Molecular species of lecithins of rat heart, kidney, and plasma

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ABSTRACT The lecithins of the heart, kidney, and plasma of the rat were isolated, and the major molecular species identified and quantitatively estimated by combined thin-layer and gas–liquid chromatographic analyses and specific enzymic hydrolyses. The lecithins of the three tissues differed significantly in the composition, positional distribution, and pairing of the fatty acids. No preferential pairing of any one saturated with any other unsaturated fatty acids was observed. The three tissues contained qualitatively the same molecular species of lecithins. The lecithin profiles of the rat heart and kidney appeared to be unrelated to that of the plasma.

SUPPLEMENTARY KEY WORDS diglyceride acetates · fatty acids · positional distribution

In natural lecithins most of the fatty acids in the 1-position are saturated while most of those in the 2-position are unsaturated (1). Since the major unsaturated fatty acids are linoleic and arachidonic, most animal lecithins are made up of dienoic and tetraenoic species. There is evidence now that the two kinds of lecithins differ greatly in metabolic turnover rate (2–4), and the differences observed have been attributed to the type of fatty acids involved. A clear need therefore exists for the determination of the acids in all lecithin species, as recognized by Renkonen (5) in the original work on the diglyceride acetates derived from glycerophosphatides.

We have recently proposed (6) a method for determining the complete structure of natural glycerophosphatides and have tested it with the lecithins of egg yolk. Egg phospholipids are, however, relatively poor in the polyunsaturated long-chain fatty acids that make up a large proportion of the fatty acids of animal tissue lecithins. Since the polyunsaturated molecules are particularly vulnerable to oxidative changes as well as subject to possible enzymic discrimination (7, 8), legitimate doubt might arise as to the suitability of the scheme for a detailed analysis of the structure of highly unsaturated glycerophosphatides.

The present study shows that the previously described method (6) is also adequate for the estimation of all major species of the lecithins of rat heart, kidney, and plasma, provided that exposure to air is minimized and enzymic digestions are taken to completion. The unavoidable minor losses in the polyunsaturates must be accurately determined and corrected for by reconstitution of the starting composition of the lipid mixture after each step of analysis.

MATERIALS AND METHODS

The solvents, chemical reagents, and enzyme preparations as well as the general methods of analysis were as previously described (6). No antioxidants other than a nitrogen atmosphere were employed with any of the solvents or reagents. Phospholipase A (venom of *Crotalus atrox*) was obtained from Pierce Chemical Co., Rockford, Ill.

Male albino rats (220–240 g) from Charles River Breeding Laboratories, Oakland, Mass., were maintained on a commercial choline-deficient diet (General Biochemicals, Chagrin Falls, Ohio), supplemented with 0.4% choline chloride, for 10 days prior to sacrifice. The tissues were collected after overnight fast. Under diethyl ether anesthesia the abdomen was opened, and the blood, completely drained through the abdominal aorta by

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; C_{14}–C_{22}, fatty acids with 14–22 carbon atoms; C_{32}–C_{46}, diglyceride acetates with a total number of fatty acid carbon atoms (including those of acetic acid) of 32–46. Fatty acids designated by number of carbon atoms:number of double bonds.

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syringe, was immediately centrifuged and the plasma collected. Next the kidney and the heart were excised and, after being drained of blood, homogenized with 0.9% NaCl and chloroform–methanol as described below. The corresponding tissues from four animals were pooled and all samples were analyzed in duplicate.

Preparation of Total Lipid Extracts

Total lipid extracts were prepared as described by Bligh and Dyer (9), except that 0.9% NaCl was substituted for water in the initial stages and the phases were separated by centrifugation. The procedure varied somewhat with the water content of the tissue, but the volumes of chloroform, methanol, and water, before and after dilution, approximated the proportions 1:2:1 and 1:1:1, respectively, as recommended.

At least one aliquot of each lipid extract was worked up immediately (as described below), while the rest of the total lipid extract was stored at −20°C for a maximum of 1 wk.

Isolation of Lecithins

Total lecithins were isolated by TLC on Silica Gel G as described before (6). About 0.25-mm thick layers were used and 20–50 mg of total lipid containing 3–5 mg of lecithin was applied per plate. The cleanly resolved lecithins were eluted from the silica gel with chloroform–methanol–water 50:40:10 containing about 1% of formic or acetic acid. The extracts were washed free from acid and dissolved silica with distilled water. Aliquots of the extract were then taken for immediate transmethylation or digestions with phospholipases. The lecithins were shown to be free from readily detectable contaminants by rechromatography in the TLC systems described by Skipski, Peterson, and Barclay (10), and by the spot tests they suggested.

Preparation of Diglyceride Acetates

Diglycerides were prepared by hydrolysis of the lecithin fractions with phospholipase C, and purified by TLC (6). Since the recovery of the lecithins from the silica gel was complete when the acidic eluent was used, there was no need to perform these digestions in the presence of silica gel as we did previously. A determination of the fatty acid composition of the purified diglycerides showed that all the fatty acids were recovered in the ratios in which they occurred in the original lecithins; this indicated that the enzyme hydrolyses had been complete, or at least representative.

The diglycerides were converted into the acetates by treatment with acetic anhydride and pyridine as described previously (6), except that we insured that the reaction would be complete by heating the tightly closed vial at 80°C for 1 hr. After the mixture had been cooled to about 10°C, the excess acetic anhydride was decomposed by dilution with methanol while the temperature was allowed to rise to ambiance. The diglyceride acetates were recovered after a further dilution of the reaction mixture with water and extraction with petroleum ether. The acetates were purified by TLC (6).

Determination of the fatty acid composition of the diglyceride acetates showed that all acids were recovered in the ratios in which they occurred in the original lecithins, which indicated that the acetylation and additional purification of the sample were not accompanied by selective losses of the polyunsaturates.

Resolution of Diglyceride Acetates

The diglyceride acetates were resolved on the basis of total number and distribution of double bonds per molecule by argentation TLC (6). To the eluates of each zone we added 50–200 μg of tridecanoin in chloroform and reduced the final volume to 0.25 ml. Appropriately weighted summation of the fatty acids recovered for each zone gave acid proportions similar to those obtained for the total diglyceride acetate and hence the original lecithin mixture, which again indicated the absence of significant losses of the polyunsaturated components.

The molecular weight distributions of the total diglyceride acetates and the diglyceride acetates of various degrees of unsaturation were determined by GLC on a Beckman GC-4 gas chromatograph (6). The columns were stainless steel tubes (50 cm × 3 mm O.D., 0.5 mm wall) filled with 3% JXR on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories Inc., State College, Pa.). Carrier gas was nitrogen at 100–150 ml/min. The temperatures were: injector, 300°C; hydrogen flame ionization detector, 340°C; oven, temperature-programmed as shown in the figure. The carbon numbers of the diglyceride acetates were determined by comparison with the acetates of 1,2-dipalmitin (C_{31}) and 1,2-distearin (C_{38}), and by assessment of the fatty acid composition of the component diglyceride acetates.

Quantitative results with standard triglyceride mixtures (trilaurin through tristearin, and interesterified trimyristin and triolein) agreed with the weight proportions, showing relative errors of less than 2% for triglycerides up to tripalmitin, and less than 10% for tristearin, triolein, and those components which made up less than 10% of the total mixture.

Positional Analysis of Fatty Acids

The positioning of the fatty acids in the original lecithin mixture was determined by hydrolysis with phospholipase A (11). The substrate was introduced into the buffered enzyme solution in diethyl ether (5–10 mg of lipid per ml of ether). The incubations were continued for 4 hr at 30°C with stirring. The total lipids were extracted

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with chloroform–methanol 2:1 after evaporation of the solvent and buffer in a rotary evaporator under nitrogen. The free fatty acids and lysolecithin were separately recovered after TLC of the extracts by previously described solvent systems (6).

The positional distribution of the fatty acids in the diglyceride acetates was determined by hydrolysis with pancreatic lipase (6). In addition to giving direct evidence of the fatty acid placement in various diglyceride acetate subfractions, these results confirmed the distribution data derived from phospholipase A hydrolyses.

Determination of Fatty Acids

The fatty acids were separated and quantitatively estimated by GLC of the methyl esters (6). Polyunsaturated fatty acids were identified by their retention times relative to the methyl esters of cod liver oil, as suggested by Ackman and Burgher (12). The chain length of the unsaturated acids was confirmed by GLC of the acids after hydrogenation (13). The identification of all fatty acids was completed by reanalyzing by GLC the various zones obtained by TLC of the fatty acid methyl esters on silver nitrate.

Calculations

The general scheme of analysis and calculations was identical with that described for the determination of the complete structure of egg yolk lecithins (6), and of the lecithin of rat liver, intestine, lung, plasma, and erythrocytes (13). The accuracy of the final estimates was tested by reconstruction of the original diglyceride acetate and fatty acid compositions from the results of the entire analysis. The proportional distribution of the fatty acids in the diglyceride acetates provided by randomization (13) served as a theoretical guide to the identification of the molecular species of lecithins. The "random" calculation is based on the over-all fatty acid composition of the lecithin mixture and the fact that two fatty acids occur per molecule of lecithin.

RESULTS AND DISCUSSION

The amounts of total lipid and lecithin obtained by the modified Bligh and Dyer extraction were of the order reported in the literature for rat plasma (14), kidney (15), and heart (15, 16). It was assumed that the isolated lecithins represented all the molecular species of lecithins that were present in the tissue.

No evidence was obtained for the presence of plasmalogenic molecules (less than 0.5%) or molecules containing alkyl ethers (less than 2%, which was the error of the analysis) in the lecithin mixture, despite a critical application of the methods proposed by Renkonen (17); however, alkyl and alkenyl ethers could be readily demonstrated by both TLC and GLC in the phosphatidyl ethanolamine fraction of rat heart lipids. Wood and Snyder (18) have recently estimated the total ether content of rat heart phospholipids to be 1.6%, which, if present entirely as phosphatidyl choline ether, could have given a maximum of 4.5% contamination of our sample. Much of this ether-containing material, however, must have been associated with the phosphatidyl ethanolamine fraction. Wood and Snyder (18) also note that their ether content is lower than that reported by previous investigators and discuss reasons for this. The ether content of individual phospholipid classes needs to be reassessed.

Using methyl heptadecanoate as internal standard, we estimated the recovery from the whole procedure, including argentation TLC, and calculating the end-product as the fatty acid methyl esters of lecithin, to be 70–75 moles per 100 moles.

Fatty Acid Composition

Table 1 gives the fatty acid composition of the lecithin mixtures isolated from the rat tissues along with the composition of the fatty acids of the diet. As judged from the fatty acids, these lecithins are characteristic for the rat since comparable acid compositions have been previously reported, at least for the phosphatidyl cholines of rat plasma (4) and heart (16). We could find no previous quantitative records of the fatty acid analysis of rat kidney lecithins, but qualitative findings similar to ours have been mentioned (19). Although all of the dietary acids are found in the lecithins, their amounts in the different tissues differ widely. The kidney lecithins contain about twice as much palmitic and one half as much stearic, arachidonic, and docosahexaenoic acid as do those of the heart. The heart lecithins contain a greater proportion (30%) of the unsaturated C_{20} and C_{22} fatty acids than those of either the kidney (20%) or the plasma (26%).

The positional distribution of the fatty acids in the lecithin molecules agrees well with the observations recorded previously (1) on other lecithins, where the unsaturated fatty acids have been found to be primarily in the 2-position. The present data for the plasma lecithins are identical with those noted for another group of rats on a similar diet (13).

Molecular Weight Distribution

Table 2 gives the molecular weight distributions of the total lecithins of rat tissues as estimated by GLC of the diglyceride acetates derived from them. In all tissues the major lecithins are those with one C_{16} and one C_{18} (C_{36}), with two C_{18} (C_{38}), or one C_{18} and one C_{20} (C_{40}) fatty acid per molecule. The variations in the proportional contributions of the lecithins of different molecular weights reflect differences in both their fatty acid composition and positional distribution. These differences are greatest

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TABLE 1 COMPOSITION AND POSITIONAL DISTRIBUTION OF FATTY ACIDS OF TOTAL LECITHINS FROM TISSUES AND IN DIET

Fatty Acid	Heart			Kidney			Plasma			Diet§
	1-*	2-†	Total‡	1-	2-	Total	1-	2-	Total	Total
					moles %					
14:0		0.8	0.4		1.1	0.5	1.2		0.4	2.4
16:0	30.8	10.4	20.5	50.2	6.4	37.7	45.0	5.0	21.0	19.9
16:1	1.5	0.9	0.7	3.8	3.0	2.8	0.6	1.4	1.0	5.8
18:0	47.1	2.7	22.4	22.2	7.8	12.4	42.2	0.5	19.5	2.1
18:1	8.8	16.5	11.3	13.8	21.0	14.1	8.0	12.0	7.5	25.4
18:2	11.3	20.0	15.1	9.9	17.7	14.5	2.0	46.0	25.5	33.3
20:0				0.1						
20:1		0.2	0.5		0.2	0.9			0.7	
20:2		0.2	0.4		0.4	0.3		1.5	0.6	
20:3		0.9	1.1		2.0	1.2	1.0	2.0	1.8	
20:4		33.3	18.7		17.1	10.6		18.6	13.5	4.2
20:5			1.5		1.4	1.4			1.0	
22:0		0.3						1.2	0.5	
22:3		0.5	0.2							
22:5		2.5	1.7		1.0	0.6		2.2	1.0	0.4
22:6		9.3	6.0		2.7	3.0		7.0	6.0	2.1

The lecithins were obtained from tissue pools from four male rats.
* Fatty acids of the lysolecithins liberated by phospholipase A.
† Free fatty acids liberated by phospholipase A.
‡ Fatty acids of total lecithins obtained by transmethylation.
§ Fatty acids of the total lipids of the diet.

TABLE 2 MOLECULAR WEIGHT DISTRIBUTIONS OF DIGLYCERIDE ACETATES DERIVED FROM LECITHINS

Diglyceride Acetates*	Heart			Kidney			Plasma		
	Reconst.†	Total‡	Random§	Reconst.	Total	Random	Reconst.	Total	Random
					moles %				
C_{32}			0.2	0.2	0.2				0.2
C_{34}	2.8	2.9	4.9	8.7	9.0	14.9	1.0	1.1	5.3
C_{36}	19.1	19.0	20.9	43.3	42.1	33.3	27.6	25.7	23.2
C_{38}	38.3	38.2	33.3	32.3	34.4	29.9	32.6	33.4	35.4
C_{40}	32.8	32.9	24.6	14.4	13.4	14.1	30.1	32.0	21.8
C_{42}	7.0	7.0	12.5	1.1	0.9	4.7	8.7	7.8	11.3
C_{44}			2.9			0.9			2.6
C_{46}			0.4			0.1			0.6

* Diglyceride acetates identified by total number of acyl carbon atoms, including that of the acetic acid residue.
† Estimates obtained by weighted summation, proportionation, and normalization of data from argentation TLC.
‡ As estimated by direct GLC of the diglyceride acetates or the free diglycerides before and after hydrogenation.
§ Random distribution calculated as previously described (13).

between the kidney and the heart, while the plasma lecithins resemble those of the heart. For all three tissues, the random distribution covers a range of molecular weights wider than actually observed, because certain types of combination, for example (20:4 20:4), do not occur in nature. Table 2 also includes the reconstitution values obtained for the diglyceride acetates by weighted summation of the data from argentation TLC. The close agreement between these values and the original total indicates that the execution of the detailed analytical routine was not accompanied by undue loss from autoxidation or otherwise.

Table 3 compares the estimated molecular weight distributions of the lecithins obtained by GLC examination of the diglyceride acetates of various degrees of unsaturation. Fig. 1 shows the GLC patterns of the major diglyceride acetate bands recovered from argentation TLC of the rat heart lecithins. As shown, only a few major diglyceride acetates or lecithins occur in any one group of molecules of uniform degree of unsaturation. This is true for both the experimental and the random estimates, although in some cases the random values cover a somewhat broader pattern. The heart and plasma lecithins contained very little (1–2%) fully saturated lecithin, while the kidney lecithins were relatively rich (7%) in these components. The random values for all three tissues were much higher (17–25%). The trienes and pentaenes accounted for 3–5% each of the total tissue

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TABLE 3 MAJOR DIGLYCERIDE ACETATES OF RAT TISSUE LECITHINS

Degree of Saturation	Carbon Number	Heart		Kidney		Plasma	
		Exptl.	Random	Exptl.	Random	Exptl.	Random
		moles %					
Saturates	32	2	1	2	57	10	1
	34	90 } 2	23 } 19	89 } 7	37 } 25	80 } 1	27 } 17
	36	8	49	9	6	10	48
	38		27				22
Monoenes	34	2	4	8	3	3	7
	36	70 } 13	45 } 11	83 } 27	71 } 16	65 } 9	46 } 8
	38	27	50	9	26	28	42
	40		2			4	4
Dienes	34		1	1	66	52	1
	36	36	42	72	34 } 18	46 } 38	49
	38	64 } 24	55 } 15	27 } 28			48 } 22
	40		2			2	1
	42						1
Trienes	36	6	4	7	12	25	8
	38	89 } 4	77 } 5	83 } 5	78 } 6	64 } 5	73 } 6
	40	5	18	11	10	11	19
	42		2				
Tetraenes	36		1		77	3	1
	38	38 } 38	42 } 19	70 } 23	23 } 13	34 } 24	67 } 18
	40	62	55	30		63	33
	42		2				
Pentaenes	36	7	9	30	12	tr.	13
	38	29 } 5	76 } 8	70 } 3	81 } 5	28 } 3	74 } 5
	40	64	15		7	62	13
	42					10	
Hexaenes	38					2	1
	40	44 } 14	50 } 11	80 } 7	85 } 6	61 } 20	77 } 12
	42	56	49	20	15	37	22
	44		1				

Footnotes as in Table 2. Degree of saturation as explained in the text. Each acetate reported as percentage of saturation class, and each saturation class as percentage of total acetate mixture.

TABLE 4 MAJOR FATTY ACIDS OF DIGLYCERIDE ACETATES OF VARIOUS DEGREES OF UNSATURATION (MOLES %)*

Source of Lecithins	Fatty Acids												
	14:0	16:0	16:1	18:0	18:1	18:2	20:1	20:2	20:3	20:4	20:5	22:5	22:6
						Saturates							
Heart	11.2	73.1		15.7									
Kidney	1	93		5									
Plasma	5	90		5									
						Monoenes							
Heart		35.9	4.2	14.9	45.0								
Kidney	0.5	42.0	6.0	7.9	43.5								
Plasma		32.5	2.0	16.5	46.0		3.0						
						Dienes							
Heart		19.0	0.7	26.1	8.0	45.6		0.6					
Kidney	0.5	37.5	1.0	11.2	1.0	49.5							
Plasma		24.3	1.7	20.9	5.7	47.5							
						Trienes							
Heart			3.5		44.4	49.1	2.9						
Kidney	1.0	16.4	2.3	18.8	23.5	18.0	0.5	6.0	11.0				
Plasma	1.0	16.0	8.0	5.0	25.0	31.0			14.0				
						Tetraenes							
Heart		18.1		28.7	1.3	5.0			1.5	45.3			
Kidney		33.0		16.5	0.5				0.5	50.0			
Plasma	1.2	11.4	1.2	28.0	6.0	8.0		0.8	1.4	42.0			
						Pentaenes + hexaenes							
Heart	0.8	15.9	0.8	17.5	7.2	8.0				13.5	3.8	7.4	25.1
Kidney		32.0	1.0	6.0	6.0	4.0	0.5	0.5	0.5	6.5	8.0	6.0	29.0
Plasma	1.0	16.0	1.5	12.0	6.2	13.0			0.9	16.0	5.0	3.2	23.6

* Diglyceride acetates were resolved by TLC on silver nitrate-treated plates. Footnotes as in Tables 1 and 3. Each fatty acid is reported as a percentage of the fatty acids with a given degree of saturation.

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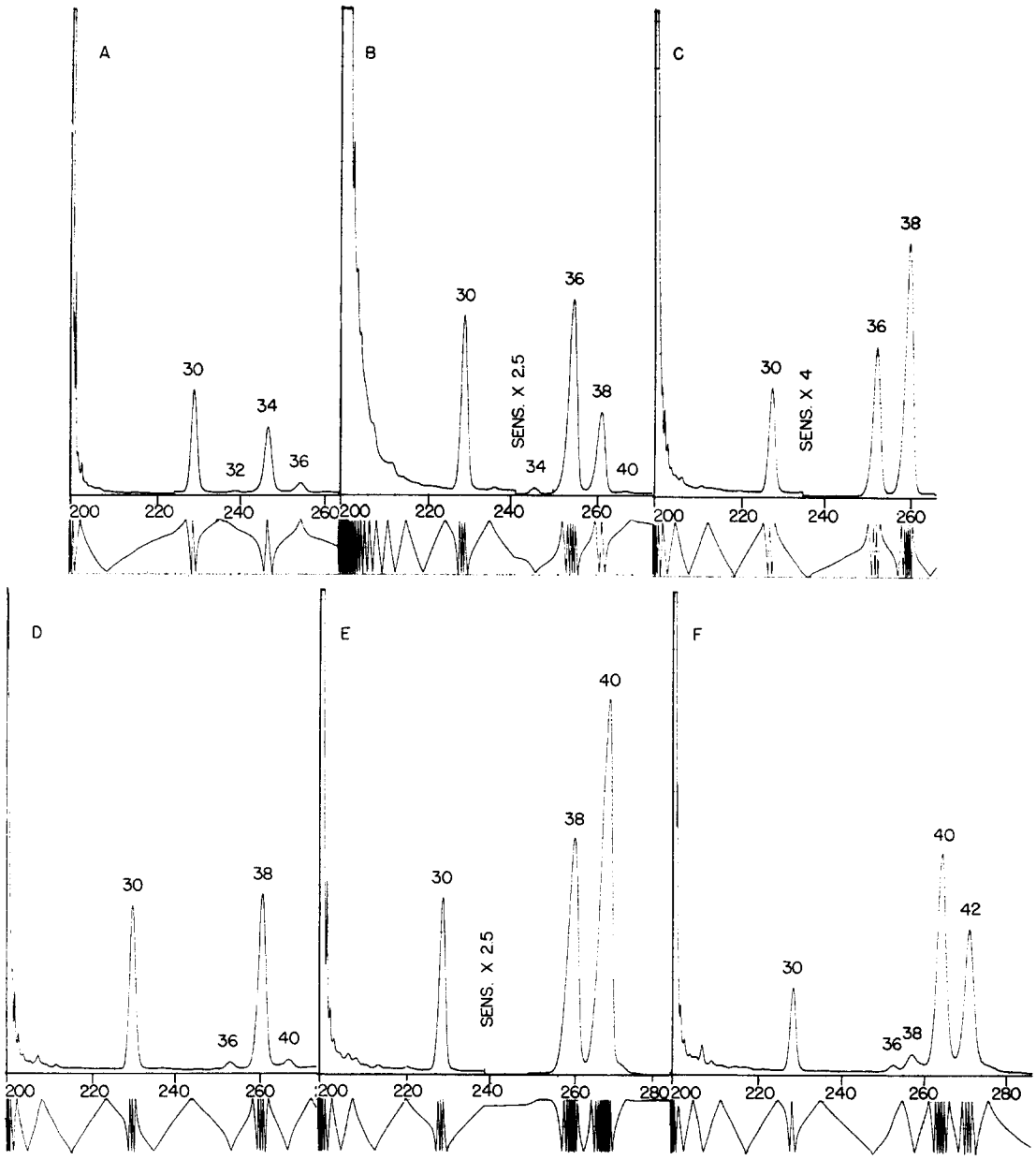

FIG. 1. GLC of major diglyceride acetates of rat heart lecithins of uniform degree of saturation. Peaks identified by the total number of acyl carbons in the molecule. Peak *30*, tridecanoin internal standard. A, saturates; B, monoenes; C, dienes; D, trienes; E, tetraenes; F, pentaenes and hexaenes. Technical details given under Methods.

lecithins. The dienes and tetraenes were the major components of all lecithins and accounted for 23–38% of the total, which greatly exceeded the random values. The monoenes accounted for about 27% of the kidney lecithins while the plasma lecithins contained 20% hexaenes.

Molecular Species

Table 4 gives the major fatty acids of the diglyceride acetates of various degrees of unsaturation. Each fatty acid is reported as percentage of the fatty acids with a given degree of saturation. Since the pentaenes and hexaenes were not cleanly resolved by TLC in all cases, a combined estimate has been entered for these componets. For identification of individual molecular species it was necessary to subtract the fatty acids that belonged to the pentaenes from those that were due to the hexaenes.

Table 5 lists the major individual lecithins of rat heart, kidney, and plasma. To conserve space, we have specified the positional distribution of the fatty acids only for the major isomer where there was more than one. The species with the reversed fatty acid placement occur in much smaller amounts, which can, however, be calculated from the fatty acid distributions reported in Table 1. The ex-

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perimental values given in Table 5 were determined by reconstitution of the over-all molecular weight distribution (Table 3) and fatty acid composition (Table 4). The saturated lecithins of all tissues are now seen to be largely made up of dipalmitoyl lecithin with lesser amounts of material containing palmitic with either myristic or stearic acids. If fatty acids were randomly distributed, most of the saturated species would consist of stearoyl–palmitoyl lecithin, which does contribute significantly to the saturated lecithins of the kidney. The major monoenes in all three tissues were (16:0 18:1) and (18:0 18:1) lecithins. The major dienes were (16:0 18:2) and (18:0 18:2) lecithins. For the kidney, calculation of the random distribution predicted about 11% of (18:1 18:1), but only 1% was found. However, the experimental and random values for the tetraenes of the heart and kidney, made up of (16:0 20:4) and (18:0 20:4) lecithins, were remarkably close, while the plasma contained twice the random amount of (18:0 20:4). The agreement between the random and experimental values was poor for the hexaenes: (16:0 22:6) and (18:0 22:6) lecithins were the expected components, but significant amounts (10–25%)

TABLE 5 MAJOR LECITHINS OF THE HEART, KIDNEY, AND PLASMA OF THE RAT*

	Fatty Acids		Heart		Kidney		Plasma	
	1-	2-	Exptl.	Random	Exptl.	Random	Exptl.	Random
					moles %			
Saturates	16:0	14:0	2	1	3		10	1
	16:0	16:0	69	22	80	56	80	26
	18:0	16:0	8 (2)	49 (19)	11 (7)	37 (25)	10 (1)	48 (17)
	18:0	18:0		26	1	6		
	18:0	14:0	21	1	5	1		
Monoenes	14:0	18:1		1	1	1		
	16:0	16:1	4	3	6	2	3	5
	18:0	16:1	4	3	6	7	3	5
	16:0	18:1	67 (13)	42 (11)	78 (27)	64 (16)	61 (9)	41 (8)
	18:0	18:1	25	48	9	20	26	39
	16:0	20:1		1		5	2	4
	18:0	20:1		2		1	4	4
Dienes	18:1	16:1	1	1	1	5	1	1
	18:1	18:1	8	9	1	11	4	2
	16:0	18:2	36 (24)	41 (15)	75 (28)	61 (18)	51 (38)	48 (23)
	18:0	18:2	53	45	22	22	42	44
	16:0	20:2	2	1	1	1		
	18:0	20:2		1				
Trienes	16:1	18:2	7	4	10	12	21	8
	18:1	18:2	88	68	60	63	50	61
	20:1	18:2	6 (4)	4 (5)	10 (5)	5 (6)	(5)	(6)
	16:0	18:3			10	15		
	16:0	20:3			10		12	12
	18:0	20:3				5	11	12
Tetraenes	16:0	20:4	35	41	65	60	25	31
	18:0	20:4	57	45	20	17	58	29
	18:1	20:3	3 (38)	1 (19)	5 (23)	6 (13)	3 (23)	1 (18)
	18:2	18:2	5	12	5	17	8	36
	18:2	20:2		1				
Pentaenes	16:1	20:4	7	6	3	12		
	16:0	22:5			30	10	17	4
	18:1	20:4	33	66	30	60	14	80
	18:0	20:5	(4)	9 (5)	3 (3)	5 (5)	11 (3)	8 (5)
	18:2	20:3				5	21	18
	16:0	20:5	26	6	30		28	8
	14:0	20:5	7					
Hexaenes	14:0	22:6					2	
	16:0	22:6	20	20	70	36	33	21
	18:0	22:6	50 (10)	20 (7)	14 (7)	10 (6)	23 (21)	19 (12)
	18:1	20:5		3			2	1
	18:1	22:5	5	3	2	5	5	1
	18:2	20:4	25	40	10	49	26	55

* Estimated best fit only, not absolute identities, or proportions. Footnotes as in Tables 1–3.

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of (18:2 20:4) lecithin were also present and accounted for about half of the values (40–55%) calculated on a random basis.

The data show that despite the considerable differences in the proportions of the various saturated and unsaturated lecithins, the same molecular species occur in all three tissues. Furthermore, the proportions and makeup of the species are closely related to the over-all fatty acid composition of the lecithin mixture. Thus, the percentages of the tetraenes and hexaenes, and even those of the monoenes and dienes, agree reasonably well with those derived from doubling the mole percentage of the major unsaturated fatty acid in the hexaenoic, tetraenoic, dienoic, and monoenoic group of lecithins. This estimate is most accurate for the polyenes, but occasionally may be in considerable error for the monoenes and dienes, the proportion of which may be more sensitive to diet.

The lack of preferential pairing of specific saturated and unsaturated fatty acids on a mass basis observed in this study does not necessarily contradict the findings of Collins (2) and Balint, Beeler, Treble, and Spitzer (4), since the present studies are static rather than dynamic. Our data also give no information on the extent to which exchanges of specific molecular species of lecithins take place between the plasma lipoproteins and the tissue membranes, although it is obvious that a complete equilibration does not occur. We hope that in the future we will be able to provide the dynamic data on both counts.

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