International Symposium on Rapid Methods and Automation in Microbiology*, 1976; J. B. Brooks, R. B. Craven, D. Schlossberg, C. C. Alley, and F. M. Pitts, *J. Clin. Microbiol.* **8**:203-208, 1978; J. B. Brooks, D. S. Kellogg, C. C. Alley, H. B. Short, and H. H. Handsfield, *J. Infect. Dis.* **129**:660-668, 1974) that bacterial metabolites might be detectable in diseased body fluids. The growth characteristics of the organisms in the animal model and fetal calf serum are discussed, and a moderately priced computer for performing data manipulations is evaluated.

Several recent studies (9, 10, 11, 12, 14) have indicated that frequency-pulsed, modulated electron capture gas-liquid chromatographic (FMPEC-GLC) analysis of derivatized extracts of body fluids and spent culture media produces data which are potentially useful for the rapid identification of some diseases and disease-causing organisms. Samples from patients with meningitis, pleural effusions, and arthritis were studied. Components detected in the studies of spent media consisted primarily of metabolic products and cellular constituents of the organism. With appropriate substrate and growth conditions, most organisms thus far reported (9, 10, 13, 18) were distinguished at the species and strain levels. The source of the compounds detected by FMPEC-GLC in body fluids is more difficult to determine than it was with the in vitro studies, and discussion about them is speculative. The

compounds detected in the in vivo studies probably are bacterial metabolic products, products of the host response to the organism, or a combination of both. The absolute value of many of these FMPEC-GLC profiles for use in disease identification is hard to fully establish because of the difficulty encountered in obtaining suitable samples from untreated patients for whom a confirmed diagnosis was made, and because of possible unknown variables such as the effect of acuteness and duration of disease. A comparison of products detected in an in vitro system using serum as a substrate and in an in vivo animal model system might help answer the following questions: (i) are bacterial metabolites detectable by FMPEC-GLC in small amounts (2 ml) of body fluid samples? (ii) is their production reproducible? (iii) how much do these metabolites contribute as FMPEC-GLC identification markers? and (iv) how similar would the in vivo and in vitro products be?

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Animal models have been established which involve the implantation of porous chambers beneath the skin of rabbits (3). After installation, the animals are treated with antibiotics, and when sufficient time has elapsed the porous chambers become aseptic, become lined with connective tissues, and are filled with a clear transudate. Organisms such as spirochetes which require an in vivo system will grow in these chambers (2, 3). This growth indicates that there exists within the chamber an in vivo type of environment. Although the chamber models offer an in vivo environment and have been demonstrated to be useful for the in vivo study of certain organisms (2), the host response to organisms isolated in the chamber is not the same as that occurring in an infectious process. This lack of host response can be seen in the ability of the organism to multiply to large numbers in the chamber without causing a typical inflammatory body response. Since the chambers are filled with a transudate which is in a dynamic state with other body fluids, one might

use this approach for FPMEC-GLC studies. We, therefore, decided to use the rabbit chamber system to grow certain organisms known to be present in transudates from humans with arthritis (4, 5, 10, 20), (i) to learn more about the growth behavior of the organisms in the chamber and (ii) to use the chamber exudates in an attempt to answer some of the questions arising from FPMEC-GLC metabolic studies of disease body fluids.

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MATERIALS AND METHODS

Organisms. The bacterial cultures obtained for use in the study were primarily from joint fluids or tissues. The Lancefield grouped streptococci (Table 1) were provided by Richard R. Facklam of the Center for Disease Control. Five bacterial isolates of *Diplostreptococcus agalactiae* were obtained from Nana Svartz

TABLE 1. *Bacterial isolates used*

Study no.	Name	Source ^a	Source no.
3A	<i>Streptococcus pyogenes</i>	Facklam	74-1506
4A	<i>S. pyogenes</i>	Brooks	B-25
5A	<i>S. pyogenes</i>	Facklam	73-0107
6A	<i>S. pyogenes</i>	Facklam	73-0501
7A	<i>S. pyogenes</i>	Facklam	73-0891
8A	<i>Streptococcus agalactiae</i>	Facklam	73-1979
9A	<i>Streptococcus</i> group G	Facklam	73-3867
10A	<i>Streptococcus</i> group G	Facklam	73-4073
12A	<i>S. pyogenes</i>	Facklam	72-0034
15A	<i>Streptococcus</i> group G	Facklam	72-0550
16A	<i>Streptococcus</i> group G	Facklam	72-0617
17A	<i>Streptococcus</i> group G	Facklam	72-0621
18A	<i>S. agalactiae</i>	Facklam	72-1538
19A	<i>S. pyogenes</i>	Facklam	72-1887
20A	<i>Streptococcus</i> group G	Facklam	71-1064
21A	<i>S. pyogenes</i>	Facklam	71-1675
40B	<i>Diplostreptococcus agalactiae</i>	Svartz	Strain 30
41B	<i>D. agalactiae</i>	Svartz	Strain BO
42B	<i>D. agalactiae</i>	Svartz	Strain Ram
43B	<i>D. agalactiae</i>	Svartz	Strain E7
45B	<i>S. agalactiae</i>	Facklam	1972 (type)
46B	<i>S. agalactiae</i>	Facklam	617 (type)
47B	<i>D. agalactiae</i>	Svartz	DSA-EK
56C	<i>Propionibacterium acnes</i>	Bartholomew	Bailey
57C	<i>P. acnes</i>	Bartholomew	La Hood
58C	<i>P. acnes</i>	Bartholomew	Filstrup
59C	<i>P. acnes</i>	Bartholomew	Kadwell
60C	<i>P. acnes</i>	Lombard	CDC-554 (type)
61C	<i>P. acnes</i>	Lombard	CDC-605 (type)
1D	<i>Staphylococcus aureus</i> PT ^b 52	Brooks	SV-120
2D	<i>S. aureus</i> PT 187	Brooks	SV-112
4D	<i>S. aureus</i> PT 3A	Brooks	SV 152
6D	<i>S. aureus</i> PT 52	Brooks	SV 218

^a See the text.

^b PT, Phage Type.

(20), King Gustaf V Research Institute, Stockholm, Sweden. Four strains of *Propionibacterium acnes* were obtained from L. E. Bartholomew (4, 5), Albany Medical College, Albany, N.Y. Reference strains of *P. acnes* were obtained from George Lombard, Center for Disease Control. Four isolates of *Staphylococcus aureus* were obtained from our own collection.

All cultures were verified by conventional biochemical (15, 16), serological (19), and phage (6) methods used by the Center for Disease Control. The cultures were preserved by lyophilization and were maintained on paraffin-sealed nutrient agar slants at 5°C or in cooked meat media at room temperature in a Brewer jar.

Media. Working cultures were maintained on blood agar base with 5% sheep, rabbit, or horse blood added. One lot of fetal calf serum (FCS) served as the primary medium for the in vitro studies.

Culture procedures and growth conditions. Organisms were cultured for 24 h in Todd-Hewitt broth and centrifuged; the sediment was resuspended in 1 ml of medium, and 1 drop from a Pasteur pipette was used as the inoculum for 10 ml of FCS. The flasks were placed in a candle extinction chamber as described (17) and incubated at 35 to 37°C for 48 h on a rotary shaker (New Brunswick Scientific) operated at about 120 rpm. An uninoculated medium control was included with each set of cultures to serve as a sterility check and as a medium control for FPMEC-GLC analysis. All studies were done in duplicate.

Animal model procedures. Golf ball implants (two per rabbit) were installed as described (2, 3). Antibiotics were administered during the period of chamber clearing. The shaved skin over the chamber was cleaned with surgical disinfectant, ethanol, and iodine before injection or sampling. Beginning with week 7 after implantation, samples were taken from the chamber every other week to check for clearing of cellular material and for possible infection. Serum samples were drawn each time the chambers were sampled.

Twenty-four-hour cultures in Todd-Hewitt broth were centrifuged, and the cells were resuspended in sterile phosphate-buffered saline to the density of a McFarland no. 3 standard; 0.5 ml of this suspension was injected with a 1-ml syringe and a 26-gauge needle through the open spaces in the right implanted golf ball. The scheme for inoculation is shown in Table 2. After inoculation, samples for FPMEC-GLC analysis were taken at 24, 48, 72, 96, and 120 h from both the inoculated (right) and the uninoculated (left) chambers. These samples were checked for turbidity due to growth by visual comparison and were cultured both aerobically and anaerobically. The bacterial isolates from the chambers were concluded to be the same as those inoculated, on the basis that they conformed to the criteria used in the initial verification.

Extraction and derivatization procedures. A 2-ml volume of sample, either spent FCS or rabbit chamber fluid, was acidified to a pH of about 2 (pH paper) by adding 0.1 ml of 50% (vol/vol) sulfuric acid just before extraction. This acidified sample was extracted by vigorous shaking with 20 ml of nanograde quality chloroform (Mallinckrodt). Trichloroethyl

TABLE 2. Rabbit chamber numbering and inoculation scheme

Rabbit no.	Chamber no.	Side installed	Organism inoculation
265	265R 265L	Right Left	<i>S. aureus</i> Uninoculated
266	266R 266L	Right Left	Group A <i>Streptococcus</i> Uninoculated
267	267R 267L	Right Left	Group G <i>Streptococcus</i> Uninoculated
268	268R 268L	Right Left	<i>P. acnes</i> Uninoculated
269	269R 269L	Right Left	Uninoculated Uninoculated
270	270R 270L	Right Left	Group B <i>Streptococcus</i> Uninoculated
271	271R 271L	Right Left	Killed streptococcal sus- pension Uninoculated
272	272R 272L	Right Left	<i>D. agalactiae</i> Uninoculated

(TCE) esters were prepared from the pH 2 extracts as described (1).

GLC analysis. For all analyses, 1.4-μl amounts of the TCE derivatives were injected into a Perkin-Elmer model 3920 gas-liquid chromatograph equipped with dual ⁶³Ni (10 mCi) electron capture detectors operated in the frequency pulse modulated mode (6, 8, 11). The instrument was operated with two coiled glass columns (0.3-cm ID by 7.3-m length). The columns were packed with Chromosorb W 80/100 mesh (AW-DMCS H.P.) coated with 3% OV-101 (Applied Science). The column oven was equipped with a liquid nitrogen subambient cool-down valve to permit rapid cooling of the column bath (2.5 min). For analysis of TCE derivatives, the instrument was programmed for a linear increase of 4°C/min from 110°C to 245°C and held for 48 min. Two Perkin-Elmer model 56 10-inch (ca. 25.4-cm) recorders were used, with an input signal of 1 mV and chart speed of 1 cm/min.

Samples were also analyzed on a Perkin-Elmer model 900 gas-liquid chromatograph fitted with a single ⁶³Ni electron capture detector operated as described above. One column was packed with a nonpolar liquid phase (OV-1) and the other column with a polar liquid phase (3% Dexsil 410). Both were coated onto Chromosorb W 80/100 mesh (AW-DMCS H.P.). Retention times from both the polar and nonpolar columns were used to establish tentative identification. Benzoic acid was identified by FPMEC-GLC and mass spectrometry. Analytical conditions are described (10). The instrument was programmed for a linear increase of 4°C/min from 120°C to 265°C, and the latter temperature was held for 32 min during analysis of the TCE derivatives on the Dexsil column.

On both instruments the temperature was 275°C for the detectors, 250°C for the manifolds, and 225°C

for the injectors. Argon-methane (95:5) was used as the carrier gas at a flow rate of 50 ml/min through each column. The detector scavenge gas was regulated so that a total of 68 ml of gas per min passed through the detector.

Computerization. The PEP-2 (Perkin-Elmer) processor was used with the Modular Software System 16. The hardware consists of a teletype data terminal with paper tape punch and reader, a processor with 16 K bit memory, and one GC interface assembly for each detector. The software allows the processor to monitor simultaneously the output signals from several gas chromatographs and prepares analytically meaningful reports which include retention times in real elapsed time, peak area (with or without skimming) in absolute units, relative retention times, normalized peak areas, and peak naming. The stored method shown in Fig. 1 indicates the parameters used by the computer. It also shows the types of acids tested for by the computer during the analysis.

RESULTS

The results of characterization of the bacteria by microscopic, biochemical, serological, and phage typing criteria were generally consistent with those reported by the various sources.

All organisms produced turbid growth in the FCS, which was chosen as a substrate because of its possible similarity to chamber fluids. A single lot of serum was used throughout the study to maintain a constant FPMEC-GLC

background profile. Maximum quantities of products were detected after 48 h of incubation; therefore, this incubation period was used throughout the study. All strains of each species tested had similar FPMEC-GLC profiles.

The courses of in vivo infection were similar with all bacterial groups except *D. agalactiae*. *Staphylococcus* and *Streptococcus* grew in the inoculated chamber within 24 h. The turbidity of the chamber fluids continued to increase until about day 3. By day 5, the infection had begun to clear. Antibiotics were administered on days 5, 7, and 16 postinfection to protect the animal. *P. acnes* followed a similar course with a slightly longer incubation period (3 days) and a longer duration (2 weeks) before the infection began to clear. The uninoculated chamber (left) on each animal exhibited an increase in erythrocyte content during the first week of daily sampling, but remained sterile. The *D. agalactiae*, however, did not appear to infect the inoculated chamber. By day 2 the only obvious cellular material was erythrocytes. Because there was no evidence of infection, the animal was not given antibiotic therapy until later. By day 23 the fluid in the inoculated chamber had become cloudy, bloody, and viscous, but was still negative when cultured on blood agar plates aerobically in Todd-Hewitt broth and anaerobically in thioglycolate broth. Another sample, taken on day 30, was turbid with growth. It was cultured in chopped meat medium anaerobically and then subcultured to thioglycolate and to blood agar plates, which were cultured both aerobically and anaerobically. An organism was isolated and was confirmed by established criteria to be *D. agalactiae*. The tests were repeated on the next 4 days, but only under strictly anaerobic conditions was this bacterium isolated; however, after only one subculture it regained its ability to grow aerobically in all five tests.

The PEP-2 computer was a valuable aid for obtaining retention time and relative retention time (in this case relative to residual TCE), naming peaks when the retention time matched the stored method, and obtaining peak areas. The computer also served as a quality control factor, since known standards analyzed at specified intervals were improperly labeled when the gas chromatograph malfunctioned. The computer resolved and obtained areas of fused peaks such as those shown in the first part of the chromatogram in Fig. 2 (F, G, H). This ability of the computer to resolve and determine areas of fused peaks due to detector overloading avoided the necessity of repeating the FPMEC-GLC analysis.

As shown in Fig. 2, the control chambers

STD CONC 0.000:			
TIMES	80,000,	3.90,	13.00, 26.00, 45.00,
THRESHOLDS	32,	4,	20.00,
UNK/AIR	1.000,	0.01,	
TOL	0.080,	0.025,	0.5,
REF PK	0.000,	0.00	0.00,
STD NAME:			
RRT	RF	C	NAME
0.001,	0.000,	0.0000,	TCE:
0.183,	1.000,	0.0000,	ACETIC:
0.381,	1.000,	0.0000,	PROPIONIC:
0.483,	1.000,	0.0000,	ISOBUTYRIC:
0.594,	1.000,	0.0000,	BUTYRIC:
0.740,	1.000,	0.0000,	ISOVALERIC:
0.871,	1.000,	0.0000,	VALERIC:
1.056,	1.000,	0.0000,	ISOCAPROIC:
1.170,	1.000,	0.0000,	CAPROIC:
1.478,	1.000,	0.0000,	HEPTANOIC:
1.726,	1.000,	0.0000,	BENZOIC:
1.780,	1.000,	0.0000,	OCTANOIC:
1.805,	1.000,	0.0000,	PHENYLACETIC:
2.077,	1.000,	0.0000,	NONANOIC:
2.361,	1.000,	0.0000,	DECANOIC:
2.893,	1.000,	0.0000,	LAURIC:

FIG. 1. Computer printout of results with the stored method used for TCE derivative analysis of GLC profiles by the GC Data System. RRT, Relative retention time; RF, response factors; C, concentration; STD, standard; CONC, concentration; UNK, unknown; TOL, tolerance; REF PK, reference peak.

FIG. 2. Gas chromatograms of TCE-derivatized chloroform extracts (pH 2). Chromatogram F was obtained with fluid from rabbit chamber 268R 96 h after inoculation with *P. acnes*; chromatogram G shows fluid from chamber 271R 72 h after injection with killed suspension of streptococci; and chromatogram H shows fluid from control rabbit chamber 271R before inoculation with an organism.

(chromatogram G and H) with or without a killed inoculum were alike and were different from the infected chamber (chromatogram F).

Table 3 shows the computer-generated areas for acids detected by FPMEC-GLC in the spent FCS. *D. agalactiae* and group A and group G *Streptococcus* (not shown) produced only acetic acid. Group B *Streptococcus* produced acetic acid and benzoic acids. *S. aureus* produced acetic, isobutyric, and isovaleric acids. *P. acnes* produced acetic, propionic, and isovaleric acids. Table 4 shows the computer-generated areas for the acids detected from FPMEC-GLC analysis of chamber fluids infected with the indicated organisms. Chamber fluids containing *D. agalactiae* had acetic acid and high concentrations of lauric acid. The tests were repeated with similar results on four different samples taken at different intervals. Lauric acid may be present as a result of host response to this organism. We have detected lauric acid in human arthritis

TABLE 3. Computer-generated area values for chromatograms A, B, C, D, and E obtained by FPMEC-GLC analysis^a

Acid	Chromatogram peak area ^b				
	A	B	C	D	E
Acetic	4.60	26.17	—	3.36	12.16
Propionic	—	—	—	—	95.81
Isobutyric	—	—	—	5.21	—
Butyric	—	—	—	—	—
Isovaleric	—	—	—	14.93	27.31
Valeric	—	—	—	—	—
Isocaproic	—	—	—	—	—
Caproic	—	—	—	—	—
Heptanoic	—	—	—	—	—
Benzoic	—	10.83	—	—	—
Phenylacetic	—	—	—	—	—
Nonanoic	—	—	—	—	—
Decanoic	—	—	—	—	—
Lauric	—	—	—	—	—

^a Analysis of derivatized extracts of organisms cultured in FCS (adjusted for background).

^b Area is measured by the computer in arbitrary units. The computer has a linear dynamic range of 2×10^6 . A, *D. agalactiae* 41B; B, group B *Streptococcus* 18A; C, FCS control; D, *S. aureus* 1D; E, *P. acnes* 57C.

specimens (unpublished data), but no organisms were isolated. Lauric acid was not detected in spent FCS and was a useful marker for differentiation of *D. agalactiae* from the other organisms. Group B *Streptococcus* was again distinguished from the other organisms on the basis of acetic and benzoic acid production. The identification of benzoic acid was confirmed by mass spectrometry. *S. aureus* produced relatively high concentrations of acetic and isovaleric acids, and production of these acids differentiated it from the other organisms tested. *P. acnes* produced propionic and isovaleric acids in large amounts, and this differentiated it from the other groups. *Streptococcus* groups A and G produced only acetic acid, which made them a single group different from the other organisms. Table 5 summarizes the acids produced in vitro in FCS and in vivo in the animal model. It is clear from the data presented in the table that the organisms tested produced many of the same types of acids in vitro and in vivo. The only exception was *D. agalactiae*, which produced lauric acid in the chamber model only. From the table it is also apparent that the organisms in the animal model could be differentiated at the genus and species levels. The only exception is the group A and G streptococci; however, *Bergey's Manual* (7) states that group G *Streptococcus* spp. are taxonomically a poorly defined group which cannot be distinguished from *S. pyogenes* (group A) on the basis of present metabolic tests.

TABLE 4. Computer-generated area values for chromatograms obtained by FPMEC-GLC analysis of derivatized extract of chamber fluids infected with the indicated organisms

Acid	Chromatogram peak area ^a						
	I	J	K	L	M	N	O
Acetic	1.37	0.9	25.93	—	8.84	2.20	2.38
Propionic	10.86	—	—	—	—	—	—
Isobutyric	—	—	—	—	—	—	—
Butyric	—	—	—	—	—	—	—
Isovaleric	11.22	—	22.73	—	—	—	—
Valeric	—	—	—	—	—	—	—
Isocaproic	—	—	—	—	—	—	—
Caproic	—	—	—	—	—	—	—
Heptanoic	—	—	—	—	—	—	—
Benzoic	—	—	—	—	9.34	—	—
Phenylacetic	—	—	—	—	—	—	—
Nonanoic	—	—	—	—	—	—	—
Decanoic	—	—	—	—	—	—	—
Lauric	—	33.69	—	—	—	—	—

^a Area is measured by the computer in arbitrary units. The computer has a linear dynamic range of 2×10^6 . I, *P. acnes*; J, *D. agalactiae*; K, *S. aureus*; L, control chamber fluid; M, group B streptococci; N, group A streptococci; and O, group G streptococci.

TABLE 5. Summary of metabolic products detected by FPMEC-GLC from TCE derivatives of spent culture medium (FCS) and animal chamber fluid samples^a

Peak	Acid	<i>P. acnes</i>		<i>S. aureus</i>		<i>D. agalactiae</i>		Streptococci:					
								Group B		Group G		Group A	
		FCS	AC	FCS	AC	FCS	AC	FCS	AC	FCS	AC	FCS	AC
A	Acetic	+	+	+	+	+	+	+	+	+	+	+	+
B	Propionic	+	+										
E	Isovaleric	+	+	+	+								
J	Benzoic							+	+				
N	Lauric						+						

^a AC, Animal chamber system; FCS, FCS system. +, The compound was detected.

Analysis of uninfected chamber fluids (implanted on the left side of the animal and opposite to the infected chamber) showed an accumulation of products similar to those detected in the chamber containing actively growing organisms; however, repeated culturing of the left (uninoculated) chamber showed that it was sterile in every case. The only explanation is that there is a buildup of products in the sterile chamber which may be due to an exchange of fluids in which the metabolites are concentrated in some selective manner. Analysis of 2-ml samples of serum taken from the rabbits with established chamber infections did not show bacterial metabolic products present in the chamber fluid to be present in the serum; however, failure to detect these products in the serum may be due to low concentration of the metabolites in the serum or to absorption of them by the serum, rather than to an absence of these compounds. Both the chamber fluids and the spent FCS were analyzed for lactic acid by FPMEC-GLC as described (8). Only trace amounts of lactic acid were detected in either case.

DISCUSSION

The data obtained from the study have special significance because they offer supportive evidence that microbial products produced in vivo may be similar to those produced in vitro in serum and can be detected by FPMEC-GLC analysis of body fluid samples when the concentration of these products is sufficient. Furthermore, the patterns obtained present possibilities for their use in the identification of the infectious agents at the genus and species levels.

Results obtained from the in vitro and in vivo studies of *D. agalactiae* were surprising in that the organism altered its physiology in the animal chamber to such a degree that it could be cultured only when taken directly from the chamber under strict anaerobic conditions. The FPMEC-GLC patterns obtained from chamber fluid analysis of fluids infected with *D. agalactiae* differed from those obtained from analysis of spent FCS in that lauric acid was present in the chamber fluid. The other organisms included in the study did not behave in this manner, and

this physiological change demonstrated by *D. agalactiae* might indicate that some organisms present in human transudates would require anaerobic conditions for isolation. For example, it is general knowledge that gonococci are difficult to isolate from human arthritic transudates, but the reason for this difficulty has not been fully established. *D. agalactiae* have been reported, mainly on the basis of serological evidence, to be the causative agent of rheumatoid arthritis (20); however, isolation of the organism from the afflicted area has not been reported.

The apparent lack of host response makes it possible to study better the bacterial metabolites produced in vivo, but in a generalized infection the inflammatory and other types of host response would be present. From published data (14) it is almost certain that these different types and degrees of body response to infecting agents could be helpful in categorizing meningitides due to viruses, bacteria, and fungi. This host response might make it, in many instances, difficult to detect bacterial products, since compounds such as lactic acid, carboxylic acids, and amines would probably be present in higher concentrations than those of most bacterial metabolites; however, some of the peaks detected by FPMEC-GLC analysis of body fluids probably are bacterial metabolites, as indicated by comparison of FPMEC-GLC profiles of infected body fluids and those obtained by in vitro analysis (14). Only by the continued FPMEC-GLC study of well-documented cases and perhaps in part through further studies with animal models can the answer to these complex questions be found. If bacterial metabolites produced in vivo could be established, in many cases it would permit the use of a more rapid procedure to detect them by FPMEC-GLC and make possible a more simplified identification scheme.

The Perkin-Elmer PEP-2 computer offers a modest means of computerizing the processing of FPMEC-GLC data. This instrument incorporates the immediate capability of reducing the chromatographic data to meaningful numerical values as shown in this study. In some instances, differences in profiles were more easily seen from the PEP-2 printout. By interfacing the PEP-2 to a large time-sharing computer for further refinement by more sophisticated software, the data can be standardized from instrument to instrument, background corrections can be made, and profiles can be matched. This will further simplify the rapid classification of bacteria by their FPMEC-GLC profiles obtained from both in vivo and in vitro analysis.

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