Serum Phospholipids of Pigs Given Different Amounts of Linoleic Acid

1. FATTY ACID COMPOSITION OF THE KEPHALIN, LECITHIN, LYSOLECITHIN AND SPHINGOMYELIN FRACTIONS

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(Received 25 September 1963)

By using silicic acid and gas-liquid chromatography it has been shown that 70–75 % of the eicosatrienoic acid that accumulates in the serum lipids of pigs reared on diets low in linoleic acid is associated with the phospholipid fraction (Leat, 1963). This fraction has now been separated further into 'kephalin', lecithin, lysolecithin and sphingomyelin fractions in an attempt to determine the effect of dietary linoleate on the fatty acid composition of the individual serum phosphatides and to determine more precisely the location of the eicosatrienoic acid.

EXPERIMENTAL

Estimation of phospholipids by using silicic acid-impregnated paper. Serum lipids were extracted by the method of Marinetti, Albrecht, Ford & Stotz (1959) and the phospholipids separated on silicic acid-impregnated paper with di-isobutyl ketone-acetic acid-iso-osmotic saline (40:20:3, by vol.) (Marinetti, 1962). The completed chromatograms were stained with Ponceau Red and the percentage compositions of the phosphatides estimated (Hooghwinkel & van Niekerk, 1960; Böttcher & van Gent, 1961).

Extraction of phospholipids from serum. The serum lipids from six pigs given different amounts of linoleic acid (Table 1) were extracted and the phospholipids separated by dialysis through a rubber membrane as described by Böttcher, Woodford, Boelsma-van Houte & van Gent (1959).

Separation of phospholipids by column chromatography on silicic acid. The phospholipids from 50 ml. of serum were chromatographed on a silicic acid column prepared as described by Böttcher & van Gent (1961). The chromatographic procedure was modified slightly in that, instead of a gradient elution, a stepwise elution involving the following solvents was used (C. J. F. Böttcher & C. M. van Gent, personal communication): fraction I, 100 ml. of chloroform; fraction II, 200 ml. of methanol-chloroform (1:4, v/v); fraction III, 300 ml. of methanol-chloroform (3:2, v/v); fraction IV, 100 ml. of methanol-chloroform-water (20:4:1, by vol.). The fractions were evaporated almost to dryness on a rotary evaporator at 40° and the remainder of the solvent was removed under a rapid stream of nitrogen. The lipids were immediately redissolved in chloroform and a suitable portion was spotted on silicic acid-impregnated paper which was then developed and stained as described above.

The paper-chromatographic separation of the total serum lipids showed three constituents staining with Ponceau Red, representing lecithin (R_F 0.60), sphingomyelin (R_F 0.48) and lysolecithin (R_F 0.40). Fraction I from the column chromatography contained an unknown component with R_F 0.80, and fraction II contained three components, with R_F 0.91 (unknown), R_F 0.70 (phosphatidylethanolamine) and R_F 0.43 (unknown). The components of fraction II are referred to below as 'kephalins'. Fraction III contained two components, with R_F 0.60 (lecithin) and R_F 0.48 (sphingomyelin), and fraction IV contained two components, with R_F 0.48 (sphingomyelin) and R_F 0.40 (lysolecithin). To obtain suitable separation, the lipid equivalent of 1 ml. of serum from each of fractions I and II, 0.03 ml. of serum from fraction III and 0.15 ml. of serum from fraction IV were used for identification on silicic acid-impregnated papers.

Isolation of the fatty acids from the chromatographic fractions of column chromatography. Fraction I was discarded as it consisted mainly of neutral lipids that had resisted dialysis. The methyl esters of the fatty acids of fraction II were prepared by refluxing for 1 hr. with methanolic 3*N*-hydrochloric acid. The fatty acids of lecithin and lysolecithin from fractions III and IV were obtained by differential alkaline hydrolysis followed by silicic acid chromatography (Böttcher & van Gent, 1961), and the methyl esters were prepared by refluxing the fatty acids for 1 hr. with methanolic 2% (v/v) sulphuric acid (Leat, 1963). The alkali-stable fraction (sphingomyelin) was transmethyalted for 3 hr. with methanolic 3*N*-hydrochloric acid to obtain the methyl esters. Quinol (5% of weight of lipid) was added to all refluxing solutions. The methyl esters were analysed by gas-liquid chromatography on poly(ethylene glycol adipate) as described by Leat (1963).

Statistical evaluation. The results from pigs 1–4 (Table 1) were compared with those from pigs 5 and 6 by using Student's *t* test.

RESULTS

The percentage composition of the serum phospholipids of pigs reared on various amounts of dietary linoleic acid as determined by chromatography on silicic acid-impregnated paper is shown in Table 1. Lecithin accounted for 60–70 % of the total phospholipid, with sphingomyelin and lysolecithin each accounting for 15–20 %. The percentage of lecithin was increased when dietary linoleate was low, which is in agreement with other work (Howard, Leat, Gresham & Bowyer, 1964).

The fatty acid compositions of the total phospholipids of serum and of the component phosphatides, lecithin, lysolecithin, kephalin and sphingomyelin are depicted in Tables 2-6 and Figs. 1 and 2.

When dietary linoleate was decreased from 3.7 to 0.1% of the calories, the following changes occurred in the composition of the fatty acids of serum phospholipids.

Total phospholipids (Table 2). The octadecadienoic acid content fell from 11.3 to 5.3% and that of eicosatetraenoic acid from 7.4 to 2.3%, but that of eicosatrienoic acid rose from 1.4 to 7.5%.

Lecithin (Table 3 and Fig. 1). The octadecadienoic acid content fell from 13.6 to 6.1% and that of eicosatetraenoic acid from 9.1 to 3.2%, but that of eicosatrienoic acid rose from 1.9 to 9.3%. About 60% of the fatty acids was unsaturated and over 90% of the eicosatrienoic acid plus eicosatetraenoic acid of the phospholipids was located in the lecithin.

Lysolecithin (Table 4). The eicosatrienoic acid content rose from 0.4 to 4.1%, but the contents of eicosatetraenoic acid and octadecadienoic acid remained unchanged. About 40% of the fatty acids was unsaturated.

Table 1. *Percentage composition of the serum phospholipids of pigs given different amounts of linoleic acid as olive oil*

Experimental details are given in the text.

	Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6
Dietary linoleate (% of total calories)	0.07	0.07	0.30	0.52	0.97	3.67
Total phospholipids (mg./100 ml. of serum)	125	150	138	130	148	150
Lecithin (%)	69.2	68.0	67.0	60.7	58.3	59.8
Sphingomyelin (%)	16.2	13.4	14.5	21.3	20.5	19.5
Lysolecithin (%)	14.6	18.6	18.5	18.0	21.2	20.7

Table 2. *Percentage composition of the fatty acids of the serum phospholipids of pigs given different amounts of linoleic acid*

Experimental details are given in the text. Some minor fatty acids have been omitted for clarity. The carbon no. was calculated by the method of Woodford & van Gent (1960). The designation of the fatty acids follows the system recommended by Farquhar, Insull, Rosen, Stoffel & Ahrens (1959). Percentage composition is calculated

as: $\frac{\text{peak area of fatty acid}}{\text{total peak area}} \times 100.$

Carbon no. on poly- (ethylene glycol adipate)	Designation	Description or trivial name	Percentage composition of fatty acids					
			Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6
12.0	12:0	Lauric acid	+	0.3	+	+	0.2	0.2
14.0	14:0	Myristic acid	0.3	0.3	0.3	0.3	0.2	0.4
16.0	16:0	Palmitic acid	19.8	23.6	20.3	19.8	21.7	19.2
16.3	16:1	Palmitoleic acid	1.4	1.6	2.1	1.5	1.7	1.6
17.0	17:0	.	0.7	0.9	0.6	0.8	0.6	0.7
17.3	17:1	.	0.3	0.3	0.2	0.1	0.1	0.1
18.0	18:0	Stearic acid	22.5	23.4	19.4	21.3	19.7	18.7
18.3	18:1	Oleic acid	27.5	29.1	26.0	25.4	25.7	27.2
18.9	18:2	Linoleic acid (octadecadienoic acid)	6.1	4.2	7.9	8.2	8.3	11.3
19.6	18:3	Linolenic acid	0.4	0.3	0.4	0.3	0.3	0.4
20.0	20:0	Arachidic acid	0.7	1.0	0.8	1.0	0.9	1.4
20.6	20:2?	.	0.9	1.0	0.5	0.3	0.2	0.1
21.0	20:3	Eicosatrienoic acid	7.7	7.2	5.6	5.0	3.0	1.4
21.2	?	.	0.6	0.8	0.9	1.0	0.9	0.3
21.5	20:4	Arachidonic acid (eicosatetraenoic acid)	2.6	2.0	3.9	5.8	7.5	7.4
22.0	22:0	Behenic acid	0.3	0.3	0.5	0.4	0.6	0.5
22.3	22:1	.	2.6	0.4	2.1	1.4	1.1	2.1
23.0	23:0	.	0.8	0.4	0.9	0.7	1.5	0.6
24.0	24:0	.	0.6	0.4	0.7	0.9	0.4	1.0
24.2	24:1	.	1.8	0.7	3.1	2.4	2.7	2.3
	20:3 20:4 ratio		3.0	3.1	1.4	0.9	0.4	0.2
	Total unsaturated acids		54.9	49.6	57.2	55.3	55.6	57.8

Table 3. *Percentage composition of the fatty acids of serum lecithins of pigs given different amounts of linoleic acid*

Experimental details are given in the text. Other details are as given in Table 2.

Carbon no. on poly- (ethylene glycol adipate)	Designation	Percentage composition of fatty acids					
		Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6
14.0	14:0	0.3	0.2	0.2	0.1	0.2	0.2
15.0	15:0	0.2	0.2	0.1	0.1	0.1	0.1
16.0	16:0	16.8	16.7	19.9	17.0	18.6	16.8
16.3	16:1	2.1	1.5	1.2	1.3	0.9	1.3
17.0	17:0	0.5	0.8	0.5	0.7	0.6	0.7
17.3	17:1	0.4	0.3	0.3	0.1	0.1	0.1
18.0	18:0	21.7	22.4	19.9	24.7	18.5	21.7
18.3	18:1	32.2	33.2	30.6	28.6	28.4	29.4
18.9	18:2	6.6	5.6	7.6	9.3	11.2	13.6
19.6	18:3	0.5	0.4	0.5	0.3	0.2	0.2
20.0	20:0	0.2	0.2	0.1	0.2	0.1	0.1
20.6	20:2	0.9	1.1	0.1	0.5	0.1	0.1
21.0	20:3	8.9	9.6	7.1	5.6	3.9	1.9
21.2	21:1?	0.9	1.0	1.2	1.4	1.3	0.9
21.5	20:4	3.4	3.0	4.5	6.3	9.7	9.1
21.8	?	0.4	0.2	0.6	0.3	0.3	0.1
22.3	22:1	0.6	0.8	0.5	0.5	0.5	0.3
23.0	23:0	0.2	0.2	0.6	0.2	Trace	0.3
23.6	?	0.5	0.2	0.5	0.4	0.7	0.5
23.9	?	0.3	Trace	0.7	0.3	0.2	0.1
24.3	24:1	1.3	1.1	1.3	1.0	1.9	1.2
24.6	?	0.8	0.8	0.9	0.6	1.1	0.9
	$\frac{20:3}{20:4}$ ratio	2.6	3.2	1.5	0.9	0.4	0.2
Total unsaturated acids		59.6	59.9	58.7	56.9	60.6	60.0

Table 4. *Percentage composition of the fatty acids of serum lysolecithin of pigs given different amounts of linoleic acid*

Experimental details are given in the text. Other details are as given in Table 2.

Carbon no. on poly- (ethylene glycol adipate)	Designation	Percentage composition of fatty acids					
		Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6
14.0	14:0	0.6	0.5	0.7	0.4	0.3	0.4
15.0	15:0	0.3	0.4	0.2	0.3	0.2	0.3
16.0	16:0	31.9	28.0	29.2	32.7	30.7	29.9
16.3	16:1	3.2	2.8	3.9	2.9	3.8	2.3
17.0	17:0	0.9	0.7	0.5	1.3	0.6	1.2
17.3	17:1	0.3	0.2	0.3	0.5	3.5	2.9
18.0	18:0	24.5	28.2	18.7	29.2	25.8	29.0
18.3	18:1	26.7	28.5	38.4	24.0	22.7	22.5
18.9	18:2	4.6	2.7	3.4	3.1	3.6	3.9
19.6	18:3	0.1	—	0.2	0.3	0.2	0.5
20.0	20:0	0.3	0.6	0.4	0.1	Trace	0.4
20.6	20:2	0.7	0.7	0.3	0.3	Trace	0.3
21.0	20:3	3.8	4.4	1.9	1.9	0.9	0.4
21.2	21:1	0.2	0.2	0.2	0.4	0.2	0.2
21.5	20:4	1.0	1.2	1.1	1.8	1.7	1.6
> 21.5		Trace	Trace	Trace	Trace	Trace	Trace
	$\frac{20:3}{20:4}$ ratio	3.8	3.5	1.7	1.0	0.5	0.3
Total unsaturated acids		41.3	41.2	50.1	35.5	36.9	35.2

Table 5. *Percentage composition of the fatty acids of serum 'kephalins' of pigs given different amounts of linoleic acid*

Experimental details are given in the text. Other details are as given in Table 2.

Carbon no. on poly- (ethylene glycol adipate)	Designation	Percentage composition of fatty acids					
		Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6
14.0	14:0	0.9	0.5	0.2	0.1	0.6	0.7
15.0	15:0	0.4	0.4	0.1	0.2	0.5	0.5
16.0	16:0	14.5	17.3	10.2	9.7	9.8	11.1
16.3	16:1	3.8	2.7	2.0	1.8	2.0	2.9
17.0	17:0	0.9	1.1	0.7	0.8	0.6	0.4
17.3	17:1	0.5	0.4	0.1	0.1	0.4	0.2
18.0	18:0	24.5	27.8	24.8	25.8	21.0	26.0
18.3	18:1	24.2	19.6	20.9	20.6	22.8	21.0
18.9	18:2	5.6	4.2	4.0	4.7	4.1	6.7
19.6	18:3	0.2	0.8	1.2	0.4	0.3	0.5
20.0	20:0	0.7	2.0	1.6	1.6	2.0	1.6
20.6	20:2	0.9	1.8	1.4	0.8	0.7	0.1
21.0	20:3	6.6	7.8	7.3	6.3	3.3	1.7
21.2	21:1	0.5	0.8	0.7	0.7	0.4	0.4
21.5	20:4	5.0	5.1	9.0	13.6	17.2	17.6
> 21.5		9.0	5.6	12.5	11.7	10.9	7.4
20:3 20:4 ratio		1.3	1.5	0.8	0.5	0.2	0.1
Total unsaturated acids		54.4	47.9	56.3	57.3	59.1	57.5

Table 6. *Percentage composition of the fatty acids of serum sphingomyelin of pigs given different amounts of linoleic acid*

Experimental details are given in the text. Other details are as given in Table 2.

Carbon no. on poly- (ethylene glycol adipate)	Designation	Percentage composition of fatty acids					
		Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6
14.0	14:0	Trace	0.6	0.7	Trace	0.5	Trace
15.0	15:0	Trace	0.4	0.2	Trace	0.2	Trace
16.0	16:0	17.6	25.3	23.4	14.9	28.5	30.0
16.3	16:1	2.4	Trace	2.4	0.9	+	Trace
17.0	17:0	0.6	1.1	0.7	0.7	0.9	0.9
17.3	17:1	0.2	Trace	0.2	0.4	0.2	0.1
18.0	18:0	10.4	11.5	9.0	9.0	12.2	8.9
18.3	18:1	8.0	4.6	5.7	3.3	5.5	3.5
18.9	18:2	1.6	0.2	0.3	1.2	1.6	0.8
19.0	19:0						
19.6	18:3	Trace	—	0.9	1.0	0.6	0.5
20.0	20:0	5.1	10.3	7.9	8.8	10.4	10.3
20.1	?	0.8	—	1.1	0.8	Trace	—
20.6	20:2	—	—	0.7	—	0.5	0.7
21.0	{ 20:3 21:0 }	2.0	1.5	1.0	1.3	1.5	1.5
21.5	20:4?	1.0	—	—	0.7	1.4	0.9
22.0	22:0	7.5	7.9	5.6	10.7	5.9	6.8
22.3	22:1	1.4	0.6	5.0	—	0.3	—
22.5	?	1.0	—	—	—	1.0	2.3
23.0	23:0	8.2	8.2	5.3	11.9	5.2	6.4
23.3	23:1?	—	0.4	1.1	—	—	—
24.0	24:0	17.2	11.1	10.3	20.4	7.8	12.0
24.3	24:1	12.2	15.6	14.8	11.5	13.3	12.1
24.6	?	2.4	—	2.7	—	1.0	1.3
24.8	?	1.4	—	0.9	1.5	0.9	0.8
Total unsaturated acids		33.4	23.2	36.9	22.6	27.9	24.9

Fig. 1. Fatty acid composition of (a) serum kephalins and (b) serum lecithins. ○, Eicosatrienoic acid; ●, arachidonic acid; ▲, linoleic acid; △, oleic acid. Experimental details are given in the text.

Kephalins (Table 5 and Fig. 1). The eicosatrienoic acid content rose from 1.7 to 7.2% and that of eicosatetraenoic acid fell from 17.6 to 5.0%. About 55% of the fatty acids was unsaturated. A large number of minor acids having retention times greater than that of arachidonic acid were also present.

Sphingomyelin (Table 6). This fraction was characterized by the presence of large amounts of long-chain fatty acids, up to 57% of the total fatty acids having retention times greater than that of arachidonic acid. About 25% of the fatty acids was unsaturated. Alteration in dietary linoleate had no effect on the fatty acid composition of the sphingomyelins.

Fig. 2. Total amounts of (a) arachidonic acid and (b) eicosatrienoic acid located in the total phospholipids (●) and in the lecithin (○) of serum. Experimental details are given in the text.

DISCUSSION

Although alterations in dietary linoleate had little effect on the total phospholipids of serum there was a change in the percentage composition of the component phosphatides: lecithin increased at the expense of lysolecithin and sphingomyelin when dietary linoleate was decreased. Collins (1962) has shown that the turnover of lecithins and kephalins in the rat liver is increased in essential-fatty acid deficiency, and this increase may be reflected in serum lecithin concentrations. The increase in the percentage of lecithin in serum phospholipids may also reflect changes in the serum lipoproteins, which are known to possess differing phospholipid patterns (Nelson & Freeman, 1960).

Fatty acids of lecithin. The fatty acids of lecithin accounted for 70–75% of the total phospholipid fatty acids, to which they thus bore a marked resemblance. The fatty acids of lecithin were more unsaturated than those of the total phospholipids, containing more oleic acid, linoleic acid, arachidonic acid and eicosatrienoic acid, and less palmitic acid. The major changes in fatty acid composition

occurred when dietary linoleate provided less than 1% of the calories (Fig. 1). The percentages of octadecadienoic acid and eicosatetraenoic acid were lower ($P < 0.05$ and $P < 0.02$ respectively) and that of eicosatrienoic acid higher ($P < 0.05$) when dietary linoleate supplied less than 1.0% of the calories than when it supplied more than 1.0%. Increasing the dietary linoleate to more than 1% of the calories had little further effect on the fatty acid composition of lecithin, a similar observation being made on the other serum lipids of these pigs (Leat, 1963). Over 90% of the eicosatrienoic acid plus eicosatetraenoic acid of phospholipids was located in the lecithin (Fig. 2). This fraction therefore contained 63–69% of the eicosatrienoic acid plus eicosatetraenoic acid of serum, as 70–75% of these acids in serum was associated with the phospholipids. The decrease in the percentage of arachidonic acid in lecithin, when dietary linoleate was decreased to less than 1% of the calories, was almost exactly balanced by the rise in that of eicosatrienoic acid, so that the sum of eicosatrienoic acid and tetraenoic acid remained constant at all amounts of dietary linoleate. Therefore, chemically, the arachidonic acid of lecithin can be replaced by eicosatrienoic acid. Between 3 and 5% of the fatty acids of lecithin had retention times greater than that of arachidonic acid, and these probably represent the highly unsaturated pentaenoic acid and hexaenoic acid detected by alkali isomerization (Leat, 1962). Klenk & Oette (1960) have shown that in linoleic acid deficiency in rats an eicosatetraenoic acid that is not arachidonic acid was found in tissue lipids. However, in my experiments it is assumed that the fatty acid having a carbon no. of 21.5 is almost entirely arachidonic acid. This appears to be justifiable as pigs reared for 6 months on diets deficient in linoleic acid contain very little fatty acid of this carbon number (less than 1%) in the serum lipids (Howard *et al.* 1964). A fatty acid of carbon no. 21.2 was noted in the phospholipid fatty acids and to a lesser extent in the other lipid fractions of serum. This acid was difficult to estimate accurately as it was incompletely resolved from the eicosatrienoic acid that had carbon no. 21.0. This acid may be one of the isomeric eicosatrienoic acids noted in liver lipids of rats reared on diets of differing fatty acid composition (Morin, Bernick, Mead & Alfin-Slater, 1962).

Fatty acids of lysolecithin. The fatty acids of lysolecithin were more saturated than those of lecithin, containing more palmitic acid and stearic acid, and less oleic acid and polyunsaturated acids. This finding is in agreement with the observations on the fatty acid composition of the lysolecithin of human plasma (Gjone, Berry & Turner, 1959). As the fatty acids attached to the β -position of the lecithin molecule are almost exclusively un-

saturated (Tattrie, 1959; Hanahan, Watts & Pappajohn, 1960), it would appear that the fatty acids of serum lysolecithin are attached predominantly to the α -position. This is supported by the finding in lysolecithin of only trace amounts of fatty acids having retention times greater than that of arachidonic acid. These acids account for 3–5% of the fatty acids of lecithin and are attached exclusively to the β -position of the molecule (Leat, 1964). The predominance of lysolecithins, esterified mainly at the α -position, is in marked contrast with the lysolecithins of aorta which are a mixture of α - and β -types (Böttcher & van Gent, 1961). This is indirect evidence that the lysolecithins of aorta are not derived as such from the plasma. Unlike the fatty acids of lecithin, those of lysolecithin were relatively unaffected by changes in dietary linoleate. The eicosatrienoic acid content tended to rise when dietary linoleate was low but linoleic acid and arachidonic acid were unaffected.

Fatty acids of kephalins. The fatty acids of the kephalin fraction were derived from at least three components, one of which was phosphatidylethanolamine. Ponceau Red does not stain such phospholipids as phosphatidylserine and phosphoinositide (Klibansky & de Vries, 1963), and thus the kephalin fraction probably contains more than the three components stained by this dye. Whether this fraction is a true component of serum is not known, but it probably is derived from platelets, which are a rich source of kephalin (Erickson, Williams, Avrin & Lee, 1939). The most notable feature of the fatty acids of the 'kephalin' fraction was the high content (17%) of arachidonic acid. When dietary linoleate supplied less than 1.0% of the calories, the percentage of arachidonic acid was lower ($P < 0.05$) and that of eicosatrienoic acid higher ($P < 0.01$) than when it supplied more than 1.0%. Increasing the dietary linoleate to more than 1% of the calories did not raise the percentage of arachidonic acid further. Although the kephalin fraction is a complex mixture it is possible that these changes in polyunsaturated fatty acids can be attributed to the phosphatidylethanolamine component, which is known to have a high content of arachidonic acid (Marcus, Ullman, Safier & Ballard, 1962). The decrease in the percentage of arachidonic acid when dietary linoleate was diminished was not balanced by a rise in that of eicosatrienoic acid; this is in contrast with the observation on lecithin. This suggests that a deficiency of arachidonic acid is not as readily replaced by eicosatrienoic acid in the kephalins as in the lecithins. Although no quantitative information is available on the components of the kephalin fraction it is tentatively suggested that the function of arachidonic acid may be concerned with the correct metabolism of kephalins and in particu-

lar phosphatidylethanolamine. The considerable changes in the percentages of arachidonic acid and eicosatrienoic acid in kephalins were not reflected in the total phospholipids of serum because the kephalins were present in very small amounts and could only be detected when the lecithin, lysolecithin and sphingomyelin of serum had been removed.

Fatty acids of sphingomyelin. The fatty acid composition of sphingomyelin, unlike that of lecithin and of kephalin, was unaffected by changes in dietary linoleate. The polyunsaturated fatty acid content was low and up to 80 % of the fatty acids was saturated. The fraction was characterized by the presence of long-chain acids containing 22, 23 and 24 carbon atoms, up to 57 % of the fatty acids having retention times greater than that of arachidonic acid. The fatty acid composition of sphingomyelin reported in the present paper agrees well with that reported by Hanahan *et al.* (1960) for sphingomyelin in human plasma.

Thus the individual phospholipids of serum have characteristic fatty acid patterns that are affected differently by essential-fatty acid deficiency. The fatty acid compositions of lysolecithin and sphingomyelin are little affected by changes in dietary linoleate, but those of lecithin and kephalin are markedly altered although the total percentage of unsaturated acids remains unchanged. In lecithin, the decrease in the percentage of arachidonic acid when dietary linoleate is decreased to less than 1 % of the calories is compensated by a rise in that of eicosatrienoic acid. However, in the kephalins the decrease in the percentage of arachidonic acid is not balanced by the rise in that of eicosatrienoic acid.

It is tentatively suggested therefore that the role of arachidonic acid is concerned with the correct functioning of kephalins, which are known to be major components of cell membranes.

SUMMARY

1. The fatty acid composition of the serum phospholipids of six pigs, reared on various amounts of dietary linoleate, have been examined.

2. In the lecithin fraction decreasing the dietary linoleate resulted in a diminution in the percent-

ages of linoleic acid and arachidonic acid, and an increase in that of eicosatrienoic acid.

3. In the kephalin fraction there was a fall in the percentage of arachidonic acid, and this greatly exceeded the rise in that of eicosatrienoic acid.

4. The fatty acids of the lysolecithin and sphingomyelin fractions were least affected by changes in dietary linoleate.

5. When dietary linoleate was decreased to less than 1 % of the calories the percentage of lecithin in serum phospholipids increased at the expense of lysolecithin and sphingomyelin.

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