scopic-agglutination tests, took into account the final 2-fold dilution of serum by antigen.

Results. Results obtained in quantitative latex-agglutination (L-A) tests of human sera are compared in Table II with those of agglutination-lysis (A-L) and microscopic-agglutination (M-A) tests conducted in other laboratories; 30 individuals are represented. L-A titers with few exceptions were higher than those of standard A-L or M-A tests, the greater sensitivity of the test in some instances making the difference between reactivity and non-reactivity, and revealing antibody before it was detectable by standard procedures. A qualitative L-A screen test in which the antigen component was pooled *L. icterohemorrhagiae, L. pomona,* and *L. canicola* was evaluated with the same sera. Although the component leptospirae were present at one-third the optimal concentration for quantitative tests, reactions(R) were observed in all 8 cases of homologous infection. Reactivity was also observed in 16 (76%) of 21 patients whose infections were caused by other leptospiral serotypes such as *australis* A or B, "Kremastos"(4), *hyos,* "Robinson"(4), and *bataviae.* The screen test failed to detect antibody in 5 of 16 cases of *australis* A or B infection. The initial negative result, however, was reversed in 3 instances by replacing the *L. canicola* component of the antigen with homologous leptospirae. There was insufficient serum to retest the others. The specificity of the latex agglutination reaction was examined in tests of 162 healthy and 14 syphilitic individuals. Three of the former reacted, but only at the lowest final dilution (1:100) tested; the remainder were seronegative. The latex-agglutination tests thus compared favorably in sensitivity and specificity with standard agglutination-lysis and microscopic-agglutination tests. They had additional advantages in simplicity and rapidity of performance, and in the use of a stable, noninfectious antigen.

Summary. The reaction of leptospiral antisera with an antigen composed of polystyrene latex particles and formalin-killed leptospirae provides the basis for a simple, macroscopic tube agglutination test for leptospirosis.

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Received July 17, 1958. P.S.E.B.M., 1958, v99.

Gas-Liquid Chromatography of Highly Unsaturated Fatty Acid Methyl Esters.* (24307)

W. STOFFEL, W. INSULL, JR., AND E. H. AHRENS, JR.
The Rockefeller Institute, N. Y. City

Biochemical studies of fatty acid metabolism have been given great impetus by the development of gas-liquid chromatography (GLC) by James and Martin(1). This method permits microgram mixtures of methyl esters of fatty acids to be resolved when these esters are volatilized and distributed between a moving carrier gas (nitrogen or argon) and a stationary liquid phase. At the high temperatures (up to 200°C or more) required for volatilization, degradation of saturated fatty acid esters has not been encountered, but the possibility that the double bond structure of highly *un*saturated esters might be altered under these conditions has not been ruled out. In view of growing interest in the biologic role of unsaturated fatty acids as integral parts of certain enzymes(2) and in human nutrition(3), it seemed imperative to investigate the stability of these acids during

* Aided in part by grants from the U. S. Public Health Service and Nutrition Fn.

TABLE I. Chemical Structure and GLC Characteristics on 2 Stationary Phases of Unsaturated Fatty Acids of Natural Origin.*

Fatty acid methyl esters		Position of double bonds†	Apparent retention vol relative to stearic acid methyl ester = 1.00 at 197°C: Apiezon M	Apparent retention vol relative to stearic acid methyl ester = 1.00 at 197°C: Reoplex 400
C_{16}	saturated		.41	.53
	monoenoic	8	.37	.60
		9	.37	.60
	dienoic	6,9	.36	.68
		9,12	.33	.74
	trienoic	6,9,12	.32	.84
	tetraenoic	6,9,12,15	.31	1.04
C_{18}	saturated		1.00	1.00
	monoenoic	9	.87	1.12
	dienoic	9,12	.76	1.35
		6,9	.76	1.35
	trienoic	9,12,15	.77	1.58
	tetraenoic	6,9,12,15	.72	1.84
C_{20}	saturated		2.44	1.90
	dienoic	‡	1.88	2.05
	trienoic	‡	1.64	2.29
	tetraenoic	8,11,14,17	1.46	2.83
	pentaenoic	5,8,11,14,17	1.46	3.60
C_{22}	saturated		5.95	3.60
	pentaenoic	7,10,13,16,19	not done	6.60
	hexaenoic	4,7,10,13,16,19	3.00	7.34

* All acids except the C_{22} group were isolated from menhaden body oil and characterized as previously described(4). C_{22} acids were generously supplied by Prof. E. Klenk, Cologne, Germany, having been isolated from herring oil.

† Numbered from carboxyl carbon.

‡ Double bond structure not yet clarified.

analysis by GLC. The present report demonstrates that highly unsaturated long chain aliphatic fatty acid methyl esters are exceedingly stable when chromatographed under James and Martin's conditions. In addition, GLC analytical data needed for identification of 17 of these esters in mixtures of biologic origin are presented.

Materials. The unsaturated long chain acids listed in Table I were isolated and their structures identified by procedures described elsewhere(4). For the most part the acids were derived from a specially prepared sample of menhaden body oil† used by us in human nutritional studies(5).

† Generously supplied by Dr. T. M. Miller, Marine Chemurgics, Morehead City, N. C.

Methods. GLC was carried out essentially as described by James and Martin(1) using the ionization chamber detector of Lovelock and James(6) vapor-jacketed at 197°. The mobile phase, argon, was applied at a pressure of 67 cm Hg. Each 4-foot column was packed with 5 g of acid-washed, alkali-treated Celite 545 (mesh 140-200). Two stationary phases were studied, the non-polar hydrocarbon vacuum grease Apiezon M(1) and the polar polyester Reoplex 400(7). The Apiezon column contained 0.8 g Apiezon; with a flow rate of 52 ml A/min this column had an efficiency of 3500 plates at methyl stearate (retention time of 60 minutes). The Reoplex column contained 2 g of Reoplex 400 and had a column efficiency of 2060 plates at methyl stearate (retention time of 21 minutes, flow rate 52 ml A/min). Both columns were conditioned at 197° by flushing with nitrogen applied at 67 cm Hg pressure for at least 3 days before analytical use. All relative retention volumes were calculated from apparent retention times (= time from air peak to center of symmetrical elution curve). Esters were recovered after chromatography by leading the effluent gas from the detector into a glass tube loosely packed with defatted cotton moistened with absolute methanol. The decomposition of organic vapor passing through this detector has been estimated to be about one in 10^9 molecules(8).

Stability studies. A. Criteria. Our stability studies have been limited to date to GLC on the non-polar stationary phase, Apiezon M. For proof of complete stability we demanded: quantitative recovery of acids applied to the column, no change in retention volume of methyl esters repeatedly re-chromatographed, no production of *trans* double bonds as shown by infrared spectroscopy(9), no formation of conjugated double bonds and no alteration in specific extinctions in the ultraviolet range after isomerization in alkali(10), and, finally, no alteration in double bond positions as shown by identification of fragments produced by ozonolytic degradation(4).

B. Results. The most highly unsaturated ester in each chain length group (Table I) was studied. Quantitative recovery of 2-10

mg of each of the 4 esters was demonstrated by microgravimetric analysis of the products applied to the column and recovered from the effluent trap. Test runs varied from 1-3 hours. Over these periods there was no detectable "bleeding" of the Apiezon M stationary phase, as shown by absence of weighable residue in traps applied to the uncharged column for 3 hours. These gravimetric analyses were considered accurate to 1% or better (with samples of 2 mg and balance sensitivity of ± 0.01 mg(11)).

Relative retention volumes of the 4 esters were determined individually by applying small charges (1/16 μl) of each together with the respective saturated homolog and methyl stearate. The unsaturated esters were trapped and re-run. There was no change in relative retention volume of each ester after 2 re-runs.

Infrared and ultraviolet spectra of each of the 4 esters, before and after GLC on Apiezon M, were almost identical. Infrared patterns showed no evidence of *cis*⟶*trans* isomerization. Ultraviolet spectra of esters in concentrated solutions (1 mg/2 ml methanol) showed only slight increases in conjugated double bonds: the C_{16}-tetraenoic fraction showed an increase of di-, tri- and tetraene conjugation of less than 1%; the C_{20}-pentaenoic acid conjugation rose from 1 to 2% in the diene region only; the C_{22}-hexaenoic acid showed an increase in diene conjugation from 6 (before) to 10% (after GLC) without conjugation in the higher polyene regions. After isomerization in alkali(10), the 4 chromatographed esters showed ultraviolet spectra which were qualitatively and quantitatively identical to those of the original esters.

Two polyenoic acids were degraded by micro-oxidative ozonolysis(4) before and after GLC on Apiezon M. The dicarboxylic acids produced were methylated(12) and chromatographed on Apiezon M at 78°. In the case of both samples of C_{18}-tetraenoic acid, adipic and malonic acid di-methyl esters were identified as sole dicarboxylic acids, while with the C_{20}-pentaenoic acid only glutaric and malonic acid di-methyl esters were found before and after GLC. These studies showed that the methylene-interrupted double bond structure of the 2 acids (divinyl methane rhythm) was not altered by GLC, and that the positions of the double bonds along the chains had not shifted.

Retention volumes relative to methyl stearate (= 1.00) are presented in Table I for all polyenes studied. On Apiezon M the unsaturated esters precede their saturated homologs through the column, but the separations of the individual polyenes from each other are not always clean. On Reoplex 400, on the other hand, the polyenes follow their saturated homologs, as shown by Orr and Callen(7,13), and separations within each chain length group are excellent.

Fig. 1 graphs the logarithms of retention volumes relative to methyl stearate (= 0) when Reoplex 400 is the stationary phase. The heavy diagonal line shows linear relationship with molecular weight in the case of the saturated homologs. Logarithms of relative retention volumes of unsaturated acid ester groups are shown in dashed lines. Five points deserve comment: 1) relationship between these logarithmic values and number of double bonds in each chain length group approaches linearity; 2) even-numbered unsaturated acids overlie the next higher odd-numbered and branched-chain acids; 3) there are overlaps between C_{16}-tetraene and normal C_{18}-saturate, between C_{18}-tetraene and normal C_{20}-saturate, between C_{20}-pentaene and normal C_{22}-saturate. 4) C_{20}-monoene would not be expected to separate from C_{20}-saturate; 5) the only isomers separated under these conditions were the two C_{16}-dienes.

The fact that both non-polar and polar stationary phases fail to accomplish complete separations of the acids studied here warns against reliance upon any single stationary phase for complete definition of the fatty acid composition of biological mixtures. However, the different chromatographic characteristics of the two phases are complementary and can be used to advantage. In this laboratory complex mixtures have been advantageously resolved by preparative runs (10 μl) on Apiezon M with trapping of effluent fractions between successive even-numbered esters. Analytical runs of these fractions (¼-1 μl) on Reoplex

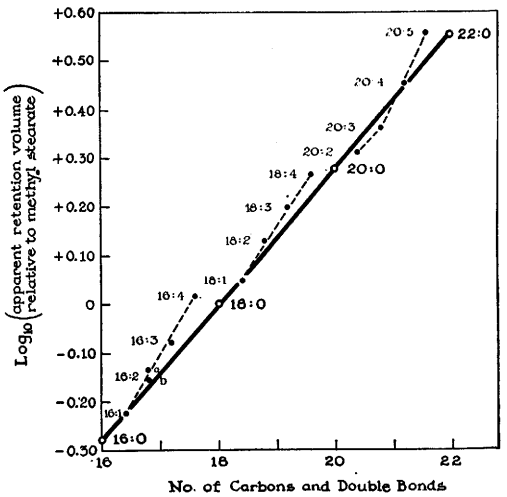

FIG. 1. Logarithms of retention volumes of long chain fatty acid methyl esters relative to methyl stearate (= 0); GLC at 197° on Reoplex 400 as stationary phase. Saturated normal homologs are shown by diagonal solid line. Groups of unsaturated acids are plotted in dashed lines. No. after colon indicates double bonds in acids of stated chain length (*i.e.* 16:4 = C_{16}-tetraenoic acid methyl ester). 16:2a = hexadeca-9,12-dienoic, 16:2b = hexadeca-6,9-dienoic acid.

400 serve to separate the mixtures in each. For example, in analysis of menhaden body oil fatty acids the fraction trapped between the trailing ends of the C_{14}- and C_{16}-saturate curves from an Apiezon M column can be collected and applied to a Reoplex 400 column. This mixture resolves cleanly into individual C_{15} and C_{16} acids, and in addition further separations in each group are based on numbers of double bonds. The success of this approach, an application of which has been described elsewhere by us(5), depends upon rigorous proof of stability of polyunsaturated fatty acid esters during GLC on Apiezon M.

Summary. Proof is presented that methyl esters of highly unsaturated long chain fatty acids are not significantly altered in chemical structure during gas-liquid chromatography with the stationary phase Apiezon M at 197°. Relative retention volumes of C_{16}, C_{18}, C_{20} and C_{22} polyenoic acids are listed for 2 stationary phases, non-polar Apiezon M and polar Reoplex 400. A system for rapid total analysis of complex fatty acid mixtures on a submilligram scale is described.

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Received July 18, 1958. P.S.E.B.M., 1958, v99.

Inactivation of Amphotericin B, Chlorquinaldol, Gentian Violet and Nystatin by Bile Salts. (24308)

S. STANLEY SCHNEIERSON, DANIEL AMSTERDAM AND M. L. LITTMAN
Department of Microbiology, Mount Sinai Hospital, N. Y. City

Bile salts exert a variety of effects upon the action of different antibiotics(1). They enhance the antimicrobial activity of neomycin and penicillin against certain bacterial species, inactivate polymyxn, ristocetin and vancomycin and produce no effect whatsoever on other antibiotics. The present report is concerned with the effect of these salts upon the