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Frequency-Pulsed Electron Capture Gas-Liquid Chromatography and the Tryptophan Color Test for Rapid Diagnosis of Tuberculous and Other Forms of Lymphocytic Meningitis

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A total of 260 samples of cerebrospinal fluid received from Egypt, the United States, Canada, and South America were examined by frequency-pulsed electron capture gas-liquid chromatography (FPEC-GLC) for tuberculous and other forms of lymphocytic meningitis. Thirty-four of the specimens were culture positive for *M. tuberculosis,* and four cerebrospinal fluid specimens of herpes meningitis were established by immunological techniques. The compound, 3-(2′-ketohexyl)-indoline, was found in about 60% of the Egyptian tuberculous specimens and none of the culture-positive American specimens. The carboxylic and hydroxy acid FPEC-GLC profiles were used effectively in conjunction with other clinical data to make the diagnosis even in the absence of 3-(2′-ketohexyl)indoline. Herpes meningitis and mixed infections of *Myeobacterium tuberculosis*-herpes, *M. tuberculosis*-leptospira, and *M. tuberculosis-Haemophilus influenzae* produced profiles different from each other and from pure culture cases. The color test for tuberculous meningitis was evaluated, and free tryptophan was eliminated as the source of color reaction. Indications are that 3-(2′-ketohexyl)indoline, in most cases, is not responsible for the positive color reaction. Differences in the clinical and FPEC-GLC data obtained from samples from different geographical regions are discussed.

Since the reports (4, 6, 9) which described the potential use of frequency-pulsed electron capture gas-liquid chromatography (FPEC-GLC) in rapidly differentiating among tuberculous, viral, and cryptococcal meningitis, we have continued to test cerebrospinal fluid (CSF) specimens referred to our laboratory for FPEC-GLC analyses for tuberculous, viral, and cryptococcal meningitis. About 96% of the specimens received were to be tested for tuberculous meningitis.

Early reports stated that a simple color test for tryptophan was beneficial in diagnosing tuberculous meningitis (1, 7). The color test used for detecting tryptophan is nonspecific and produces color with many types of compounds having an indole group, as shown by tests in our laboratory. Since we reported the detection of 3-(2′-ketohexyl)indoline (KHI) in the CSF of patients with acute tuberculous meningitis, we questioned whether the positive color tests reported from the CSF of patients with tuberculous meningitis were due to a reaction with KHI, with both KHI and tryptophan, or with neither.

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The objectives of this report are to present the results of more recent studies of FPEC-GLC as an aid in the rapid diagnosis of lymphocytic meningitis and of studies to determine whether either tryptophan or KHI was the source of the indolic group producing the color reaction. The potential usefulness of performing the color test, after the CSF sample had been extracted with organic solvents for FPEC-GLC analysis, was also investigated.

MATERIALS AND METHODS

CSF specimens. All specimens were either excess CSF remaining after routine laboratory tests were performed or CSF specimens sent to the Center for Disease Control for FPEC-GLC analysis for viral, cryptococcal, or tubercular meningitis. Control specimens consisted of the excess CSF from myelograms or from spinal epidural anesthesia and were bacteriologically culture-negative, with normal cell count, glucose, and protein.

Culture-positive CSF specimens. These specimens were taken from patients with tuberculous meningitis and were obtained from the Meningitis Ward of Abbassia Fever Hospital, Arab Republic of Egypt, in conjunction with the U.S. Naval Medical Research Unit no. 3, Cairo, Egypt. Samples were sent to us for

FPEC-GLC analysis for tuberculous meningitis from various laboratories throughout the United States, Canada, and South America. *Mycobacterium tuberculosis* infection was confirmed by culture, and herpes infections were established by serological techniques. A 2-ml amount of CSF was used in most instances; however, only 1 ml was available in some cases. Most of the samples received by the Center for Disease Control for routine analysis were taken from patients already started on chemotherapy for *M. tuberculosis*.

Extraction and derivatization procedures. The 2-ml CSF sample was placed in a 50-ml, round bottomed centrifuge tube with a Teflon-lined screw cap and acidified to about pH 2 with 0.1 ml of 50% (vol/vol) H_2SO_4. Before extraction heptanoic acid (3.04 nmol in 0.1 ml of distilled water, which was made slightly basic with NaOH to increase solubility), alpha-hydroxyisovaleric acid (1.58 μmol in 0.1 ml of distilled water), and di-*n*-butylamine (1.19 μmol in 0.4 ml of distilled water made slightly acidic to obtain solubility) were added to each sample as internal standards. After mixing, the acidified sample was extracted by adding 20 ml of nanograde chloroform (Mallinckrodt) and shaking for 5 min on a Burrell Wrist-Action Shaker at a setting of 10. This extract was then derivatized with trichloroethanol (TCE) as described elsewhere (2). The residual aqueous phase was then made basic with 8 N NaOH and reextracted with 20 ml of chloroform, as described for the acidic extraction, to obtain the amines. Next, the residual basic aqueous phase was acidified to about pH 2 with H_2SO_4 and extracted with 20 ml of ethyl ether (Fisher reagent grade stabilized with butylated hydroxy toluene) to obtain hydroxy acids. Both the basic chloroform extract and the ethyl ether extract were concentrated to about 1 ml with clean dry air and dried with either $MgSO_4$ or Na_2SO_4 as described elsewhere (3). If $MgSO_4$ is used, it is essential that it be strictly anhydrous. Next, the anhydrous samples in organic solvent were evaporated to about 50 μl and derivatized with heptafluorobutyric anhydride-ethanol-pyridine reagent (HFBA) as described previously (3). Residual chloroform was removed by heating the sample in xylene as described for TCE (2).

The carboxylic acid derivatives and amine derivatives were dissolved in 0.1 ml of xylene-ethanol (1:1), and hydroxy acid derivatives were dissolved in 0.1 ml of ethyl acetate. For analysis by FPEC-GLC, a 10-μl syringe (Hamilton) was used. A 1-μl amount of solvent was drawn into the syringe to act as a flush, then the derivatized sample was gently drawn in and out of the syringe two to three times to avoid dilution error, and 2 μl of the sample was drawn into the syringe for FPEC-GLC analysis. After the sample was injected, the syringe was cleaned by flushing consecutively with 4% HCl, distilled water, methanol, and ethyl ether.

Apparatus. The derivatives were analyzed on a Perkin-Elmer model 3920 gas-liquid chromatograph equipped with dual frequency pulse-modulated 10-mCi ^{63}Ni electron capture detectors and glass columns (0.2 cm inner diameter by 7.6 m in length). The columns were packed with 3% OV-101 on Chromosorb W 80/100 mesh H.P. (acid washed, dimethylchlorosilane treated). Argon-methane (95:5) was used as the carrier gas. Flow through the column was 50 ml/min, and flush gas increased the flow from the packed column through the detector to 67 ml/min. For analysis of TCE derivatives, the instrument was programmed at 4°C/min from 100 to 265°C and then held isothermally. For amines and hydroxy acids, the column was held 8 min isothermally at 90°C, then programmed at 4°C/min to 265°C, and then held isothermally. Instrument temperatures were as follows: injector, 225°C; manifold, 250°C; and detector, 275°C. The standing current was 2 and the attentuation was 512. Two Perkin-Elmer recorders and two Hewlett-Packard strip-chart recorders were operated simultaneously with a signal input of 1 mV and a chart speed of 10 mm/min for the Perkin-Elmer and 8 inch (ca. 254 cm) per h for the Hewlett-Packard. The temperature programmer, recorder, and computer interface were activated simultaneously by pressing a foot pedal switch at the time of sample injection. The small strip-chart chromatogram was attached to the computer printout for reference.

The sensitivity of the electron capture detector was set near prescribed limits. We monitored the electron capture detector response at installation and every 3 months thereafter. We then modified the response by attentuation and standing current selection until a 2-μl injection of xylene containing 2.5 pmol of tetrachloroethylene gave between 15 and 30% full-scale deflection when analyzed isothermally at 100°C.

A Perkin-Elmer programmable processor (PEP-2) equipped with the Modular Software System (MS-16, revision B) collected data from the gas chromatograph, analyzed chromatograms according to a stored method, and prepared a report. An internal standard analysis was done on the data by using either heptanoic acid, di-*n*-butylamine, or 2-hydroxy-isovaleric acid as the internal standard (2).

Color test for indolic compounds. A color test was performed for indolic-type compounds as described (7). The test was modified later by applying it to samples that had been previously extracted with organic solvents under both acidic and basic conditions. Tryptophan was added to distilled water and carried through the procedure (7) as a control. Several other indolic compounds, including tryptamine, melatonin, and indole, were also tested. To test for a color reaction with KHI, we divided a 4-ml CSF sample which was culture positive for *M. tuberculosis* into two 2-ml aliquots. The color test for tryptophan was done on one aliquot (7). The other 2-ml aliquot was extracted once under acidic conditions with chloroform, twice under basic conditions with chloroform, and a fourth time under acidic conditions with ethyl ether. The two basic extracts were derivatized with HFBA and analyzed by FPEC-GLC to determine whether KHI was removed by solvent extraction; the residual CSF sample after extraction was tested for the presence of a color-producing agent.

FPEC-GLC test for tryptophan in CSF. Known quantities of tryptophan in 0.1 ml of water, 0.1 ml of normal CSF with added tryptophan, and 0.1 ml of a color test-positive *M. tuberculosis* CSF sample were derivatized for FPEC-GLC analysis by preparing heptafluorobutyrl propyl derivatives as described else-

where (8). Mass spectrometry was performed on the known standards as described (2) to verify derivatization of tryptophan. FPEC-GLC analysis was performed as described for amines.

RESULTS

We have now examined 36 CSF samples taken from patients that were culture positive for *M. tuberculosis* and 224 other CSF samples sent to our laboratory for FPEC-GLC analysis for tuberculous and for other types of lymphocytic meningitis. The results are summarized in Table 1. The culture-positive samples were from Egypt (31 cases), South America (1 case), and the United States (4 cases). The remainder of the specimens (217 cases) were from the United States and Canada. About 60% of the untreated culture-positive samples from Egypt and South America contained KHI (Fig. 1C). About 2% of the samples received from the U.S. and Canada were culture positive for *M. tuberculosis,* but none contained KHI. However, most of these patients had been treated for 2 to 3 weeks before the sample was taken for FPEC-GLC evaluation. One sample was taken from a United States patient who had received no therapy but whose case was culture positive. KHI was not detected in the sample. The absence of KHI in the specimens from the U.S. may have resulted from the patients having received treatment or from the specimens having been obtained at a less-acute stage of the disease.

Earlier studies of specimens from Egypt (4, 6) indicated that KHI would be present in almost all specimens from patients with acute tuberculous meningitis. Further studies have shown that this is not true. The problem of diagnosing tuberculous meningitis by the profiles obtained by FPEC-GLC in the absence of KHI was solved by combining clinical data with results obtained by analyzing the CSF samples by FPEC-GLC for carboxylic and hydroxy acids as shown (Fig. 1A and B). For purposes of identification, large quantitative and qualitative differences and, in some cases, peak ratios are used. Several of the same peaks are commonly found in CSF from patients with different types of meningitis. In the FPEC-GLC profile of tuberculous meningitis (Fig. 1A) the entire chromatogram, including the height of the internal standard, was observed, but the blackened peaks iC5, C8, PAA, C9, C18:1, C18, and C20:4 were the key peaks considered in the total pattern for diagnosing the tuberculous meningitis. The blackened peaks, when compared with an internal standard with about the intensity shown for C7 (Fig. 1), were found in essentially the same quantity as that shown from case to case. In very acute cases all peaks, excluding the internal standard, may be increased, and in convalescent cases the peaks will diminish. Thus far in cases of lymphocytic-type meningitis, such as that caused by viruses, cryptococci, and cancer, PAA and C20:4 have only been associated with tuberculous meningitis. The same is true for peaks 3 and 4 (Fig. 1B) and for KHI (Fig. 1C). This does not mean that

TABLE 1. *CSF samples analyzed for lymphocytic meningitis by FPEC-GLC*

No. of specimens	Source	Therapy	Culture results (*M. tuberculosis*)	Other factors or organisms involved	GLC[a] results for *M. tuberculosis*	Comments (abnormal profile or other type disease detected by GLC)
33	Egypt	No	+		+	Yes
1	Egypt	Yes	−[b]	5 wk of isoniazid and streptomycin	±	Yes
1	Egypt	Yes	+	*H. influenzae*	+	Yes
1	South America	Yes	+		+	Yes
1	United States	Yes	+	Insufficient sample	−	Yes
1	United States	No	+	Derivatization problem	±	Yes
1	United States	Yes	+	Herpesvirus	−	Yes, viral meningitis
1	United States	Yes	+	Leptospirosis	±	Yes
1	United States	Yes	−	Chronic mumps	−	Yes, viral meningitis
1	United States	Yes	−	Cytomegalovirus	−	Yes, viral meningitis
1	United States	Yes	−	Granulomatous meningitis	−	Yes, matched one type of cancer profile
217	United States Canada	Most	−	Unidentified	−	Yes, unidentified, viral meningitis, cryptococcal

[a] The number of false positives by GLC was none.

[b] This was the sixth sample in a series. The first sample, which was not available for GLC, was culture positive.

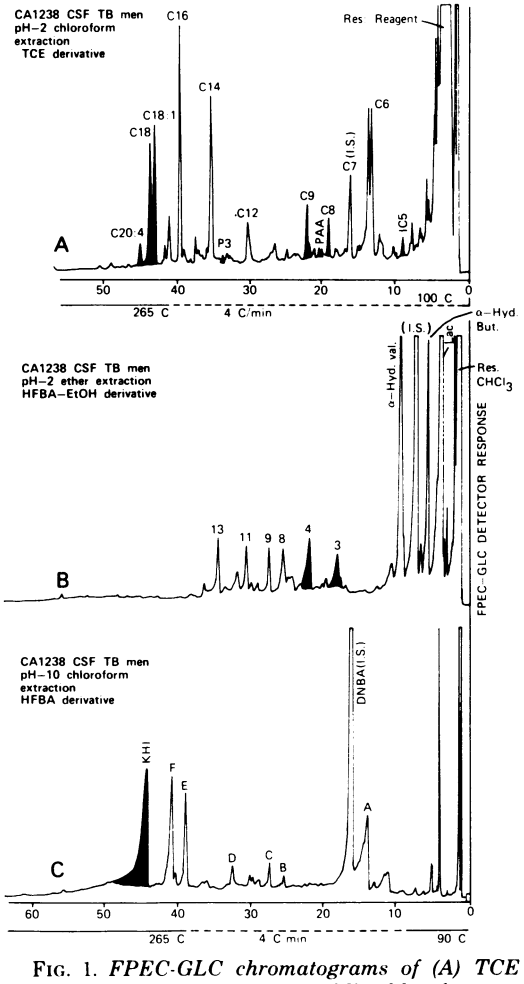

FIG. 1. *FPEC-GLC chromatograms of (A) TCE derivatives prepared from an acidic chloroform extract of CSF taken from a patient with culture-positive tuberculous meningitis; (B) Heptafluorobutyl ethyl and heptafluorobutyl esters (HFBA) prepared from the same CSF sample extracted with ethyl ether after extraction with chloroform under both acidic and basic conditions; (C) HFBA derivatives prepared from a basic chloroform extraction of the same CSF sample in A.*

these peaks will never be associated with other types of bacterial meningitis, but most of the major causative agents will not present a clinical picture of predominant lymphocytes, and cultures of the CSF specimen will generally show growth in 24 h. Lactic acid was easily quantitated by using the internal standard computer method. The sample was diluted 1:10 and rerun when precise quantitation was desired. In general, lactate in the CSF was elevated in samples from acute tuberculous meningitis cases above that found in control samples, but in cases involving patients on effective therapy, early semiacute cases, and cortisone-treated patients, the lactate concentration was normal.

Dual infections were encountered on three occasions, and a review of the literature showed that dual infection with *M. tuberculosis* is occasionally recognized (10). One case involving herpesvirus and *M. tuberculosis* gave an FPEC-GLC profile different from profiles obtained from cases involving herpes meningitis. Figure 2A through C show profiles of herpes meningitis, herpes-tuberculous meningitis, and a typical normal control. The peaks are blackened to in-

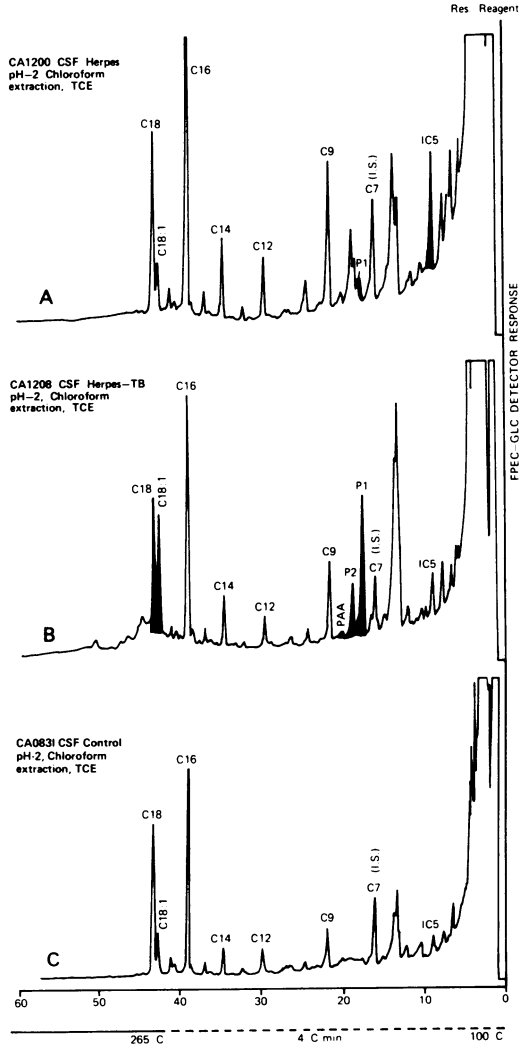

FIG. 2. *FPEC-GLC chromatograms of TCE derivatives prepared from acidified chloroform extracts of CSF taken from (A) a patient with herpes encephalitis, (B) a patient with meningitis caused by both M. tuberculosis-herpesvirus, and (C) a normal control.*

dicate those which are significant in differentiation. If the blackened peaks of Fig. 1A are compared with those of Fig. 2A and B, it is evident that there are qualitative differences (peaks labeled P1 and C20:4) and large quantitative (iC5, C14, and C18:1) differences between the single and dual infections. In another case involving leptospirosis and tuberculous meningitis, isocaproic acid (not previously seen in other types of lymphocytic meningitis) was half-scale. In a third case involving *Haemophilus influenzae* and *M. tuberculosis,* the FPEC-GLC profile was similar to profiles from cases of tuberculous meningitis, but the patient was on effective antibiotic therapy for *H. influenzae* 2 days before the sample was taken, and this probably changed the profile from what it would have been before treatment. One difference was that peak 3 (Fig. 1B) was increased above full scale. Only one case of each mixed infection was available, but it is evident that mixed infections can alter the FPEC-GLC profile commonly associated with tuberculous meningitis infections. Figure 3A and B show differences (blackened peaks) obtained in the hydroxy acid profiles of herpes meningitis and herpes-tuberculous meningitis. Considerably more lactic acid was obtained in the case with mixed infection. Peaks 1, 2, and 3 (Fig. 3A and B) also represent substantial differences in the two cases. Chromatogram A, with the exception of alpha-hydroxybutyric acid, peak 2, and the peak at 53 min is like a normal profile. The amine profile (Fig. 3C) from a patient with herpes meningitis is different from that of the patient with tuberculous meningitis (Fig. 1C); however, when KHI is absent, differences in the amine profiles are not so apparent, and the acid profiles must be used for differentiation.

The results of the tryptophan analysis are shown in Fig. 4A through C. The electron capture detector was extremely sensitive to the derivatized tryptophan (low picomoles), but components present in the spinal fluids limited its detection (without clean-up) to about 10 nmol. Even with the background components present (Fig. 4B and C), the FPEC-GLC analysis for tryptophan was about 50 times as sensitive as the color test, and had tryptophan been present in the tuberculous sample in quantities sufficient for a positive color test, it would have been a large peak. Therefore, the absence of tryptophan in Fig. 4C indicates that free tryptophan is not responsible for the positive color test results obtained from the CSF specimen. We also eliminated the possibility that KHI was responsible for the color reaction by demonstrating that KHI was completely removed by basic

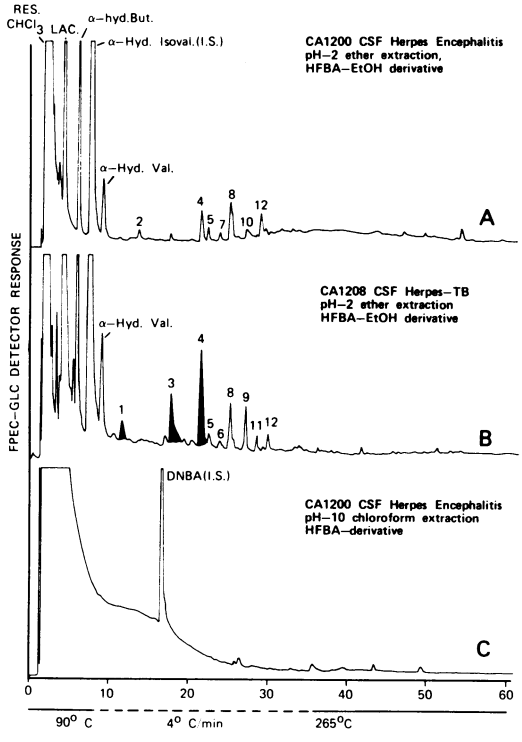

FIG. 3. *FPEC-GLC chromatograms of HFBA derivatives (A) prepared from an acidified ethyl ether extraction (after a chloroform acid and basic extraction) of CSF taken from a patient with herpes encephalitis; (B) same type of extraction as (A) except taken from a patient with both herpes and tuberculous meningitis; and (C) prepared from a basic chloroform extraction of CSF from a patient with herpes encephalitis.*

extraction with chloroform, but the residual material after extraction gave a strong positive color test.

Table 2 shows the clinical information, color test results, and KHI detection on a group of specimens obtained from Egypt, South America, and the United States. Table 2 shows that many of the culture-positive samples gave no color. Some samples were analyzed by FPEC-GLC before we began the color testing. None of the *M. tuberculosis* culture-positive United States specimens gave color. In addition, a positive color test was obtained from a herpes CSF sample, from a sample diagnosed at necropsy as being from a case of granulomatous meningitis, and from the CSF of a patient with syphilis. Other workers (7) reported positive color tests from patients with general paresis, neurological disorders, and cases of purulent meningitis. On the basis of both false negatives and false positives, we concluded that the color test described

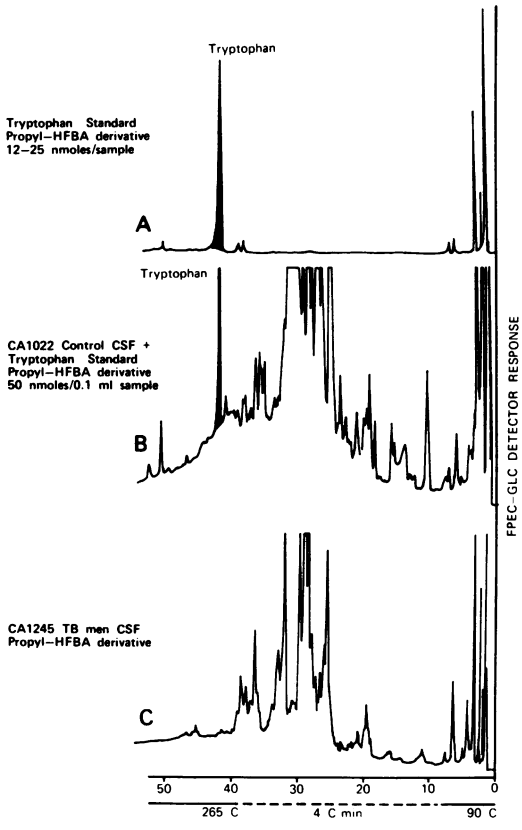

FIG. 4. *FPEC-GLC chromatograms of HFBA-propyl derivatives (A) tryptophan standard, (B) a control CSF sample with tryptophan added, and (C) a CSF specimen taken from a patient with proven tuberculous meningitis who had a positive color test.*

(7) was of little value for the early diagnosis of tuberculous meningitis.

DISCUSSION

Early diagnosis of tuberculous meningitis and lymphocytic meningitis in general presents a problem that is worthy of attention. Tuberculous meningitis in the technologically advanced countries has generally diminished, but changing trends in the epidemiology of tuberculosis in these countries have added to the diagnostic problem (10). Herpesvirus infections also present diagnostic problems (5), and with new drugs that appear to be effective in the treatment of herpes, early diagnosis becomes more important. The clinical findings typically associated with tuberculous meningitis (lymphocytosis, elevated protein, and depressed sugar levels) are often puzzling to the physician. A good example is the Egyptian cases, where elevated levels of polymorphonuclear leukocytes in the CSF samples of both children and adults predominated in a large percentage of those specimens culture positive for tuberculous meningitis.

As indicated in earlier reports (4, 6, 9), FPEC-GLC could be a valuable aid for rapid diagnosis of tuberculous and other forms of lymphocytic meningitis. Other laboratory findings, especially a clinical picture of lymphocytosis along with failure to culture other types of bacteria in a 24-h period, are a valuable aid to use in conjunction with the FPEC-GLC data. We have now examined enough specimens by FPEC-GLC to be optimistic about its use in the diagnosis of tuberculous meningitis, and since our reports on diagnostic specimens were made before cultural findings, the findings were equivalent to a coded study. Most of our problems in recognition have been traced to changes in the FPEC-GLC profiles caused by mixed infections and to treatment of the patient before the sample was taken for FPEC-GLC analysis. Both of these problems should be solved by developing a series of FPEC-GLC profiles representative of the most common infections found to be associated with tuberculous meningitis and by setting up specific protocols for taking the sample for FPEC-GLC analysis as follows: (i) take 2 ml free of blood before or shortly after treatment, (ii) either analyze by FPEC-GLC immediately or freeze until analysis, and (ii) do not add chemicals to the CSF or expose to acid not more than 30 min before extraction. We have not yet acquired enough data on herpes meningitis (four documented specimens) to have the same confidence that we have developed in diagnosing tuberculous meningitis, but the data that we have look promising.

Aiello (1) reported that a positive color test for tuberculous meningitis was due to the presence of free tryptophan, which was separated from the fibrin in the CSF, and that its release was due to autolytic enzymes accompanying the disease. The data presented here strongly suggest that this is not the case. Our results also indicate that the color test is unreliable in diagnosing tuberculous meningitis and that KHI is not responsible for the color obtained in extracted samples. We have not shown that KHI would not produce a color reaction in nonextracted CSF samples containing KHI, but even if KHI gave color, it would not affect our conclusions regarding free tryptophan, since tryptophan is not extractable under the stated conditions. If KHI did react to give a positive color test, only two additional samples would have

TABLE 2. *Summary of clinical data, KHI, and color test results from patients with tubercular meningitis*

Patient no.	Days since onset	Total cells	%P/%L[a]	Glucose	Protein	AFB[b] Culture	Age (yr)	Color test	KHI	Rx at time sample was taken[c]
CA1098	90	ND[d]	ND	16	1,230	+	35	ND	+	NT
CA1099	21	2,080	85/15	4	500	+	1	ND	+	3 days pre-adm on tetracycline
CA1100	15	240	90/10	20	304	+	2.5	ND	−	NT
CA1101	10	1,360	80/20	58	145	+	20	ND	+	3 days pre-adm penicillin and chloramphenicol
CA1236	60	50	0/100	38	45	− (6)	43	−	−	15 days pre-adm tetracycline and chloramphenicol, 5 weeks INH and streptomycin
CA1237	25	370	20/80	34	615	+	30	+	−	Rx started same day sample taken
CA1238	40	30	40/60	30	50	+	12	−	+	Rx started same day sample taken
CA1240	30	330	10/90	30	158	+	9.5	+	−	Rx started same day sample taken
CA1244	7	3,500	50/50	13	365	+ (2)	25	−	+	Rx started same day sample taken
CA1245	30	2,000	98/2	14	184	+	55	+	+	30 days pre-adm tetracycline and chloramphenicol
CA1258	90	370	90/10	23	390	+	10	+	−	30 days pre-adm tetracycline, penicillin, and chloramphenicol off and on
CA1251	Chart missing									
CA1255[e]	7	550	98/2	12	328	+	5	+	+	2 days pre-adm tetracycline, penicillin, and chloramphenicol, *M. tuberculosis* and *H. influenzae*
CA1257	30	2,000	98/2	14	194	+	5.5	?	−	
CA1348	15	130	20/80	11	136	+	2.5	−	−	Pre-adm tetracycline, sulfadiazine
CA1349	3	550	60/40	11	157	+	17	+	+	Rx started same day sample taken
CA0975	ND	129	0/100	75	300	+	ND	ND	+	
CA1207	ND	101	3/97	ND	ND	+	27	−	−	
CA1208[e]	ND	200	1/99	75	136	+	43	−	−	Herpes-*M. tuberculosis* no Rx
CA1209[e]	ND	ND	ND	ND	ND	+ (2)	43	−	−	Herpes-*M. tuberculosis* 2 days INH, EMB, Rif
CA1210[e]	ND	ND	ND	ND	ND	+ (3)	43	−	−	Herpes-*M. tuberculosis* INH, EMB, Rif
CA1401	42	190	10/90	50	150	+	62	−	−	INH, Rif. started same day
CA1407[e]	64	393	35/65	12	158	+	9	−	−	*M. tuberculosis*-Leptospirosis, Rx 14 days streptomycin EMB, ethionamide

[a] %P/%L, %Polymorphonuclear leukocytes/% lymphocytes.
[b] AFB, Acid-fast bacilli. Number in parentheses indicates the sampling sequence number.
[c] Rx, Therapy; NT, no treatment; INH, isoniazid; EMB, ethambutol; Rif, rifampin; pre-adm, pre-administration.
[d] ND, No data.
[e] Mixed infections.

been positive, and the fact remains that color was obtained in the sample that was devoid of KHI.

Some interesting facts, possibly related to geographic differences in tuberculosis, can be seen from the data (Table 2): (i) the age range (mostly adults in the United States), (ii) the ratio of polymorphonuclear leukocytes to lymphocytes (a much higher percentage of polymorphonuclear leukocytes in the Egyptian samples), and (iii) the fact that there was KHI in the CSF in most Egyptian samples. The shift in age to older populations in developed countries was also observed (10), and it is important that physicians be alert to the possibility of *M. tuberculosis* meningitis involving older age groups. There are no obvious reasons for the last two differences; however, when a meningitis patient has a predominance of polymorphonuclear leukocytes, suspicion of *M. tuberculosis* involvement is diminished, and the knowledge that KHI can be missing in positive cases of tuberculous meningitis is important when FPEC-GLC data are used to diagnose tuberculous meningitis. As stated previously (6), KHI disappears with effective therapy, which indicates an association with the disease. Not many United States specimens were culture positive (Table 1), and KHI may be found when more specimens are analyzed. We found KHI in two samples from South America (the only two tuberculous meningitis specimens that we received). The patient's age did not seem to have a bearing on the production of KHI (Table 2). The FPEC-GLC acid profiles (Fig. 1A and B) obtained from the American and Egyptian samples were similar and constitute a basis for identification of tuberculous meningitis by FPEC-GLC even in the absence of KHI.

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