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REVIEW

GAS—LIQUID CHROMATOGRAPHY—FREQUENCY PULSE-MODULATED ELECTRON-CAPTURE DETECTION IN THE DIAGNOSIS OF INFECTIOUS DISEASES*

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1. INTRODUCTION

The rapid diagnosis of infectious diseases continues to be of great importance to the clinician and the diagnostic laboratory. Even with advances in the development of diagnostic methods, however, many diagnostic problems still exist such as in pneumonia, lymphocytic meningitides, arthritis, pleural effusions, and parasitic and viral diseases. Moreover, the cultural, biochemical, immunochemical, and serologic diagnostic techniques used in these areas are sometimes slow or unreliable.

Gas—liquid chromatography (GLC) offers a very sensitive and rapid technologic concept that has been applied to the study of microorganisms and to the diagnosis of infectious diseases [1—8]. The term GLC—chemotaxonomy [9] has been used to describe the application of GLC methods to the study of microbial cells, extracts, and/or metabolic products found in culture media to identify or classify a microorganism on the basis of chromatographic profiles of carboxylic acids, hydroxy acids, alcohol, and amines or fingerprints.

Although specific GLC analytical schemes have been proposed and demonstrated for use in specific diagnostic microbiology and infectious diseases studies, we believe that the GLC methodology described holds the greatest promise for use in the differentiation and identification of the etiology of infectious diseases caused by bacteria, viruses, rickettsiae, fungi, and parasites by the analysis of a variety of body fluids or excretion products of the infected host. In addition, the technique has been used [10] to identify bacterial fatty acids and metabolic products from in vitro cultures for use in taxonomic and epidemiologic studies [10—15, 30].

The technique relies on (1) the extraction of metabolic products of the microbe, the host response to the microbe, or a combination of the two; (2) the preparation of functional-group specific electron-capturing derivatives for GLC examination; (3) GLC analysis using a very specific and sensitive frequency pulse-modulated (constant current) electron-capture detector (FP-ECD); (4) high resolution columns; and (5) manual and automated analysis of the chromatographic fingerprints to establish the identity of the etiologic agent or the infectious process. Our purpose in this presentation is not to evaluate the work of other scientists in relation to the identification of bacteria through the GLC analysis of spent media or cellular materials, but to review a promising technique for aiding in the rapid diagnosis of disease by GLC—ECD analysis of body fluids, to briefly discuss its potential for application to the analysis of spent culture media and cellular materials, and to discuss in detail some aspects of the procedure.

2. EXPERIMENTAL

2.1. Source material

Synovial (SF), pleural (PF), and cerebrospinal (CSF) fluids; blood, plasma, and serum; purulent exudates; urine from well documented and culture-proven cases of infections, and spent bacteriologic, rickettsial, fungal, or viral cell culture media served as the experimental source material. Organic compounds were then extracted, concentrated, derivatized, and analyzed. Fig. 1 summarizes the extraction technique, and it should be noted that as little as 1 ml of body fluid can be adequate for many analyses.

Microbial structural components were studied. Washed bacterial cells were saponified with 5% sodium hydroxide in 50% aqueous methanol for 30 min at 90—100°C [8, 10]. After saponification, the methanolysates were acidified to approximately pH 2 with 8 *N* sulfuric acid and then extracted immediately or neutralized and stored at —20°C for further study. Carboxylic acids were transesterified [10] to trichloroethanol esters, Fig. 1, method 1, and hydroxy acids

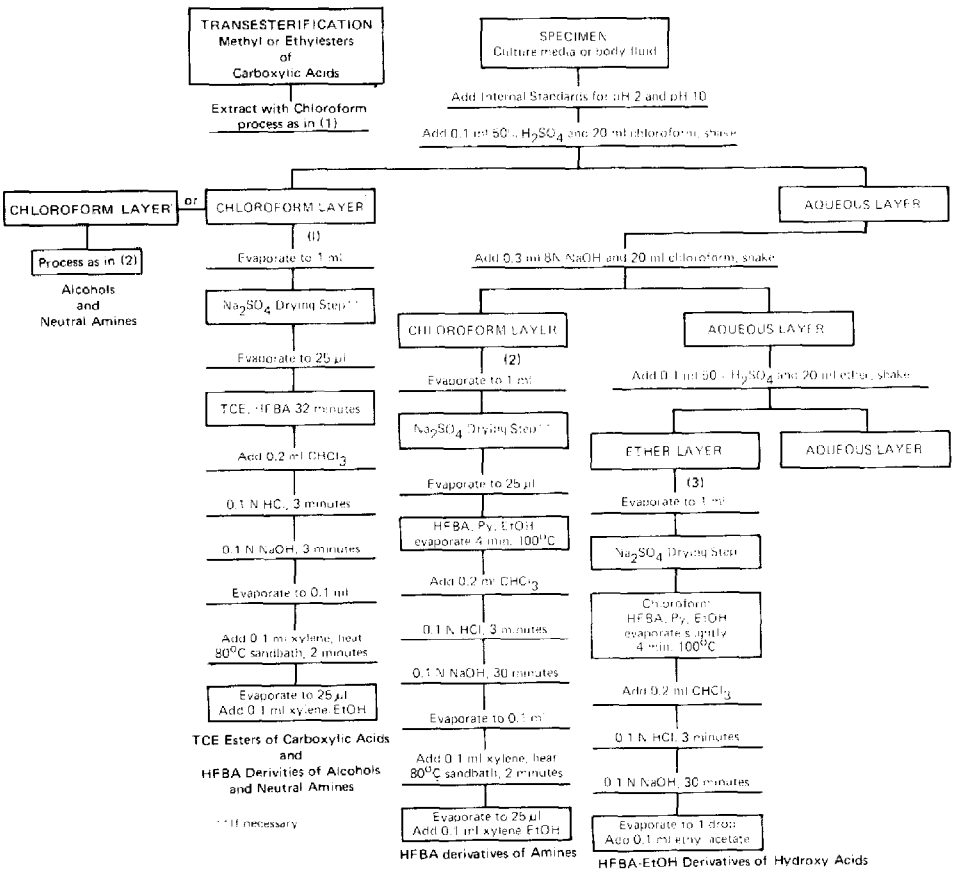


Fig. 1. Flow-chart for preparing specimens for GLC—ECD analysis. There should be *three* derivatives to be analyzed from one specimen.

TABLE 1

DETAILED PROCEDURE FOR PREPARATION OF ELECTRON-CAPTURING DERIVATIVES OF FATTY ACIDS, HYDROXY ACIDS AND ALCOHOLS, AND AMINES

I. 2,2,2-Trichloroethanol (TCE) derivatives of extracted organic acids or transesterification of esters

A. Chloroform layer (pH 2), 20 ml, in a 50-ml beaker.
B. Evaporate to 1 ml with clean, dry air.
C. Transfer 1 ml to a new, clean, 12 × 75 mm tube (can be stored at −20°C, cork stoppered, for future derivatization).
D. Remove any water by taking the 1 ml of chloroform up in a Pasteur pipette and depositing the chloroform in a clean, dry tube.
E. Evaporate to one drop with clean, dry air.
F. Add one drop TCE—chloroform (1:9) and six drops HFBA, cork.
G. Let the mixture stand 32 min at room temperature.
H. Add 0.1 ml chloroform and six drops 0.1 *N* hydrochloric acid, shake, cork, let the mixture stand 3 min.
I. Take up chloroform—hydrochloric acid mixture in a Pasteur pipette and redeposit chloroform layer in the same tube.
J. Add six drops of 0.1 *N* sodium hydroxide, shake, cork, let the mixture stand 3 min.
K. Take up the chloroform—sodium hydroxide mixture in a Pasteur pipette and redeposit chloroform layer in a clean, dry tube and discard the sodium hydroxide layer (repeat twice to remove all traces of water).
L. Add 0.1 ml xylenes and place tube in an 80°C sand bath or heating block for 2 min, uncorked, to remove all traces of TCE and most of the chloroform.
M. Evaporate to one drop, but *not to dryness*.
N. Add 0.1 ml xylenes—ethanol (50:50) as a final solvent.
O. For GLC—FP-ECD analysis use 1.4 μl (cultures) or 2.0 μl (body fluids).

II. Heptafluorobutyric anhydride (HFBA) derivatives of extracted hydroxy acids and alcohols and extracted amines

A. Diethyl ether (hydroxy acids and alcohols) extract (pH 2), 20 ml; or chloroform (amines) extract (pH 10), 20 ml, each put in a 50-ml beaker.
B. Evaporate to 1 ml with clean, dry air.
C. Transfer the 1 ml to a new, clean 12-X 75-mm tube (can be stored at −20°C, cork stoppered, for future derivatization).
D. Dry with anhydrous sodium sulfate, centrifuge, decant the ethyl or chloroform layer, add 1 ml of diethyl ether or chloroform, recentrifuge, and combine the diethyl ether or chloroform washes.
E. Evaporate to one drop with clean, dry air (for diethyl ether extract add 0.1 ml chloroform and reevaporate to one drop with clean, dry air).
F. Add one drop pyridine—chloroform—ethanol (2:4.4:1.6) solution and six drops of HFB.
G. Evaporate to one drop (to remove chloroform), cork, heat in a boiling water bath for 4 min and cool in running tap water.
H. Add 0.1—0.2 ml chloroform, six drops of 0.1 *N* hydrochloric acid, shake gently, cork, and let the mixture stand at room temperature for 3 min.
I. Take up the chloroform—hydrochloric acid mixture in a Pasteur pipette and redeposit the chloroform layer in the same tube, discard the aqueous layer.
J. Add six drops 0.1 *N* sodium hydroxide to the chloroform, shake gently, cork, and let the mixture stand for 30 min at room temperature.
K. Take up the chloroform—sodium hydroxide mixture in a Pasteur pipette and redeposit the chloroform layer in a clean dry tube; discard the aqueous layer (repeat several times to remove all traces of water).

TABLE 1 (continued)

L.	Add 0.1 ml xylenes, place tube in an 80°C sand bath or heating block for 2 min, uncorked, to remove most residual chloroform (for amines only).
M.	Evaporate to one drop (amines) or near dryness (hydroxy acids and alcohols) with clean, dry air.
N.	Final solvent for the pH 2 diethyl ether extract, 0.1 ml ethyl acetate. Final solvent for the pH 10 chloroform extract, 0.1 ml xylenes—ethanol (50:50).
O.	For GLC—FP-ECD analysis use 1.4 μl (cultures) or 2.0 μl (body fluids).

were further esterified [13] with heptafluorobutyric anhydride, Fig. 1, method 2.

2.2. *Extraction and derivatization*

Regardless of the source, all samples were either first esterified then extracted or extracted with appropriate solvents to yield fractions that contained organic acids. The samples of body fluids, exudates, urine or spent media were further extracted to yield fractions that contained hydroxy acids, alcohols, and amines. The process is summarized in Fig. 1. Organic acids alcohols or esters were extracted from the acidified (pH 2) sample in a 50-ml centrifuge tube equipped with a PTFE-lined screw cap by adding 20 ml of nanograde chloroform (Mallinckrodt) and shaking on a wrist-action shaker for 5 min. To obtain amines, we made the residual sample (pH 10) and again extracted with 20 ml of nanograde chloroform as stated for acids. The residual sample was reactified (pH 2) and extracted for a third time with 20 ml of reagent grade diethyl ether (Baker or Fisher) stabilized with butylated hydroxytoluene to obtain hydroxy acids. This particular acid—base—acid extraction is the beginning of the selective process to detect specific chemical components. The extraction methodology is summarized in Fig. 1.

Electron-capturing derivatives, one of the two critical components of the procedure, were then made after the samples had been extracted. The extracting solvent was reduced in volume by evaporation with clean dry air, and any residual water was removed with anhydrous sodium sulfate. Trichloroethanol—heptafluorobutyric anhydride (TCE), derivatives of carboxylic acids, and heptafluorobutyric anhydride—pyridine—ethanol (HFBA—EtOH), derivatives of hydroxy acids, and alcohols and amines were the specific electron-capturing species formed. The final solvent for the TCE and HFBA-amine derivatives is 0.1 ml of xylene—ethanol (50:50), and 0.1 ml of ethyl acetate was used as a final solvent for the HFBA—EtOH esters of hydroxy acids. It should be noted that if alcohols or neutral amines are present in the initial acid fraction extracted with chloroform to obtain carboxylic acids, they will be derivatized with the HFBA. Hydroxy acids which are obtained in the second acid fraction extracted with diethyl ether will form a diester derivative with the HFBA and ethanol in the derivatization reagent mixture. The derivatization scheme is listed in Fig. 1 and Table 1.

2.3. Gas—liquid chromatography

The second critical component of this analytical scheme was a gas—liquid chromatograph equipped with a frequency-pulsed modulated ECD and high resolution columns. We have used the Perkin-Elmer Model 3920B instrument with dual columns and temperature programming capability, but instruments of other manufacturers have also been used successfully, e.g. Hewlett-Packard Model 5830. The instrument was equipped with either one or two 7.3 m × 2 mm I.D. coiled glass columns packed with 3% OV-101 liquid phase coated on Chromosorb W (80—100 mesh, acid washed, and dimethylsilane treated; Applied Science Labs., College Station, PA, U.S.A.).

The following operating conditions apply to all analyses: (1) injection port temperature, 225°C; (2) detector temperature, 275°C; (3) detector attenuation, 512 with a standing current of 2 or less, but in actual use response obtained from standard mixtures will determine optimum setting of the standing current, with attenuation set at 512 or lower; (4) manifold temperature setting (Perkin-Elmer 3920B only), 250°C. The 5% methane in 95% argon carrier gas mixture was regulated to 50 ml/min, as measured at the detector vent with a soap-bubble flow meter, and an additional 17 ml/min of flush or purge carrier gas was introduced between the column exit fitting and before the detector (Perkin-Elmer 3920B and 900 only).

The following column oven operating temperature programming parameters were used. For the TCE (fatty acid) derivatives, the initial oven temperature was 100°C with a 4°C/min program to 265°C with a final hold of 16—32 min. The HFBA (hydroxy acid, alcohol, and amine) derivatives were analyzed with an initial oven temperature of 90°C held for 8 min, 4°C/min increase to 265°C, and the final temperature was held for 32 min or less.

In addition to the use of high resolution packed glass columns, fused-silica capillary columns are available in lengths up to 100 m with the liquid phase coated directly on the inside wall. These capillary columns, used in conjunction with the purged-splitless injectors, are now being used for specialized analyses and are also being evaluated as a replacement for the packed glass columns [16]. These fused-silica capillary columns improve component separation and can be used to shorten the analysis time by up to 50%. The carrier gas used for the capillary columns is helium and the purge or flush gas was 5% methane in 95% argon. The column flow was 3 ml/min and the combined column and flush gas flow-rate through the detector was 50 ml/min.

2.4. Chromatogram analysis and interpretation

Data generated from the GLC—FP-ECD analysis of body fluids, excretion products, and spent culture media, are complex and interpretation requires specific skills and acumen. However, it does not take long to master the skills necessary for interpretation and with completion of computer programs, now under development, interpretation of data should be made easier. However, when new columns are used, several instruments are employed or columns are repacked in the existing instruments, retention times, separation characteristics, and response characteristics will differ between instruments. In our studies

normalization of these differences has been addressed. Routine procedures were used by the Perkin-Elmer PEP-2 (or Sigma 10) microprocessor equipped with Modular Software System (MS-16 revision B) to accumulate data from the instrument, analyze the data according to a stored method, and prepare a report. Then these stored data from several instruments with differing retention characteristics were transmitted by telephone to a larger time-sharing computer. The data were stored and manipulated by this larger computer, and the data points were standardized through the analysis of standard mixtures which were then processed by a spline-fit programming technique on the time-shared system. This procedure then changed retention times to straight-chain hydrocarbon equivalence numbers for standardization of data between instruments.

A second software program is under final development and testing that will take the normalized or standardized data from individual samples and analyses with a known etiology and create a representative profile for each particular infection or organism, and report an identification match for an unknown sample based on the best possible correlation with the representative GLC—FD-ECD computer profile. In the future we hope to make this software system available to diagnostic laboratories and other interested investigators.

3. RESULTS AND DISCUSSIONS

Early developmental studies by Brooks and colleagues introduced a derivatization and high resolution flame ionization GLC technique as an alternative GLC technologic component in the anaerobic bacteria identification scheme developed by the Anaerobe Laboratory, Virginia Polytechnic Institute and State University [17]. Brooks and co-workers [11, 18] continued this work later at the Centers for Disease Control and examined clostridia further by flame ionization and nonfrequency-pulsed mode GLC—ECD. In subsequent studies the taxonomic usefulness of the GLC—FP-ECD technology was demonstrated in the differentiation of additional *Clostridium sporogenes* [12].

Two main events contributed to the perfection of the GLC—FP-ECD technique presently in use: (1) advances in ECD development (frequency-pulsed mode), and (2) the development of specific derivatives to exploit the extreme sensitivity and selectivity of the FP-ECD.

3.1. Septic arthritis

Septic arthritis was the first infectious process to be examined by GLC—FP-ECD. Examination of synovial fluids (SF) from infected patients and uninfected controls showed conclusively that the chromatographic profile of fluid from an infected patient differed significantly from that of an uninfected one [7, 19]. In addition, GLC—FP-ECD profiles of SF obtained from patients with different types of infecting bacterial agents were distinguishable from each other, Fig. 2.

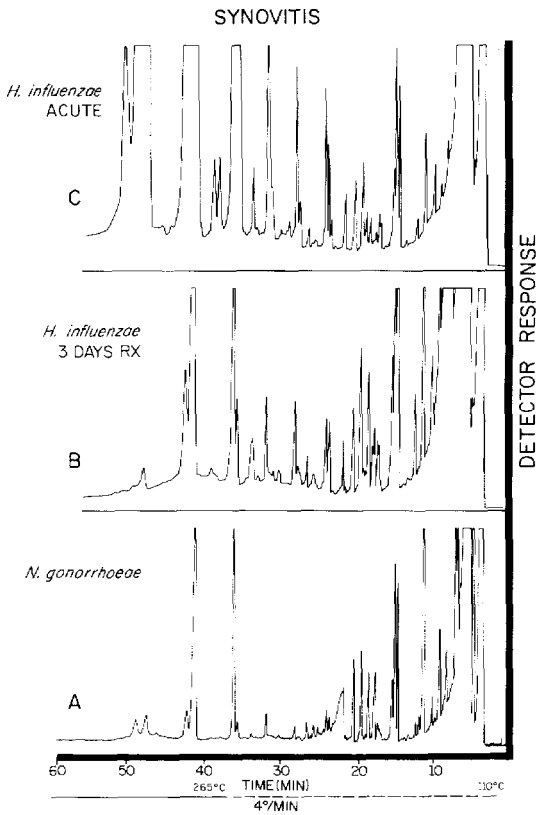


Fig. 2. GLC—FP-ECD profiles of TCE derivatized acidic chloroform extracts of synovial fluids. Etiologies of synovites are indicated in the figure.

3.2. Cystitis

Cystitis was studied concurrently with septic arthritis and a remarkable array of compounds were present in urine samples from infected patients, Fig. 3 [20]. It was also shown that some of the compounds that were present in the urine from patients were produced by the bacterium isolated from the urine sample. When the etiologic agent, *Proteus mirabilis*, was introduced into a sterile urine sample from a previously infected and antibiotic-treated patient, the GLC—FP-ECD profile was very similar to the pattern seen in the natural infection. In addition, this study also showed that *P. mirabilis* produced N-nitrosodimethylamine, a member of the highly carcinogenic nitrosamine group of compounds, both in vivo and in vitro [14, 20].

Urine was suspected to be a less than ideal body fluid or excretion product for study by GLC—FP-ECD because there was a possibility that it would undergo unpredictable composition changes due to diet, medications, etc. This proved to be partially true, and subsequent studies on urinary tract infections were temporarily suspended. Efforts were redirected toward finding a more homogeneous body fluid with which to demonstrate the diagnostic potential of the GLC—FP-ECD analytical scheme. Based on earlier work utilizing non-

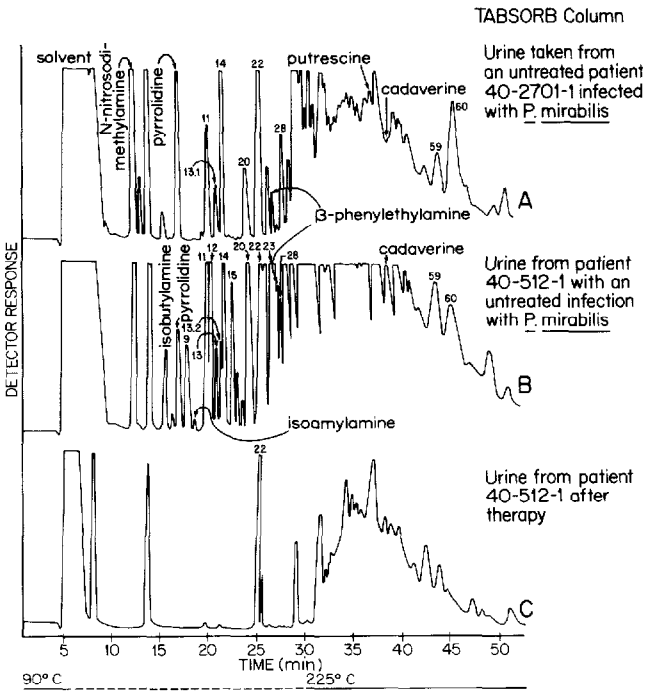


Fig. 3. Gas chromatograms of amines extracted from basic chloroform extracts of urine and derivatized with heptafluorobutyric anhydride (HFBA). (From ref. 20 with permission.)

selective detectors, such as the flame ionization detector, concerns were also raised about the composition and changes in whole blood, plasma, or serum, and these were not extensively examined in early GLC studies [16].

3.3. Meningitis and encephalitis

Attention was subsequently focused on a body fluid whose composition in the healthy individual is controlled within very rigid bounds, specifically cerebrospinal fluid (CSF). The choice of CSF and changes in CSF in meningitis cases as subjects of study was considered very relevant because meningitis and encephalitis are infections where a specific and rapid diagnosis is desirable and in some cases essential for the well-being of the patient. When conventional microscopic, cultural, or immunochemical diagnostic techniques are negative for bacterial meningitis and there is a central nervous system (CNS) infection which tests show to have a lymphocytic pleocytosis and an equivocal CSF glucose, the physician would benefit greatly from a diagnostic technique that would give a rapid definitive diagnosis.

Lymphocytic meningitides, which include tuberculous meningitis (TBM), and fungal, parasitic, and asceptic meningitis, and viral meningoencephalitis, were infections chosen to exploit. Control CSF specimens were obtained from patients receiving myelograms studies or spinal epidural anesthesia. These specimens from uninfected patients served as controls; they were relatively free

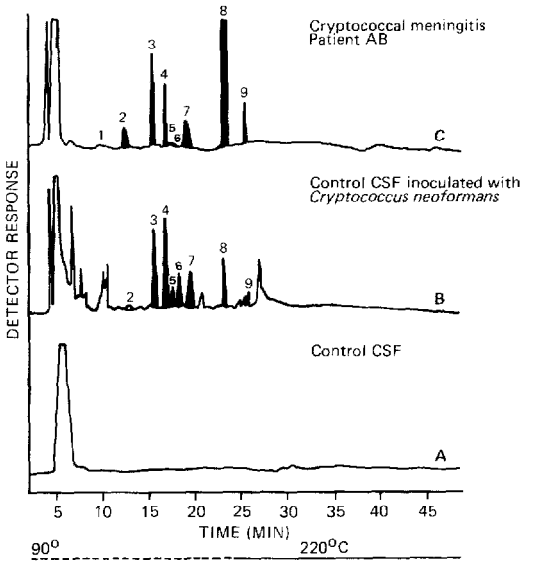


Fig. 4. GLC—ECD of HFBA derivatives from the basic chloroform extracts of CSF from *Cryptococcus neoformans*, and a normal uninfected CSF specimen obtained from a patient undergoing a diagnostic myelogram. (From ref. 21 with permission.)

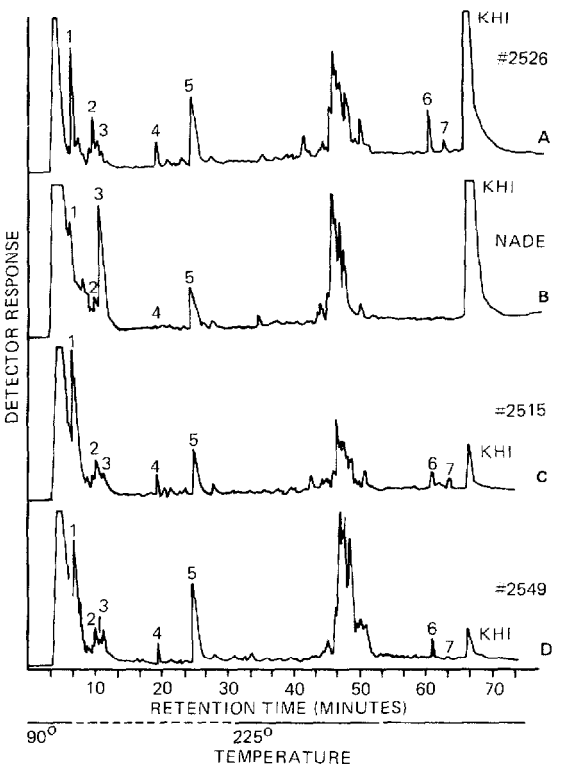


Fig. 5. GLC—FP-ECD of HFBA derivatives of the basic chloroform extracts (pH 10) from four patients with tuberculous meningitis. The peak labeled KHI has been identified by mass spectrometry as 3-(2'-ketohexyl)indoline and is a novel amine compound found in many bacterial infections. (From ref. 22 with permission.)

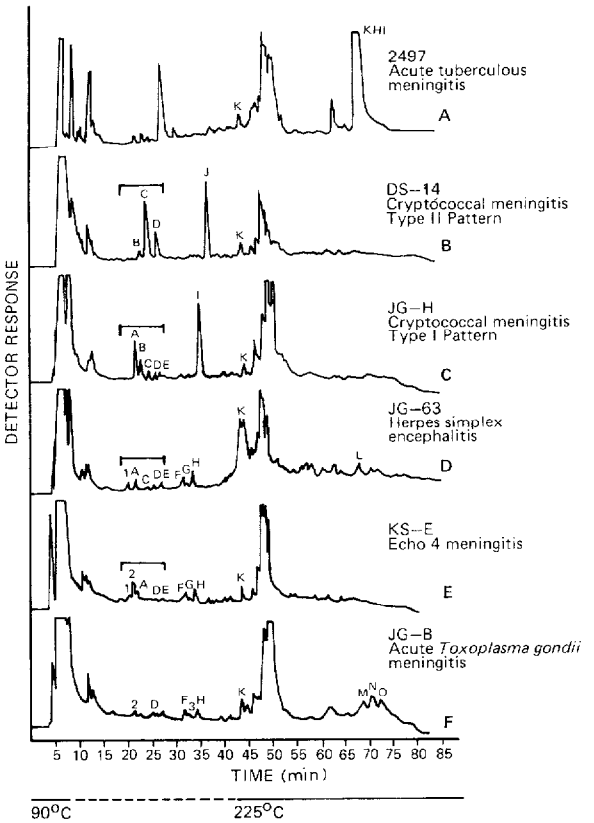


Fig. 6. Comparison of GLC—FP-ECD profiles of HFBA derivatized basic chloroform extracts of CSF samples taken from patients with the diseases indicated in the figure. (From ref. 22 with permission.)

of compounds capable of being detected by the GLC—FP-ECD procedure, and yielded a clean GLC—FP-ECD profile.

The initial infections examined were from culture-proven cases of cryptococcal (*Cryptococcus neoformans*) meningitis and aseptic meningitis caused by various echoviruses [21]. The cryptococcal specimens, though limited in number, were easily distinguishable from the echoviral ones when the basic chloroform extract HFBA derivatives (amines) were examined. Many of the compounds detected in the clinical specimens were also present when *C. neoformans* was inoculated into a sterile CSF specimen containing a supplement and incubated for seven days at 35° C. These findings suggested the possibility that some of the compounds detected were microbial metabolic products, Fig. 4.

Studies of lymphocytic meningitides were expanded with the major efforts directed toward the rapid diagnosis of TBM. Additional studies were made of cryptococcal, and various viral CNS infections [22]. A reproducible GLC—FP-ECD pattern of amines was obtained when culture-proven *M. tuberculosis* CSF specimens from Egypt were examined, Fig. 5, and this pattern was different from the patterns of the cryptococcal meningitides and viral CNS infections,

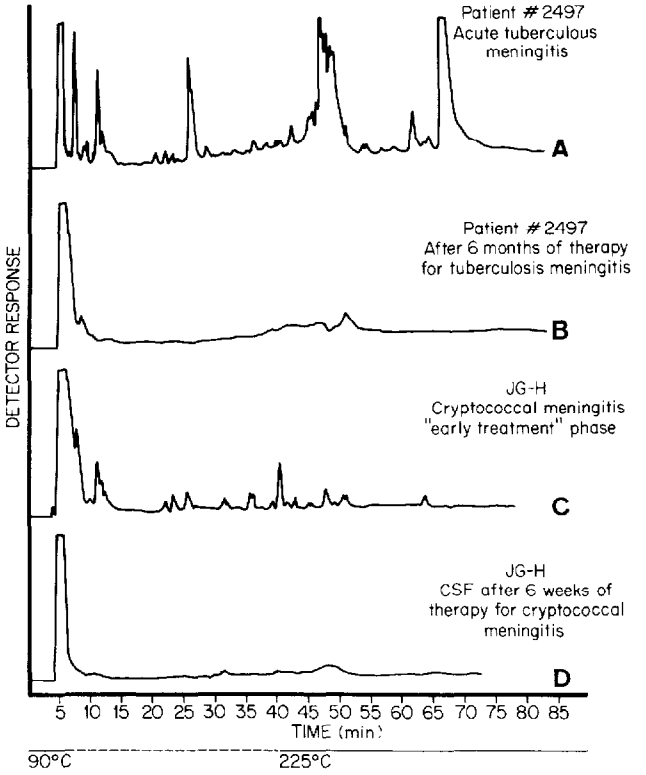
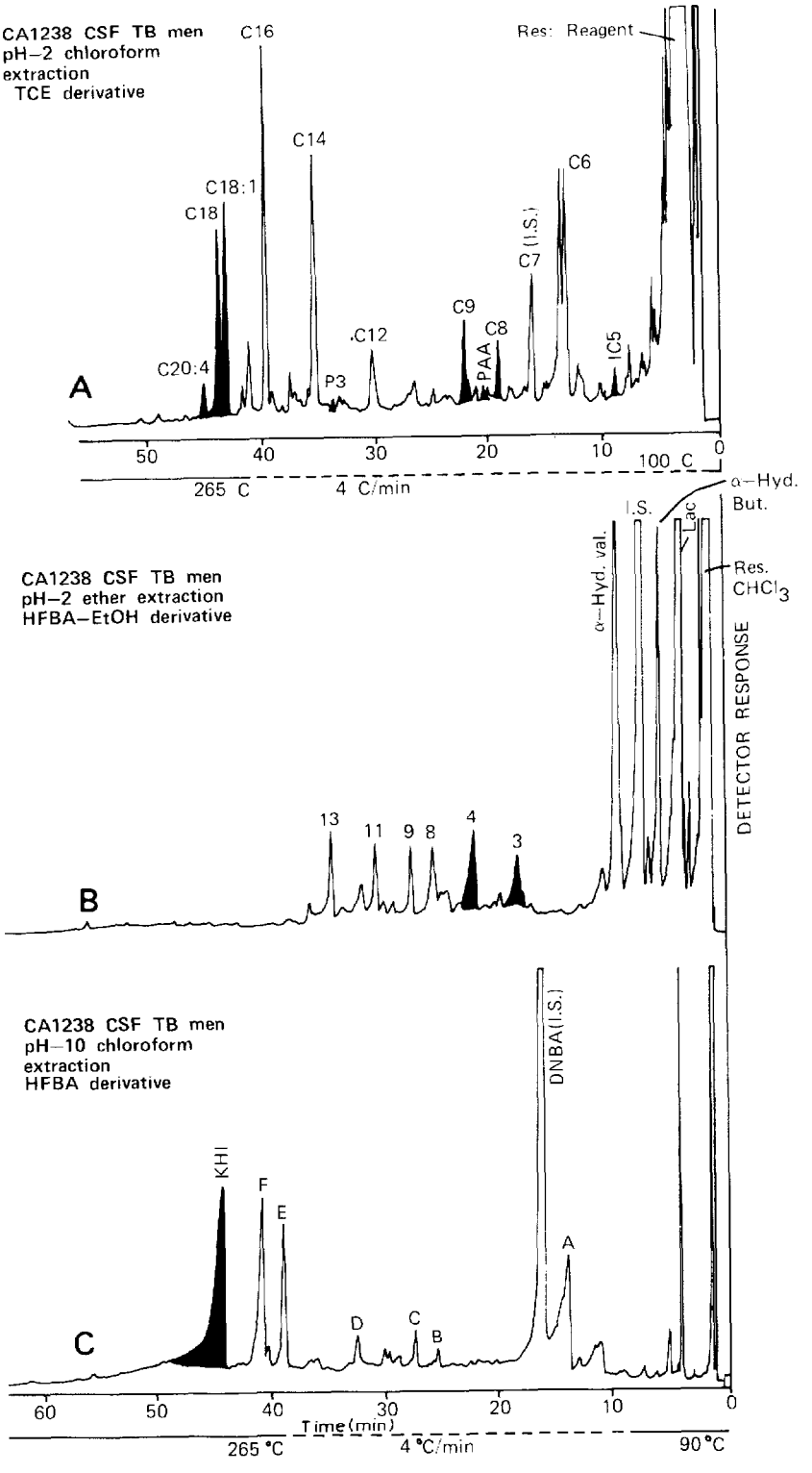


Fig. 7. GLC—FP-ECD profiles of HFBA derivatized basic chloroform extracts of CSF samples taken from patients with the diseases indicated in the figure. (From ref. 22 with permission.)

Fig. 6. In addition, when serial CSF specimens from Egyptian patients with TBM were obtained and analyzed during specific antituberculous therapy, the compounds initially detected diminished and eventually disappeared with time, Fig. 7.

The CSF specimens from acutely ill Egyptian patients yielded rather large amounts of a new amine compound which disappeared after effective therapy. Gas chromatography—mass spectrometry was eventually used to identify this amine as 3-(2′-ketohexyl)-indoline (KHI) [23]. KHI has not been previously reported in biologic materials nor had it been synthesized chemically. Interestingly, in subsequent studies KHI was not detected in CSF samples from patients with TBM in the United States [24]. The function, origin, and significance of KHI has yet to be established, but its structure is similar to certain neurotransmitters and it may play a role in the sequelae frequently seen in meningitis patients. Initially, KHI was thought to be unique to TBM and as

Fig. 8. GLC—FP-ECD profiles from the tuberculous meningitis CSF samples: (A) TCE derivatives of acidic chloroform extracts; (B) HFBA—EtOH derivatives of acidic diethyl ether extracts (third extraction); and (C) HFBA derivatives of basic chloroform extracts. (From ref. 24 with permission.)


CA1238 CSF TB men
pH–2 chloroform
extraction
TCE derivative
C16
Res: Reagent
C14
C18:1
C18
C6
C7
(I.S.)
C9
C8
C12
C5
C20:4
PAA
P3
A
50
40
30
20
10
100 C
0
265 C
4 C/min
α–Hyd.
But.
I.S.
Lac
α–Hyd. val.
Res.
CHCl3
CA1238 CSF TB men
pH–2 ether extraction
HFBA–EtOH derivative
DETECTOR RESPONSE
13
11
9
8
4
3
B
CA1238 CSF TB men
pH–10 chloroform
extraction
HFBA derivative
DNBA(I.S.)
KHI
F
E
A
D
C
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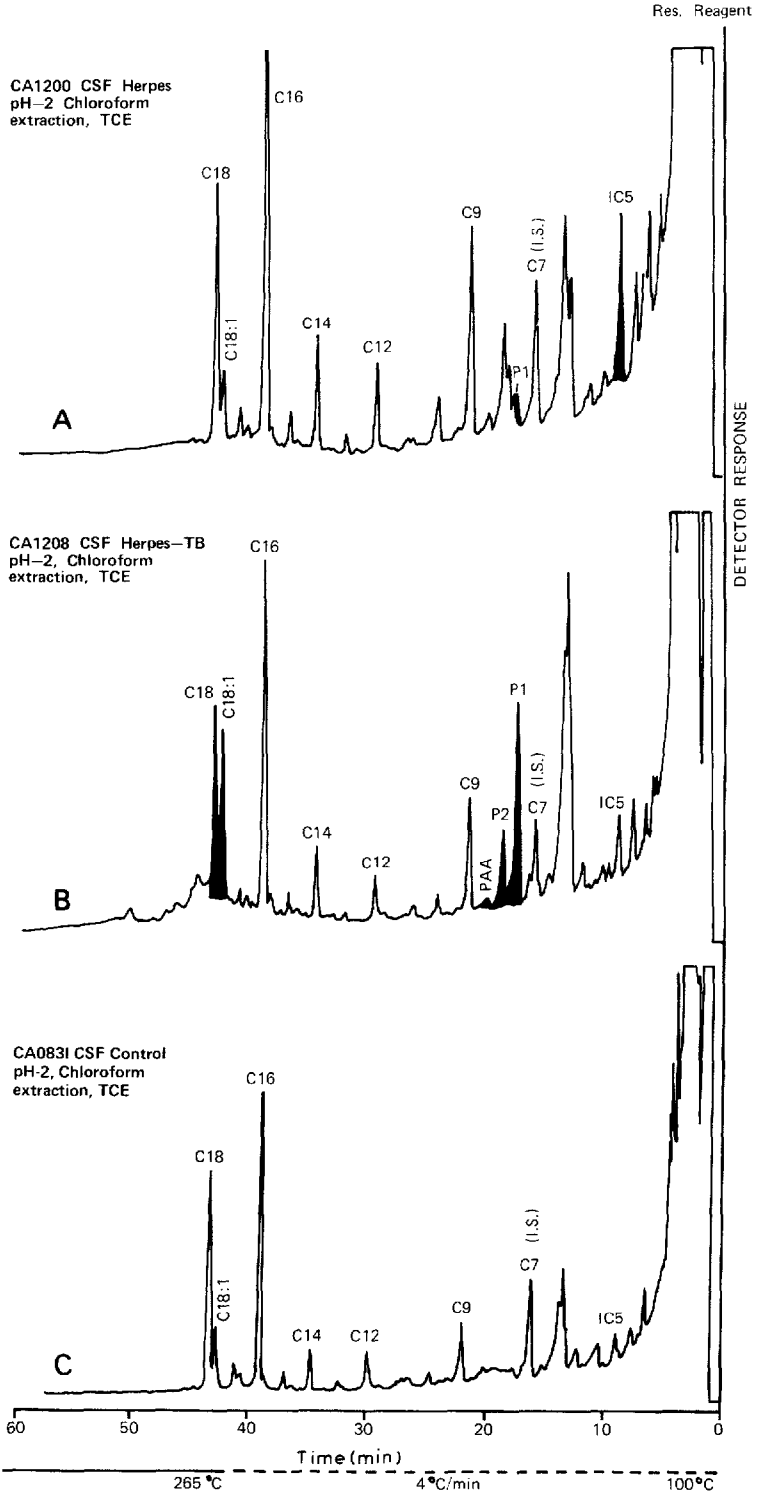
60
50
40
30
20
10
0
Time (min)
265 °C
4 °C/min
90 °C


Res. Reagent
CA1200 CSF Herpes
pH–2 Chloroform
extraction, TCE
C18
C16
C18:1
C14
C12
C9
C7 (I.S.)
P1
IC5
A
DETECTOR RESPONSE
CA1208 CSF Herpes–TB
pH–2, Chloroform
extraction, TCE
C16
C18
C18:1
P1
C9
P2
C7 (I.S.)
IC5
C14
C12
PAA
B
CA0831 CSF Control
pH-2, Chloroform
extraction, TCE
C16
C18
C18:1
C7 (I.S.)
C9
IC5
C14
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Time (min)
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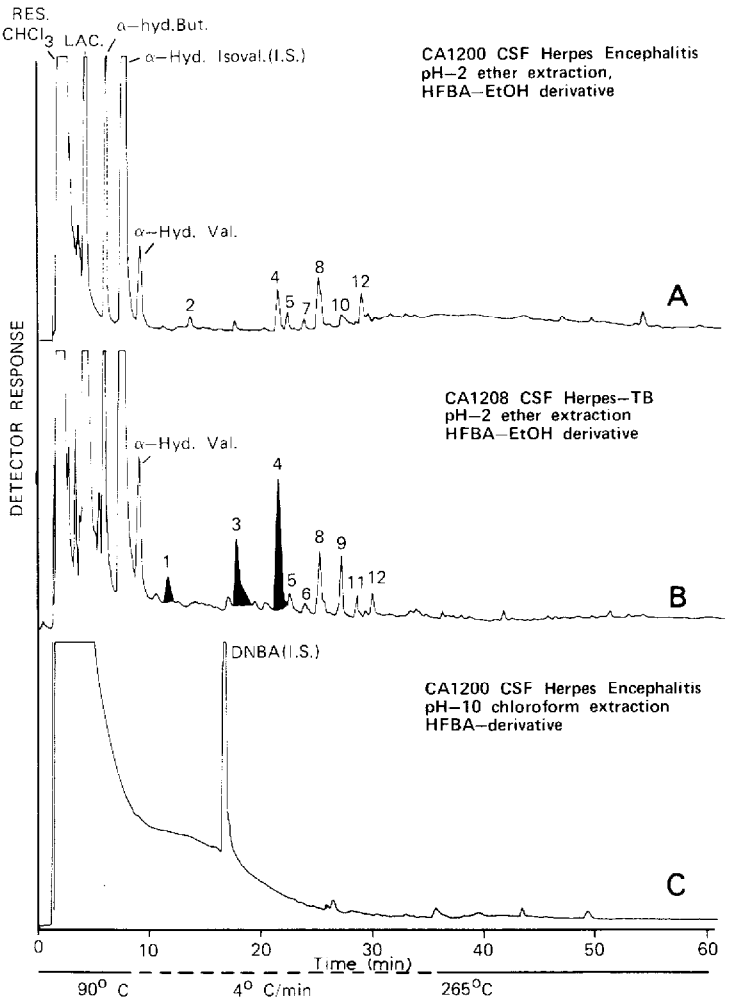

Fig. 10. GLC—FP-ECD chromatograms of: (A en B) HFBA—EtOH derivatized diethyl ether extracts of CSF taken from patients with the disease indicated in the figure, and (C) from an HFBA derivatized basic chloroform extract of CSF taken from a patient with herpes encephalitis. (From ref. 24 with permission.)

such it might be of diagnostic significance. This has not held true because KHI has since been found in CSF samples from Egyptian patients with meningitides caused by some common bacterial agents, i.e., *Haemophilus influenzae* [25], *Streptococcus pneumoniae* [16], and *Neisseria meningitidis* [16]. KHI has also been shown (Brooks et al., unpublished data) to be present in the sera of Egyptian patients with the above type infections in the United States. KHI has been detected in the serum of a child with a bacterial infection and in the serum and synovial fluid of a race horse with an acute bacterial infection. The

Fig. 9. GLC—FP-ECD profiles of TCE derivatized acidic chloroform extracts of CSF taken from patients with the diseases indicated in the figure. (From ref. 24 with permission.)

compound has thus far only been detected in bacterial infections, never in viral or parasitic infections.

Subsequent studies of additional cases of lymphocytic meningitides established that certain carboxylic and hydroxy acids and selected easily obtainable clinical data can be used effectively to rapidly diagnose TBM even in the absence of KHI, Fig. 8 [24]. This study also showed that in a well documented case the GLC—FP-ECD patterns of herpes virus meningitis/encephalitis (HVM), mixed TBM—HVM, were different, Figs. 9 and 10. This study established that KHI is not responsible for the positive color reaction in the tryptophan color test used for many years in the diagnosis of TBM. In our laboratory more than 400 specimens have been analyzed for tuberculous meningitis, and based on final reports obtained from the physician, the test has been accurate over 90%.

3.4. Pleural effusions

During the studies of meningitides, similar GLC—FP-ECD analyses were performed on a series of pleural effusions (PF) from patients with sepsis,

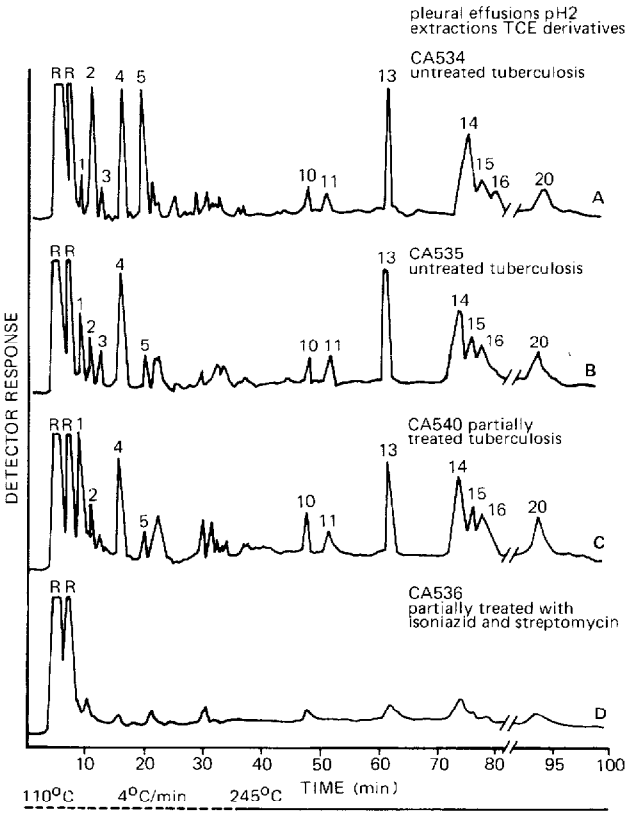

Fig. 11. GLC—FP-ECD chromatograms of TCE derivatized acid chloroform extracts of pleural fluids taken from patients with the type of disease indicated in the figure. (From ref. 26 with permission.)

congestive heart failure, malignancies, uremia, and systemic lupus erythematosus [26]. GLC—FP-ECD profiles were assigned to various groups before we received clinical information and then later we compared the profiles with the tentative diagnoses. GLC—FP-ECD analysis detected some errors made in the tentative diagnosis. The GLC—FP-ECD profiles from different types of effusions differed markedly and they may be of diagnostic value, Fig. 11 [26].

3.5. Diagnostic potential of serum

In all the above studies CSF, SF, PF, or urine were used for GLC—FP-ECD analysis. It would be very useful, however, to use a body fluid that is more easily obtainable than CSF, SF, or PF. Serum can be obtained in larger amounts with less discomfort to the patient.

A recent study used GLC—FP-ECD to analyze serum specimens from human controls and patients with the following diseases: (1) Rocky Mountain spotted

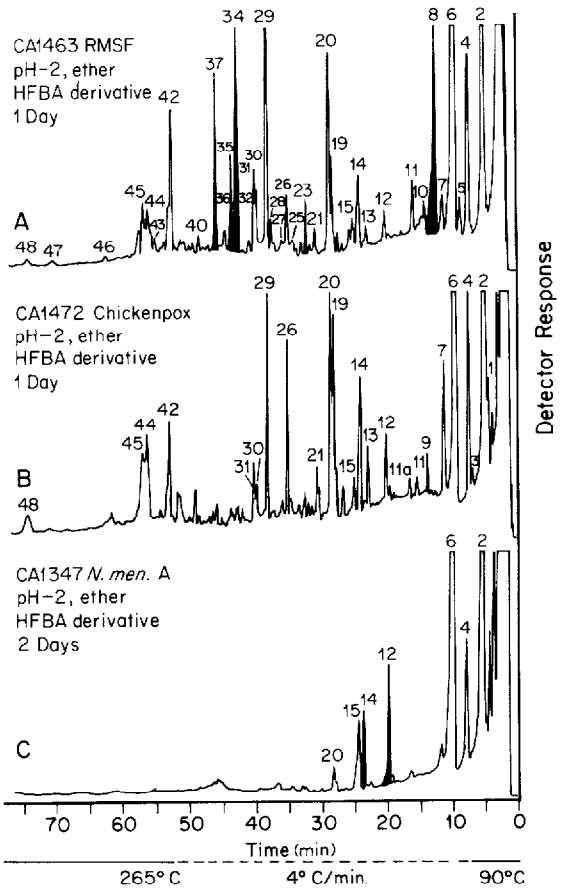

Fig. 12. GLC—FP-ECD chromatograms of HFBA—EtOH derivatized acidic diethyl ether extracts of sera taken from patients with the type of disease indicated in the figure. (From ref. 27 with permission.)

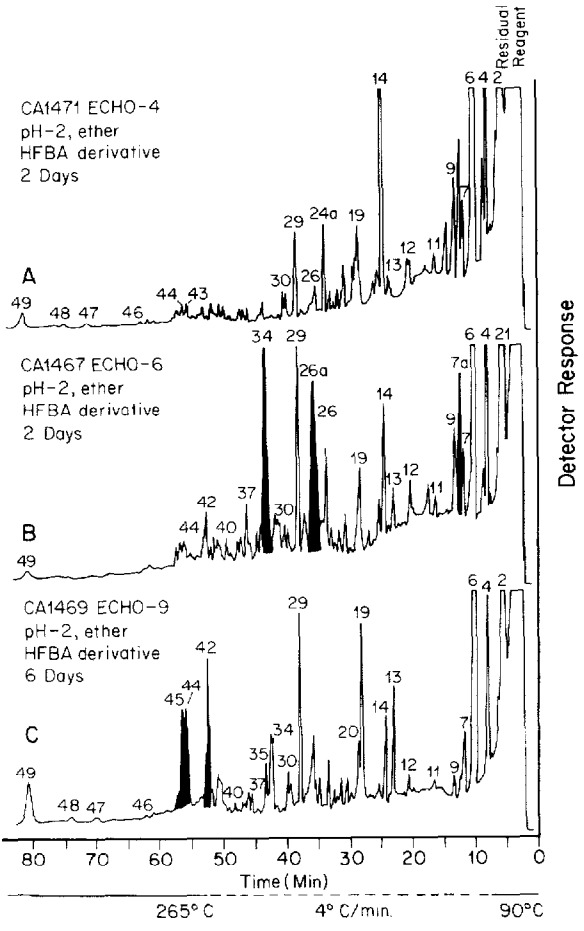

Fig. 13. GLC—FP-ECD chromatograms of HFBA—EtOH derivatized acidic diethyl ether extracts of sera taken from patients with the indicated ECHO viral infections. (From ref. 27 with permission.)

fever (RMSF), a rickettsial disease caused by *Rickettsia rickettsii*, (2) chickenpox caused by herpes virus, (3) measles, rubeola and rubella, enterovirus infections, and (4) *Neisseria meningitidis* infection. All of these diseases produce a rash and can be confusing diagnostically. GLC—FP-ECD profiles of hydroxy acids were most useful for rapidly differentiating these clinically similar diseases during the early stages (1—5 days) of infection, Figs. 12—14 [27]. A prospective study is necessary to determine the diagnostic efficacy of the procedure; however, the fact that early and acute phase sera are best for analysis is fortuitous because early diagnosis is essential in treating and managing RMSF patients.

Recent studies of sera from Egyptian patients with both *Schistosoma mansoni* and *S. hematobium* infections showed that body chemistry changes were detectable by GLC—FP-ECD using capillary columns and splitless injection [28]. Further studies are in progress to determine the reproducibility of these changes and to detect other changes in body chemistry that might

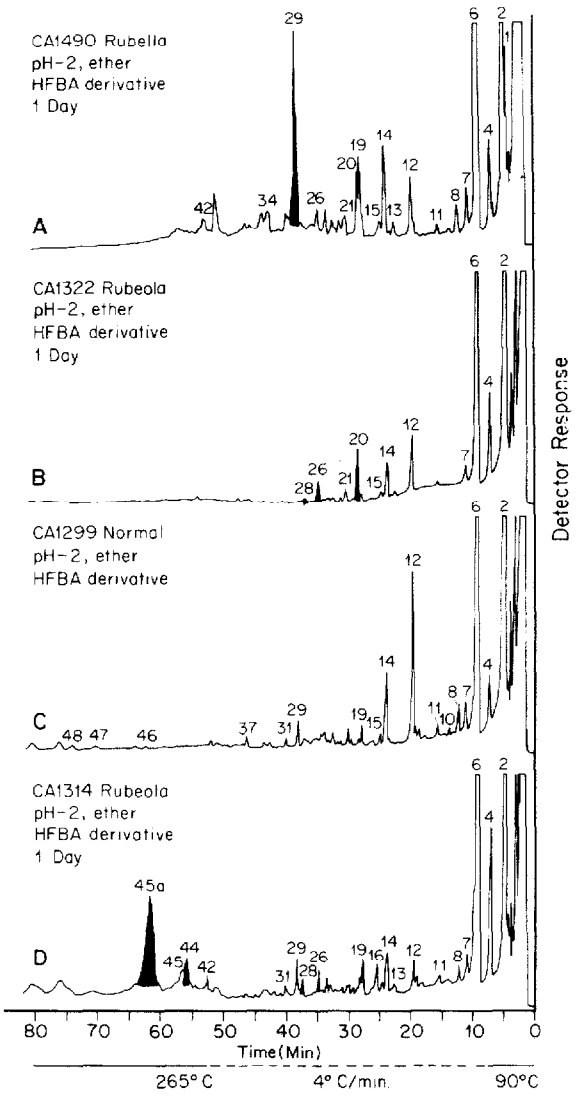

Fig. 14. GLC—FP-ECD chromatograms of HFBA—EtOH derivatized acidic diethyl ether extracts of sera taken from patients with the disease indicated in the figure. (From ref. 27 with permission.)

occur during schistosomiasis infection. These findings are important since this parasitic disease affects millions of the world population.

In addition to the analysis of CSF and sera from patients with the viral infections reported above have focused on the GLC—FP-ECD examination of monkey kidney cell culture supernatants infected with recent serum isolates of dengue virus and also stock isolates of the various dengue virus serotypes. The resulting GLC -FP-ECD fingerprints of the derivatives from the extracts of the infected cell culture medium were definitive enough to suggest GLC—FP-ECD could be used as an aid in the grouping of these viruses [29]. Further

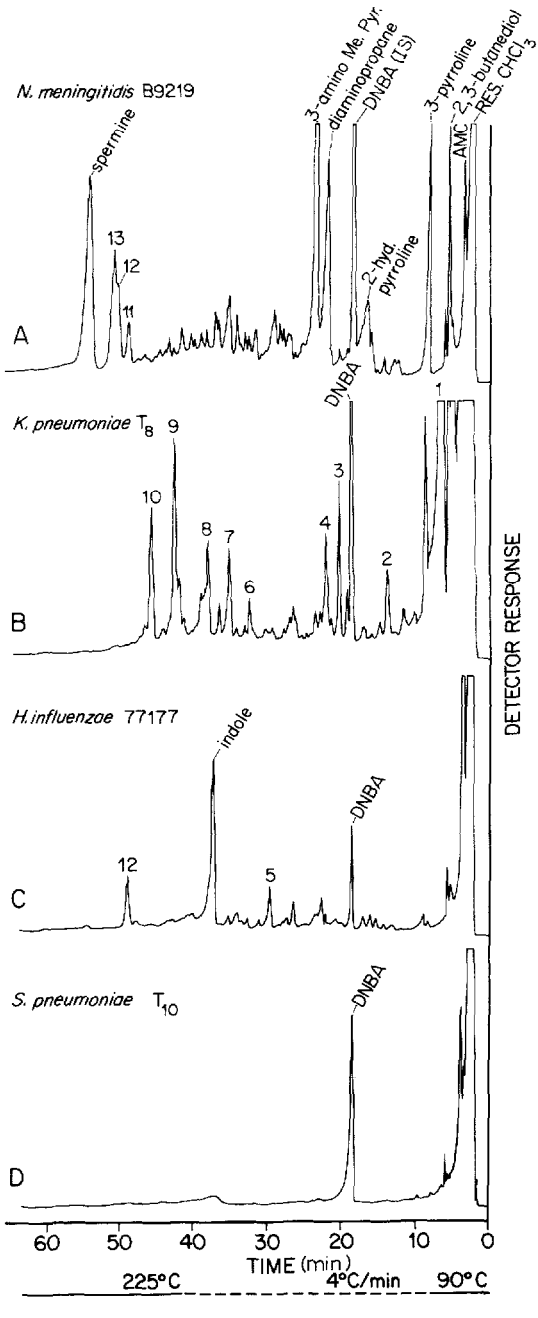

Fig. 15. GLC—FP-ECD chromatograms of HFBA derivatized basic chloroform extracts of spent bacterial culture medium, Neisseria defined medium. The type of organisms are indicated in the figure. Peaks: RES, residual; AMC, acetylmethylcarbinal; hyd, hydroxy; DNBA (IS), di-*n*-butylamine (internal standard); Me. Pyr., methylpyrrolidine. (From ref. 30 with permission.)

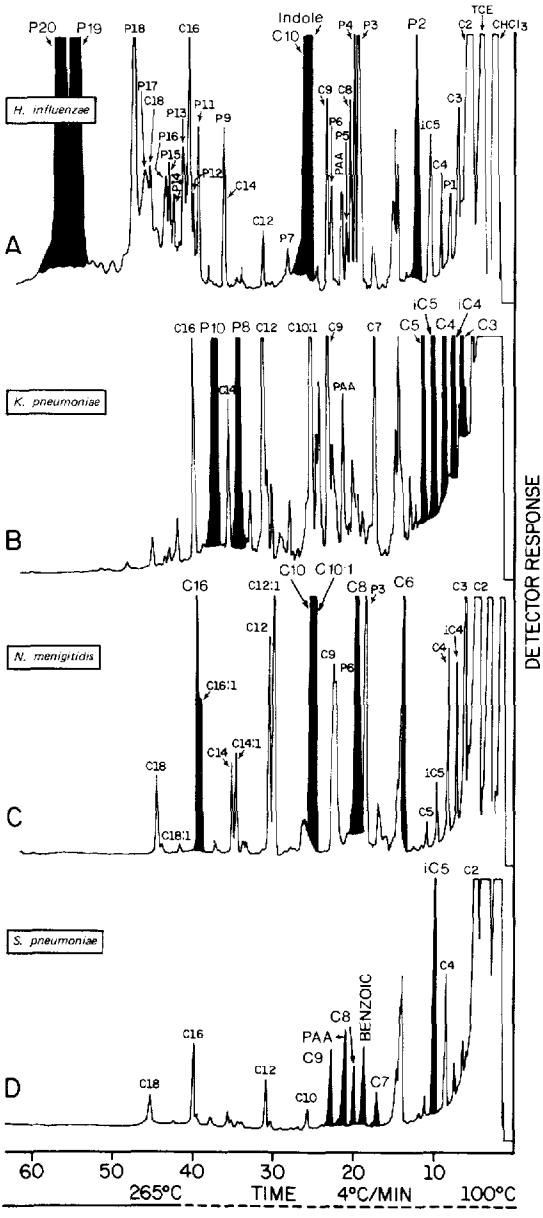

Fig. 16. GLC—FP-ECD chromatograms of TCE derivatized acidic chloroform extracts of spent Neisseria defined medium. The types of organisms are indicated in the figure. Most important peaks are blackened. C followed by a number indicates a straight chain carboxylic acid with the chain length indicated by the number. i indicates iso, and a colon between two numbers indicates unsaturation. (From ref. 15 with permission.)

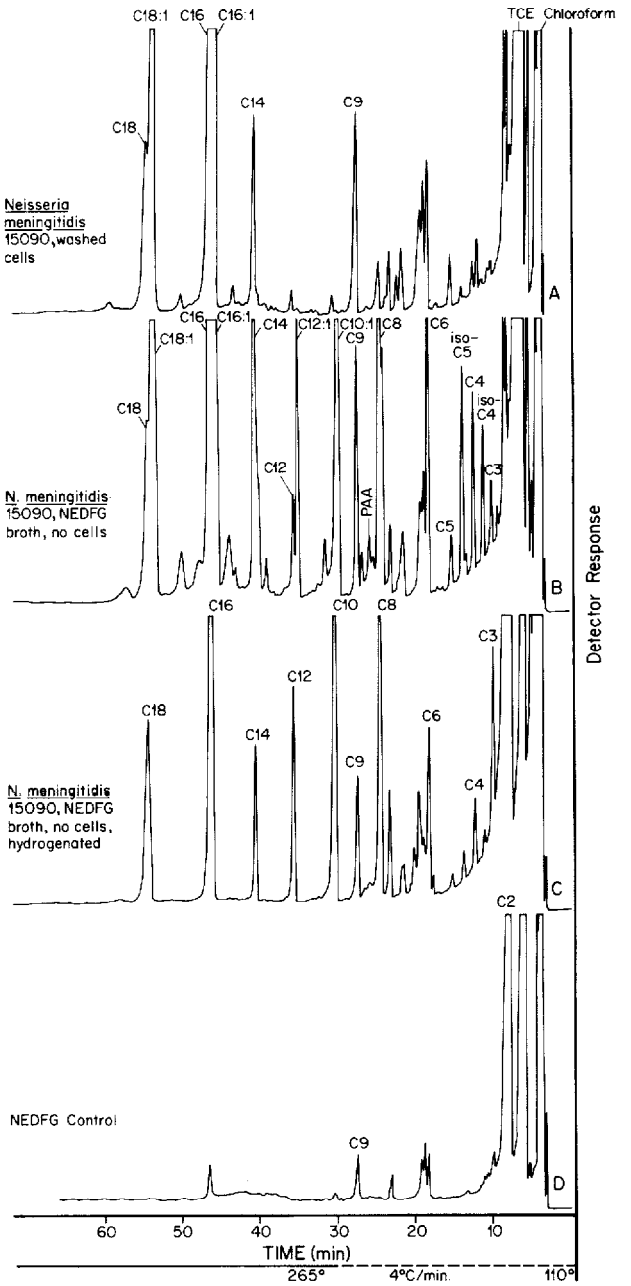

Fig. 17. GLC—FP-ECD chromatograms of TCE derivatized and TCE transesterified acidic chloroform extracts of methyl esters and free carboxylic acids prepared from (A) saponified washed cells, (B) spent culture medium with cells removed after hydrogenation; and (D) Neisseria defined medium control. The organism is indicated in the figure. (From ref. 10 with permission.) PAA = phenyl acetic acid.

studies on dengue virus infected cell culture supernatants and sera from patients with dengue fever are in progress to determine if GLC—FP-ECD has any diagnostic potential in dengue virus infections.

3.6. Metabolic and structural analysis of microorganisms

Although the GLC—FP-ECD techniques described here were developed primarily as a rapid diagnostic tool, the analytical scheme is not limited to body fluid, exudate, or excretion product analyses. It has proved to be a very useful tool for examining the metabolic activity of microorganisms in culture [10—15, 30], and for determining some of the carboxylic acid components of the microbial cell envelope [10], e.g. Figs. 15—17.

4. TECHNICAL NOTES

It is important to reiterate that the GLC—FP-ECD procedure was designed to make easy the preparation of electron-capturing derivatives of fatty acids, hydroxy acids, alcohols, and amines, to exploit the FP-ECD, and take advantage of the high temperature stability of liquid phases such as OV-101. Many established techniques, although effective for certain types of analyses, require several types of column packing materials and liquid phases, very different chromatographic conditions and detectors, or vastly different and frequently hard to prepare derivatives to be able to detect the classes of compounds that can be examined by the GLC—FP-ECD technique [1]. Even then, the selectivity and sensitivity of those procedures and instruments do not approach that of the GLC—FP-ECD procedure or the FPEC detector. We feel that the selectivity, and extreme sensitivity, to 10^{-12} *M* or greater, and potential for high resolution of chemical mixtures make the GLC—FP-ECD technique very versatile for examining chemical changes in living systems.

Secondly, the introduction of the purged splitless injector for capillary column trace analyses and the fused-silica capillary column, which can withstand stress without breakage in conjunction with the FP-ECD will allow even better separation and will reduce analysis times. It should be noted that peak retention times can vary with splitless injection if the amount of sample injected is not kept fairly constant. Also, column overloading is a factor that cannot be overlooked when capillary columns are used.

5. CONCLUSIONS

The data acquired to date indicate that the very sensitive and selective process of extraction, derivatization, and analysis by GLC—FP-ECD can detect changes in body fluids during the diseased state. These changes seem reproducible enough, to aid in the rapid identification of certain diseases. The rationale of the GLC—FP-ECD technique is different from past approaches to disease diagnosis where only one or several preselected components, such as toxins, antigens, antibodies, or a narrow spectrum of microbial or host derived products are sought and detected by a variety of frequently diverse biochemical

and immunologic procedures. Although the GLC—FP-ECD procedure relies on the detection of limited classes of organic molecules, it is used in an identical manner in all diagnostic applications, and provides data to construct a composite profile representing a particular etiology. It will probably be used initially as an adjunct for difficult diagnostic problems for which the research indicates application.

The change to the use of GLC—FP-ECD can and will be made more attractive, less expensive, and less labor intensive by the use of automated derivative preparation equipment, automatic injectors, and microprocessor based instrument controllers and data analyzers. Considerable developmental work remains to be done before many of the potential applications of the GLC—FP-ECD are standardized and the limits of the procedure are known.

Care must be taken in acquiring and handling the specimens before analysis. For example, samples should be taken before therapy is initiated; CSF should not be contaminated with blood as evidenced by red blood cells, and the sample should be frozen in a clean sterile container, preferably glass, if there is a delay in immediate analysis. One should be aware that more than one type of disease may be present and disease combinations, infectious or noninfectious, may affect or even change the GLC—FP-ECD profiles. In addition, the quality control of the GLC—FP-ECD instrumentation should be assessed at least once a month for resolution and sensitivity by analysis of standard mixtures.

If the scope of GLC—FP-ECD analyses can be limited to specific diseases, the data analysis and diagnostic usefulness are more meaningful and simple. For example, in the study of RMSF we limited the study to diseases associated with a rash which often makes early detection of RMSF difficult. We are confident that the contributions of other scientists in the utilization, testing, and improvement of the various components of the GLC—FP-ECD procedure will play a major role in its further development, acceptance, and usefulness in the diagnostic laboratory.

6. SUMMARY

The extremely sensitive and selective gas—liquid chromatography—frequency pulsed-modulated electron-capture detection (GLC—FP-ECD) procedure has been applied to the diagnosis of bacterial, fungal, viral, rickettsial, and parasitic diseases by the examination of various body fluids, effusions and exudates, and excretion products. Carboxylic acid and alcohol, hydroxy acid, and amine product profiles of microbial or host-response origin, have been used to establish specific etiologies, these profiles are reproducible, and can be used to aid in the diagnosis of infections. In addition, we have used the GLC—FP-ECD procedure to analyze microbial metabolic products in vitro and to provide data for identification and classification. We also explored computer time-sharing for data analysis, profile library comparison, and eventual profile matching for diagnosis.

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