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THE PHOSPHOLIPIDE AND PHOSPHOLIPIDE-FATTY-ACID COMPOSITION OF HUMAN SERUM LIPOPROTEIN FACTORS

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UCRL-8930
Biology and Medicine
TID-4500 (15th Ed.)

UNIVERSITY OF CALIFORNIA
Lawrence Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

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Printed for the U. S. Atomic Energy Commission

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Donner Laboratory of Biophysics and Medical Physics
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ABSTRACT

The phospholipide compositions of human serum lipoproteins have been determined on ultracentrifugally separated serum lipoprotein classes by silicic acid column chromatography and infrared spectrophotometry. The lipoproteins were separated into three classes; $s_f^o 20-400$, $s_f^o 0-20$, and HDL_{2-3}. The phospholipides were separated into a fraction containing phosphatidyl-ethanolamine and phosphatidyl serine, a lecithin fraction, and a sphingomyelin fraction. All phospholipides are present in each lipoprotein class, but their distributions in each class are sufficiently different to be characteristic. Analyses were run on sera from five individuals and only small deviations from the average values were observed.

In a few cases the fatty acids of the separated phospholipides were analyzed by gas chromatography.

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INTRODUCTION

Previous studies on human serum phospholipides have dealt with concentration in whole serum.[1,2,3] When the concept of lipoproteins[4] and lipoprotein classes[5] was introduced it became of interest to study the phospholipides of serum as structural units of these macromolecules. Although there is now agreement on the relative proportions of the phospholipide species in whole serum, it has not previously been known how the individual phospholipides are distributed in the lipoprotein classes. While this work was in progress, Phillips reported such a study.[6] His results are discussed in some detail in the appropriate section.

It has been generally assumed that the major phospholipides of serum are phosphatidyl ethanolamine, lecithin, and sphingomyelin.[7] However, Phillips reported the isolation and determination of 7% lysolecithin in human serum phospholipides.[8] The minor components are plasmalogens, inositol phosphatides, and phosphatidyl serine. The total of these minor compounds is not believed to exceed 1 or 2% of the total phospholipides.[8]

In the course of this work a gas chromatography unit became available in this laboratory and a corollary study of the fatty acid complement of the phospholipides was started. Some preliminary results are presented here.

Studies on the fatty acids of phospholipides have been primarily restricted to samples from tissues. Luddy et al. reported values for whole serum phospholipides as a single class, using alkali isomerization techniques, and found 40% saturated fatty acids and 60% unsaturated.[9] The unsaturated acids were primarily oleic (35%), linoleic (15%), and arachidonic (18%), with traces of other unsaturated acid on the order of 1%, which is the limit of error of their method. The saturated group was not characterized further.

* This work was supported in part by the United States Atomic Energy Commission and is taken from the thesis of Gary J. Nelson, submitted to the Graduate Division of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy (1960).

James and Lovelock also reported fatty acid analyses on total serum phospholipide fractions by gas chromatography.[10] They found a range of values for sera from ten subjects. The average values were palmitic, 30%; palmitoleic, 2%; stearic, 20%; oleic, 20%; linoleic, 20%; and arachidonic, 8%. Mukherjee et al., using alkali isomerization, reported the total phospholipide fraction fatty acid composition of rat serum.[11] They found rat serum phospholipides to be highly saturated, approximately 75%. In spite of these few instances, our knowledge of fatty acids of serum phospholipides is very incomplete. No data are available on the fatty acid composition of the individual phospholipides of human serum, or on the fatty acid composition of the phospholipides of the lipoprotein classes.

EXPERIMENTAL PROCEDURE

Blood was drawn in 250-ml quantities from donors on whom previous routine serum lipoprotein analyses had been performed as part of routine physical examinations. All donors were employees at the Livermore section of the Lawrence Radiation Laboratory. Subjects with high serum lipide values were selected for study so that adequate quantities of the respective serum phospholipides would be obtained. Although it was realized that this would bias the data, it was considered necessary because of limitation on the amount of blood obtainable.

The blood was drawn from the subjects not less than 3 hours after breakfast. This is not considered a fasting sample, but rather postabsorptive. No visible lipemia was present in any case, and the s_f^{20}-400 lipoproteins were not elevated above these subjects' normal levels.

Drawing was at approximately 10:00 a.m. and the blood was allowed to clot by standing for 6 hours at room temperature. After this time the clot was removed and the remaining cells were removed by centrifugation at 2000 rpm for 20 minutes in an International Model L centrifuge with a number 250 rotor. The serum, usually slightly hemolyzed, was then transferred to 6-ml Lustron preparative ultracentrifuge tubes. The tubes were capped and placed in a Spinco series 12-G rotor, designed for rotation at 40,000 rpm.

ULTRACENTRIFUGATION

The method of separation of the serum lipoprotein was developed in this laboratory by Dr. Frank Lindgren and Dr. Alex Nichols and is to be published in further detail elsewhere. It is a system utilizing NaBr solutions, containing a constant content of NaCl, (0.195 molal) but presents no radical departure from the standard lipoprotein separation by flotation in the preparative ultracentrifuge.[12] All centrifugations were carried out in a Spinco Model L ultracentrifuge at 20°C.

This procedure separated the lipoproteins of serum into three classes -- Class I, consisting of molecules with standardized flotation rates greater than $s_f^\circ 20$;[13] Class II, consisting of those molecules in the range $s_f^\circ 0$-20; and Class III, consisting of the high-density lipoproteins, HDL_{2-3} -- as determined by analytical ultracentrifugation.

The unaltered serum was centrifuged for 24 hours at 40,000 rpm. Unaltered serum has a density of 1.006 g/ml. At this density the result of centrifugation of the unaltered serum for 24 hours at 40,000 rpm is that the top 1 ml of the prep tube contains lipoproteins floating with s_f° rates greater than 20. All material denser than 1.006 g/ml sediments to the lower 3 ml of the tube. The second ml is void of all macromolecules. The top ml was collected in a vial and placed under refrigeration at 4°C to prevent denaturation of the lipoproteins until they could be extracted. The second ml was also collected in the same manner. A special pipetting technique, developed in this laboratory, was used for the removal of fractions from the preparative tubes.[14] The remaining 4 ml of serum in the preparative tube was brought to a density of 1.070 g/ml by the addition of 2 ml of a previously prepared solution of NaBr. The tubes were then respun for 24 hours at 40,000 rpm. Under these conditions the top 1 ml of the preparation at a density of 1.062 g/ml contains all lipoproteins with flotation rates greater than $s_f^\circ 2$. The denser materials sediment to the bottom 3 ml of the preparative tube, while the second ml from the top is free of macromolecules. The top ml was collected in a vial and stored in the cold, as was the second ml also. To the remaining 4 ml was added 2 ml of a previously prepared NaBr solution, which brought the density of the solution to 1.218 g/ml. The tubes were then respun for 36 hours at 40,000 rpm.

Following the final centrifugation the top ml of the preparatory tube at a density of 1.203 g/ml contains the high-density lipoproteins (HDL_{1-2-3}); the second ml is clear of macromolecules, and all the lower portion contains the serum residue, consisting principally of serum albumin and globulins. After this final recentrifugation the top 1 ml and the next 2 ml were separately collected and stored as described before. The serum residue was discarded. The high-density lipoproteins obtained in this manner are the HDL_{1-2-3}. However, HDL_2 is considered to be closely related chemically to the s_f^0-20 lipoproteins.[15] Thus, it was considered desirable to remove the HDL_1 from the HDL_{2-3} molecules. To do this, the top ml of the HDL isolation step was diluted to a density of 1.075 g/ml and respun at 40,000 rpm for 48 hours. This procedure floated the HDL_1 to the top ml of the preparative tube while the HDL_{2-3} fraction was concentrated in the bottom ml of the tube. The intermediate 4 ml was generally clear of macromolecules. For analytical purposes, however, the top ml was collected in one vial, the next 2 ml in another, the fourth and fifth in another, and the bottom ml in still another, so that analytical runs could be made in order to determine the exact lipoprotein distribution in each fraction.

For characterization of the lipoprotein fractions obtained in this procedure, all collected fractions from the preparative runs were analyzed by ultracentrifugation in a Model E Spinco analytical ultracentrifuge. Further, these analytical runs were analyzed to obtain values for lipoprotein concentrations in the serum. The solutions were then diluted to an appropriate volume so that 10-ml aliquots could be taken for extraction of the lipides.

EXTRACTION AND CHROMATOGRAPHY

The extraction and chromatography were carried out as described previously.[16] Briefly, the extraction was a modification of the procedure of Sperry and Brand,[17] using a mixture of chloroform and methanol, 2 to 1. The chromatography was performed on silicic acid columns, with increasing concentrations of methanol in methylene chloride used to elute the phospholipides from the column. The analyses of the chromatographic fractions were made by infrared spectrophometry. For further details the reader is referred to the original publication.

GAS CHROMATOGRAPHY

The fractions recovered after the infrared runs were then subjected to a transmethylation procedure to obtain the methyl esters of the constituent fatty acids for the purpose of gas-chromatographic analysis.

Approximately 5 to 10 mg of phospholipides was placed in a 10-ml glass-stoppered tube. Then 8 ml of a 1% H_2SO_4 solution by weight in MeOH was added. The tube was then tightly stoppered and heated at 75°C for 2 to 3 hours. After this time it was removed from the heat and the volume of methanol reduced by evaporation under nitrogen to 1 ml. The methanol was transferred to a small separatory funnel with a Teflon stopcock, and 4 ml of distilled H_2O was added to the methanol. The solution was then neutralized with an excess of solid $NaHCO_3$ and extracted four times with 10 ml of petroleum ether (30° – 60°C boiling range). The petroleum ether phases were pooled and evaporated to dryness by nitrogen. The collected methyl esters were placed in a 2-ml vial and dissolved in a known amount of n-hexane (purified by redistillation). The vial was then securely stoppered.

The esters were applied to column in 0.005-ml aliquots in hexane by use of a Beckman micro injector. The gas chromatographic unit[18] was designed and built in this laboratory and uses an ionization detector of the type designed by Lovelock (using a Sr^{90} source of beta particles).[19] The stationary phase was LAC-728[20] absorbed on Chromosorb,[21] 48 to 65 mesh. The column temperature was 190°C, and the carrier gas was argon at a flow rate of 80 to 90 ml per minute. The column length was 4 ft. Typical gas chromatograms obtained from this column are shown in Fig. 1.

FATTY ACID CALCULATION

The fatty acid compositions of the individual phospholipides were determined by measuring the resultant areas of the elution peaks recorded on the readout recorder of the gas chromatographic unit, a Brown recorder. The linearity of the response was previously proven, and the unit calibrated by passing known amounts of highly purified fatty acid methyl esters (obtained from the Hormel Foundation) through the column. Figure 2 shows the curve of a standard mixture run on the column. It was found that the total area obtained by summing the areas of the individual peaks was adequate to represent

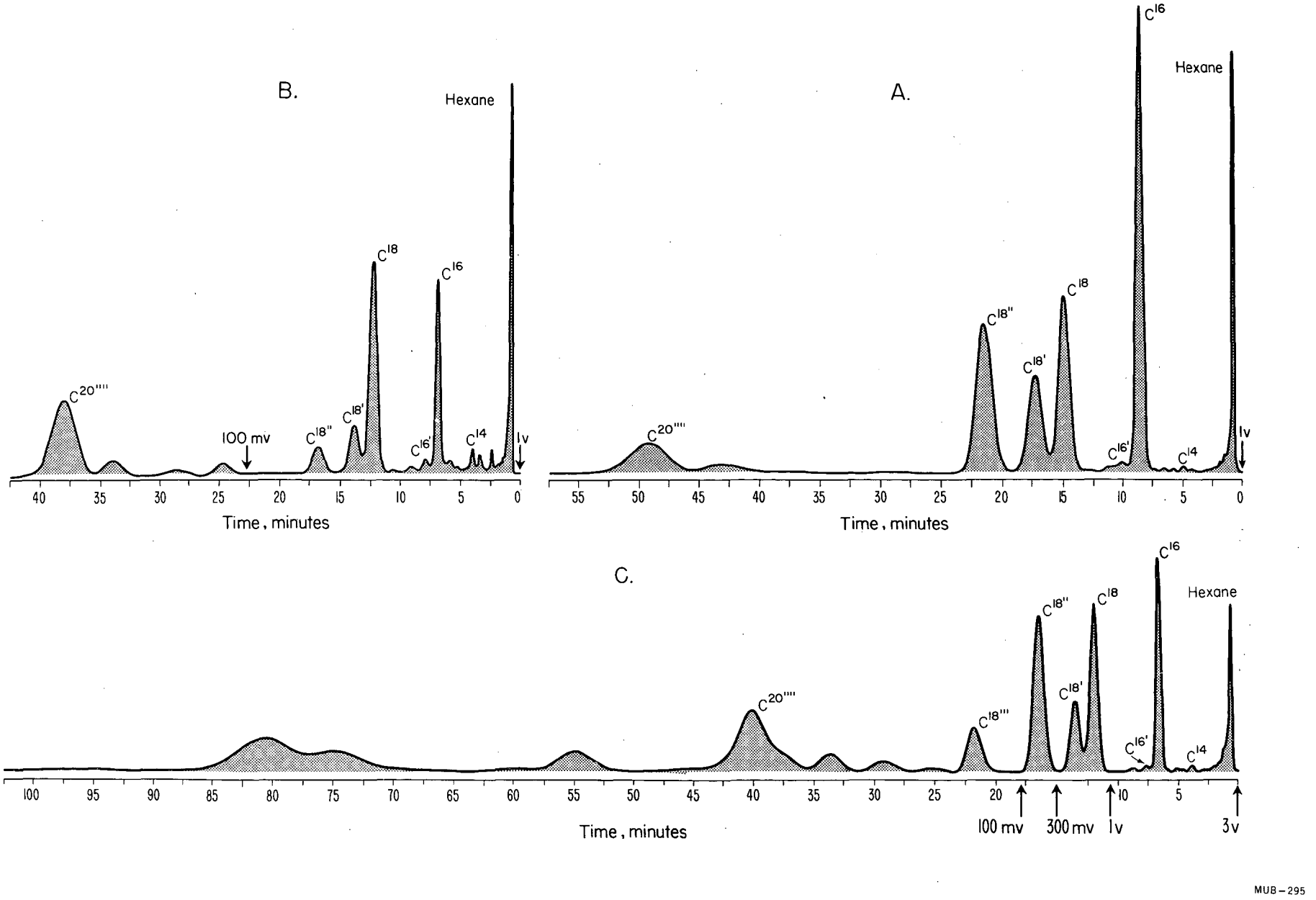

Fig. 1. Typical gas chromatograms of fatty acid methyl esters obtained from serum phospholipides. Operating conditions identical to those described in calibration run shown in Fig. 2. Curve A: se0-20 lecithin fatty acid esters. Curve B: total serum phosphatidyl ethanolamine-serine fatty acid esters. Curve C: total serum sphingomyelin fatty acid esters. Fatty acids are indicated by numbers of carbon atoms in the chain, and numbers of double bonds are indicated by primes. Thus $C^{18'}$ is an 18-carbon fatty acid with a single bond, or oleic acid. Recorder sensitivity changes are as indicated.

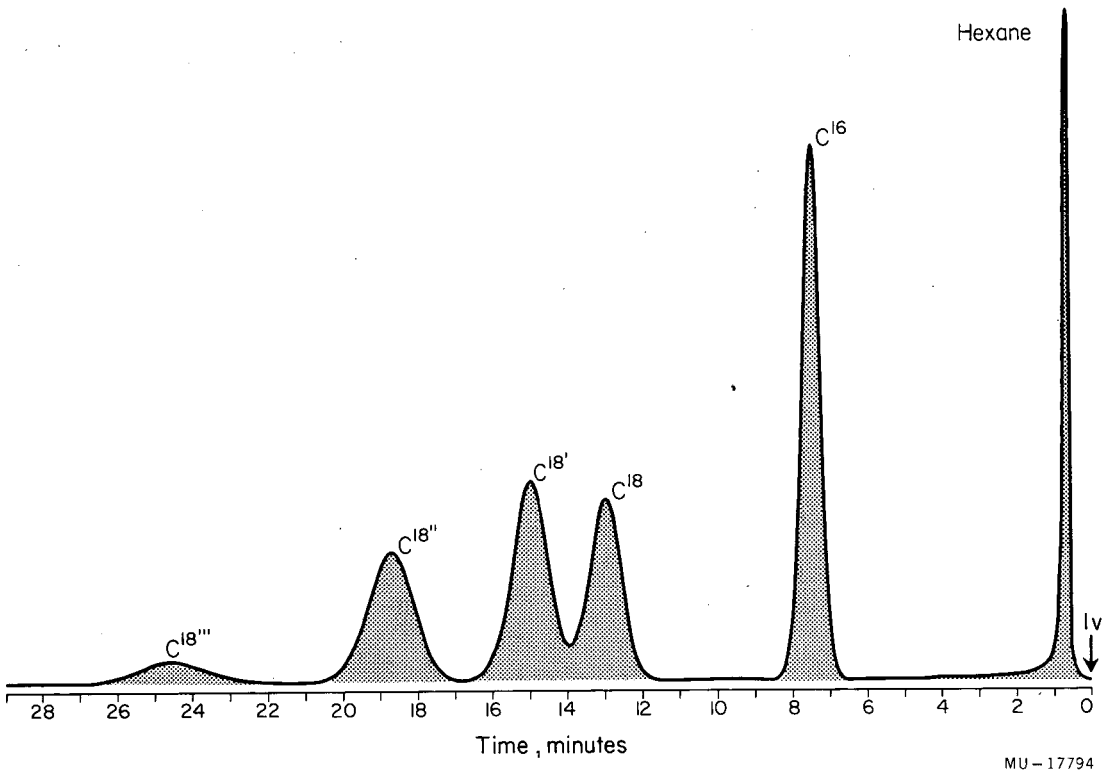

Fig. 2. The elution curve of a known mixture of methyl esters of fatty acids (obtained from the Hormel Foundation). The stationary phase was LAC-728 on Chromosorb maintained at 190°C. The flow rate was 80 ml of argon per min. Column length was 4 ft. Samples were injected in n-hexane solution. Total sample weight, 80 μg. Recorder sensitivity was 1v, full scale. Fatty acids are identified as indicated in Fig. 1.

the total amount of material added to the column if corrections were applied for differences in molecular weight of the individual fatty acid methyl esters. This is in agreement with the observation by Lovelock that the response of a beta-ionization detector is linear with number of organic molecules present in carrier gas.[19] Thus the percentages of the various fatty acids present were obtained by dividing the corrected areas of the individual elution peaks into the corrected total area of the chromatogram.

Time sequence of the elution peaks from the column was taken as the criterion of identification of the particular fatty acid.[22] The elution characteristics of the column were previously determined by the same standardization procedure used for calibration of the column with standard methyl esters. A semilog plot of elution time versus carbon chain length for saturated fatty acid esters is seen in Fig. 3, as well as a plot for degree of unsaturation in the normal-chain C-18 fatty acids, oleic, linoleic, linolenic. Notice the striking linearity of these curves.

PRESENTATION OF RESULTS

Table I presents the average individual values of the total serum phospholipides obtained in 10 normal males (A series) in an earlier study related to the development of the analytical method. These data were obtained on whole serum that had not been fractionated ultracentrifugally into lipoprotein classes. Also included in this table are the total serum phospholipide values for the five cases (B series) in which the three lipoprotein classes were analyzed separately. These values are reconstituted from the values obtained on the separate lipoprotein classes by using average values for concentrations of the lipoproteins in their sera. Although there is considerable individual variation, the average values for the A series and the B series agree quite well.

Table II presents the results of this study on the phospholipide distributions of the three lipoprotein classes. The percentage of phospholipide of the toal lipide extract in each of the three classes is remarkably constant. Only in the HDL_{2-3} is there any difference that might be greater than experimental error. This difference may arise because the HDL_2 and HDL_3 molecules have different percentages of phospholipides and the concentration of each class of molecules is independently variable.

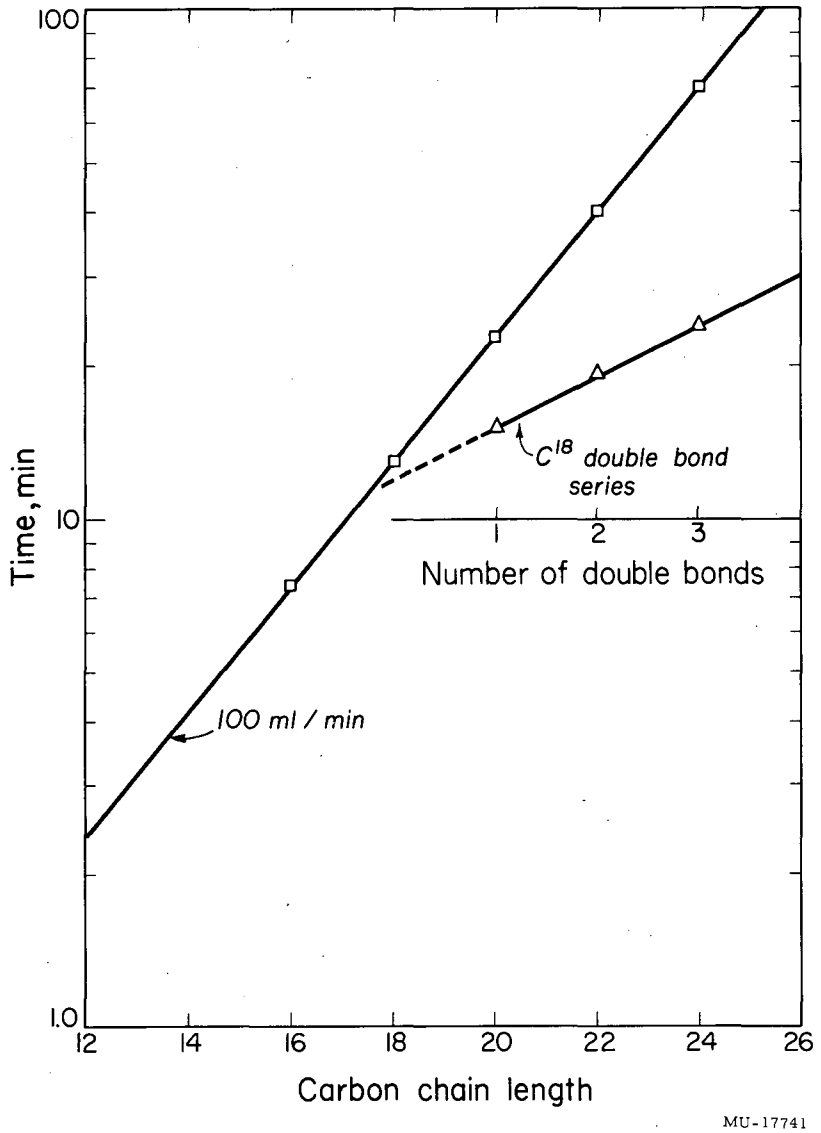

Fig. 3. A semilog plot of retention time of the methyl esters of the saturated fatty acid extending from C-12 to C-26 versus number of carbon atoms in the chain. Operating conditions identical to those described in Fig. 2 except that flow rate was 100 ml/min. Also shown is log of retention time of the normal-chain C-18 unsaturated methyl ester series as a function of the number of double bonds present in the molecule.

Table I

Distribution of total serum phospholipides

Run No.	Subject	Age	Sex	Total Lipide (mg/ml)	Phospholipide of total lipide extract (%)	Phospholipides as per cent of the total phospholipides		
						PE-S[a]	Lecithin	Sphingomyelin
1A	R.H.	-	M	8.60	24.9	4.9	76.8	17.4
2A	J.D.	46	M	6.50	26.4	6.4	73.4	20.1
3A	J.B.	34	M	4.40	28.4	5.9	71.0	23.1
4A	M.M.	33	M	3.69	29.8	6.5	75.6	17.9
5A	G.C.	50	M	6.08	29.9	5.4	70.4	24.1
6A	L.S.	37	M	6.48	29.6	5.0	76.6	18.4
7A	M.V.	55	M	6.79	28.2	4.6	71.5	23.9
8A	J.K.	34	M	8.24	30.1	7.0	82.6	10.4
9A	M.F.	40	M	6.49	29.2	6.7	73.1	20.2
10A	W.C.	46	M	4.83	33.0	8.4	73.3	18.3
Average Values, A Series					29.0	6.1	74.4	19.4
1B	L.M.	37	M	10.80	28.8	5.5	73.1	21.4
2B	F.D.	37	M	13.07	30.1	7.6	71.4	21.0
3B	H.G.	39	F	9.14	33.4	7.6	72.6	19.9
4B	D.G.	49	F	8.33	26.3	5.7	74.6	19.7
5B	R.G.	48	M	8.94	26.9	6.1	75.0	19.0
Average Values, B Series					29.1	6.5	73.3	20.8

[a] Phosphatidyl ethanolamine-serine

Table II

Summary of data for serum lipoprotein phospholipides

	Run No.	Phospholipides as per cent of total phospholipides			Per cent phospholipide of total lipide extract
		PE-S[a]	Lecithin	Sphingomyelin	
Class I $s_f^0$20-400	1B	7.3	74.5	18.2	18.2
	2B	8.2	73.3	18.5	18.7
	3B	8.6	71.2	20.2	19.8
	4B	7.8	75.3	17.0	18.3
	5B	6.6	75.9	17.6	19.3
Class I Average		7.7	74.0	18.3	18.9
Class II $s_f^0$0-20	1B	4.7	68.4	26.9	24.6
	2B	6.9	66.2	26.9	24.9
	3B	6.3	69.6	25.1	25.9
	4B	4.7	69.9	25.4	24.4
	5B	5.5	70.7	23.8	25.5
Class II Average		5.6	68.8	25.6	25.0
Class III HDL_{2-3}	1B	6.3	80.1	13.6	40.0
	2B	8.4	79.4	12.1	39.2
	3B	9.3	80.1	10.7	43.2
	4B	6.1	82.3	11.4	41.6
	5B	6.9	81.8	11.4	42.9
Class III Average		7.4	80.8	11.8	41.4

[a] Phosphatidyl ethanolamine-serine

The distribution of the phospholipides is not identical in major lipoprotein classes. Every phospholide appears in every lipoprotein class, but with different distributions in each. No differences were observed with regard to sex, either by us or by Phillips.[6] The significance of the difference between lipoprotein classes is discussed below.

Table III presents the results of the gas chromatographic analysis of the phospholipide fatty acids. The gas unit used in this experiment was undergoing modification and additions during the course of the work so that it was impossible to run complete analyses on all samples, and pending more detailed studies the results must be regarded as preliminary. However, certain conclusions can be drawn which are valid.

The phospholipide fatty acids are not highly unsaturated. Allowing for individual variation, it appears that palmitic and stearic make up more than 50% of the total fatty acids. Palmitic is the primary fatty acid of lecithin and sphingomyelin, while stearic is the primary fatty acid of the phosphatidyl ethanolamine-serine fraction.[23] The primary unsaturated fatty acids are palmitoleic, oleic, linoleic, and arachidonic, with linolenic almost completely absent. There are as many as ten other components in amounts usually less than 1% that have not been definitely identified at present. However, it is likely that the C-10, C-12, C-14 n-chain saturated fatty acids occur in small amounts in all fractions. In the sphingomyelin fraction (see Fig. 1C) there are several slow-moving components following arachidonic that have not been identified. They are not seen in the phosphatidyl ethanolamine-serine or lecithin fractions.

Two total serum phosphatidyl ethanolamine-serine samples were run. There was not enough of this fraction obtained in any of the lipoprotein classes to run fractions separately. Phosphatidyl ethanolamine-serine fractions were pooled for purposes of analyses of the fatty acids after the infrared spectra were obtained on the separate fractions.

The separate runs on the lecithin fractions from the lipoprotein classes indicate that there is no variation in the composition of the fatty acids in respect to the lipoprotein class. A total sphingoymelin fraction and one Class II fraction were run; they appear more saturated than the lecithin but less than the phosphatidyl ethanolamine-serine fraction.

Table III

Principal fatty acids of human serum phospholipides from whole serum and lipoprotein fractions analyzed by gas chromatograph (weight %)

Phospholipide	Subject	Fraction	Fatty acids as per cent of total fatty acid							
			C-16	C-16'[a]	C-18	C-18'	C-18''	C-18'''	C-20''''	Unindentified
Phosphatidyl ethanolamine-serine	H.G.	Total	17.9	1.3	35.0	9.4	9.4	0.4	17.9	8.5
	D.G.	Total	22.1	1.7	37.6	12.1	7.7	-	4.7	13.5
Lecithin	H.G.	20-400	28.4	0.6	19.7	12.5	22.3	-	11.4	4.4
	H.G.	0-20	27.4	0.6	23.7	12.3	24.4	-	9.0	2.5
	H.G.	HDL_{2-3}	29.1	0.6	19.5	12.9	24.2	-	10.6	3.1
	H.G.	Total	28.3	0.6	21.0	12.6	23.6	-	10.3	3.3
	D.G.	20-400	41.4	2.3	21.1	15.6	11.7	-	-	7.8
	D.G.	0-20	42.3	2.0	25.4	15.9	8.9	-	2.8	2.8
	D.G.	HDL_{2-3}	46.4	3.6	25.0	14.3	5.4	-	-	5.4
	D.G.	Total	43.4	2.6	23.8	15.3	8.7	-	0.9	5.3
Sphingomyelin	H.G.	Total	33.9	0.9	18.3	13.8	21.6	-	6.7	4.6
	D.G.	0-20	47.0	2.0	20.5	10.8	8.6	1.0	3.2	7.6

[a] The ' refers to the number of double bonds present in fatty acid molecules.

DISCUSSION

The results of Phillips's study[6] agree well with this work in view of the differences between experimental procedures. Each class of lipoproteins shows a distinct phospholipide spectrum. In Phillips's study the distinction between Class I and II is less than shown in this work. The explanation for this may be found in the ultracentrifugal lipoprotein separation. In Phillips's procedure, taken from the method of Havel et al.,[24] Class I contains lipoprotein with s_f^o rates above 10 or 12, whereas $s_f^o 20$ was the divisional point used in our work. The amount of material transferred in this manner, while not large, would be enough to produce the observed differences, as the sphingomyelin concentration is highest in the $s_f^o 0\text{-}20$ region. It is not altogether impossible, albeit unlikely for the $s_f^o 10\text{-}20$ lipoproteins to have a unique phospholipide distribution. There is no evidence for such an assumption, however.

This same type of argument can be applied to the sphingomyelin concentration in Class III. In Phillips's procedure, HDL_{1-2-3} and the ultracentrifugal residue are included in the third class, while only HDL_{2-3} were analyzed in this work. If HDL_1 approximates Class II, then sphingomyelin will be higher in Phillips's study than in this one. Experimental error may be the primary cause, but this is considered unlikely.

There is a significant difference between the amount of phosphatidyl ethanolamine-serine reported in Phillips's and in this study. This can only be attributed to a systematic error in one of the procedures used, but it is not possible to ascertain to which work the error should be assigned. The amount of phosphatidyl ethanolamine-serine is small in either case and subject, therefore, to the largest error. This fraction shows the largest contamination from non-phosphorus-containing materials. Phillips did not estimate the inherent error in his procedure. It is felt that the phosphatidyl ethanolamine-serine fraction is subject to an error of more than 10% in the method used in this study, however.

Phillips also reported the presence of lysolecithin in all fractions.[6,8,25] The average value reported for total serum phospholipides was 7%. The distribution of the lysolecithin was nonuniform, and the largest percentage was found in the high-density residue, $\rho > 1.21$ g/ml.[25] In Class III, Phillips reported 14% lysolecithin. In Class I and II, lysolecithin accounts for no more than 5% of the lecithin value.

The distribution of the phospholipides in the lipoprotein classes presents no unusual features, although definite differences exist. Class I contains 20% of the phospholipides in normal serum and has a phospholipide composition not unlike serum. The phosphatidyl ethanolamine-serine fraction is slightly elevated. Class II contains more than 50% of the total serum phospholipides and more than 70% of the total serum sphingomyelin, while the percentages of phosphatidyl ethanolamine-serine and lecithin are reduced. Class III contains the lowest concentration of sphingomyelin and the highest concentration of lecithin. The phosphatidyl ethanolamine-serine fraction is similar to Class I.

The values reported by Phillips concerned individuals with average serum lipide levels considerably below the cases studied in this work. As there is no significant variation that is not traceable to some other cause, the distribution of serum phospholipides, both in total serum and in the three classes, must be unrelated to their concentrations in the serum. This, indeed, is to be expected on the basis of earlier studies of serum lipides in which there was found no variation of the total lipide composition of serum lipoproteins with total serum lipide.[26] In this work, individuals with total serum lipide values varying from 13.1 to 8.8 mg/ml were studied and were shown to have similar phospholipide distributions.

In this respect the work of Petersen is of interest.[27] Petersen studied the distribution of total serum phospholipides in starvation. He found a rise in total serum phospholipides that could be accounted for entirely on the basis of a selective rise of the sphingomyelin content of serum. This does not seem possible if the present concepts of lipoproteins are correct. Petersen's experimental methods, unfortunately, are not considered reliable in view of present knowledge. The rise in total serum phospholipide was not more than 10% of the fasting values and was of transient nature. It has been shown that serum lipoprotein levels in $s_f^0$12-400 range increase during long fasting periods (7 days).[28] This produces a general rise in the total serum lipide and phospholipide. A selective rise of one phospholipide would not be expected in this phenomenon, however. Thus, it is likely that the report by Petersen was subject to experimental error.

Judging from the limited data available, the fatty acid composition is roughly the same for the various lipoprotein classes from a given serum. This is consistent with the hypothesis that lipoproteins undergo transformations from one class to another.[15] If the phospholipides in the core of a Class I lipoprotein are eventually found in Class II or III, then the fatty acid distribution must be similar in the different classes. The fact that they are indeed similar does not prove that transformations occur. It could be that all serum phospholipides in a given individual at any one time have the same fatty acid composition regardless of function or location.

The primary fatty acid of phosphatidyl ethanolamine appears to be stearic. This acid is not usually predominant in compounds isolated from humans. In general the degree of saturation found in this study was higher than that reported in the few previous studies,[9, 10] but the samples selected were so small that it could be due to individual variation. Further studies are contemplated.

Evidence was obtained for the presence in sphingomyelin of fatty acids having more than twenty carbon atoms (see Fig. 1). This is in agreement with reports on tissue that find a higher percentage of such higher fatty acids in the sphingolipide fraction[29, 30] than in the lecithin or phosphatidyl ethanolamine-serine fraction. In our study no attempt was made to identify those compounds. In all fractions analyzed, evidence was obtained for shorter-chain fatty acids in small amounts. The amounts were too small to be accurately measured by using the size samples available. Generally the chromatograms obtained indicated that all normal-chain saturated fatty acids with even numbers of carbon atoms from C-8 to C-18 or higher are present in serum phospholipides, as are also many of their unsaturated analogs. Usually, short-chain components, C-8 to C-14, were present in amounts of less than 1% of the total fatty acids.

The distribution of fatty acids in the phosphatidyl ethanolamine-serine differed significantly from either the lecithin or sphingomyelin. It was also more highly saturated than the other fractions. Lecithin appeared to be the most unsaturated phospholipide, but still more than 50% saturated. The sphingomyelin fatty acid composition approximates the lecithin composition, except for the very-long-chain (or hydroxy) fatty acids already mentioned.

ACKNOWLEDGMENTS

We wish to thank Drs. Frank Lindgren and Alex Nichols for their very helpful advice on the ultracentrifugal and gas chromatographic aspects of this work, and Dr. John W. Gofman for his continued interest and support.

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FOOTNOTES AND REFERENCES

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