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Fatty Acid Composition of the Serum Lipids of Pigs Given Different Amounts of Linoleic Acid

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Nunn & Smedley-MacLean (1938) were the first to show that in essential-fatty acid deficiency a trienoic acid accumulated in liver lipids. This has since been identified as an eicosatrienoic acid derived mainly from oleic acid (Mead & Slaton, 1956; Fulco & Mead, 1959).

During an investigation of the requirement of the pig for essential fatty acids, by using the trienoic acid:tetraenoic acid ratio as an index of deficiency (Holman, 1960), it was noted that although the lipids of serum, liver and heart had high contents of trienoic acid when diets low in linoleic acid were given, those of adipose tissue had very low amounts (Leat, 1962b). This suggested that as adipose tissue consists mainly of triglycerides the trienoic acid was associated with lipid fractions other than the triglycerides. That one of these fractions is probably phospholipid is indicated by the accumulation of high amounts of a trienoic acid in the serum phospholipids of dogs on low-fat diets (Wiese, Hansen & Baugham, 1957).

As large volumes of serum were available from pigs in various stages of essential-fatty acid deficiency it was considered of value to investigate further the distribution of eicosatrienoic acid and other fatty acids by more specific methods of separation and analysis. In this way changes in fatty acid pattern caused by gradual changes in dietary linoleate could be studied. A preliminary report has been published (Leat, 1962a).

EXPERIMENTAL

Animals. Six pigs were reared from 10 to 200 lb. live weight on diets containing respectively 0.07, 0.07, 0.30, 0.52, 0.97 and 3.67% of the calories as linoleic acid (Leat,

1962b). Olive oil provided the linoleate in the last four diets, replacing an isocaloric weight of carbohydrate (Table 1). At slaughter after overnight deprivation of food, the blood was collected, defibrinated and centrifuged to obtain the serum.

Extraction. A portion (100 ml.) of serum was poured, with stirring, into 500 ml. of ethanol-ether (3:1, v/v) and allowed to stand for 1 hr. in the dark with occasional shaking. The extract was filtered through a sintered-glass funnel and the protein precipitate extracted twice by a similar procedure using 200 ml. of solvent. Finally the protein cake was suspended in 100 ml. of methanol-chloroform (1:1, v/v) and allowed to stand overnight before filtering. The combined filtrates were evaporated almost to dryness at 40° in a rotary evaporator and the lipids dissolved in 5 ml. of methanol-chloroform (1:2, v/v).

Separation of lipids. The lipid extract was purified and separated into four fractions (as described by Böttcher, Woodford, Boelsma-van Houte & van Gent, 1959): (i) non-esterified fatty acids; (ii) sterol esters; (iii) triglycerides and free cholesterol; (iv) phospholipids. The fractions, with the exception of the phospholipids, were evaporated to dryness under a stream of nitrogen and were maintained in the dry state for the shortest possible time required to weigh them. They were then immediately redissolved in methanol-chloroform (1:2, v/v) and stored at 0° in the presence of quinol (5% of weight of lipid). The sterol ester and triglyceride fractions were saponified by refluxing for 30 min. with methanolic 0.5N-potassium hydroxide solution. The non-saponifiable fraction was removed by shaking twice with redistilled light petroleum (b.p. 40–60°). The fatty acids were liberated with N-sulphuric acid and extracted with light petroleum (3 × 20 ml.). The bulked light-petroleum extracts were then washed free of acid with water and dried over anhydrous sodium sulphate. The solvent was removed under a stream of nitrogen and the fatty acids were converted into their methyl esters by refluxing for 1 hr. with methanolic 2% (v/v) sulphuric acid. The methyl esters of the phospholipid fatty acids were obtained by refluxing for 3 hr. with methanolic 3N-hydrochloric acid. Quinol was added to all refluxing solutions to minimize oxidation.

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Gas chromatography. The methyl esters were separated at 190° on a Pye Argon Gas Chromatograph incorporating a strontium detector (W. G. Pye and Co. Ltd., Cambridge), with a column containing 10% of poly(ethylene glycol adipate) on 100–120 mesh Celite. Peak areas were determined by triangulation (Keulemans, 1957). Individual fatty acids were identified by (a) carbon numbers (Woodford & van Gent, 1960) on poly(ethylene glycol adipate) mainly but also with occasional use of Apiezon L columns, (b) hydrogenation or bromination followed by chromatography, (c) chromatography with mixtures of known fatty acids as internal standards. Structural assignments are according to Farquhar, Insull, Rosen, Stoffel & Ahrens (1959).

Total fatty acids. The total fatty acids of serum were saponified and extracted and estimated by the method of Pikaar & Nijhof (1958) and the methyl esters analysed by gas-liquid chromatography.

Cholesterol. The method of Abell, Levy, Brodie & Kendall (1952) was employed.

Phospholipids. Phosphorus was estimated by the method of Allen (1940) and the value for total phospholipid obtained by multiplication by 25.

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

RESULTS

The composition of the serum lipids of pigs reared on different dietary concentrations of linoleic acid is shown in Table 1. Increasing the percentage of olive oil in the diet had little effect on the individual serum lipids, with the possible exception of the concentration of cholesterol, which tended to rise.

The percentage composition of the total fatty acids of serum and of the fatty acids of the various lipid fractions are depicted in Tables 2–7 and Figs. 1 and 2.

Table 1. *Composition (mg./100 ml.) of serum lipids of pigs given varying amounts of linoleic acid as olive oil*

Pig no.	...	...	...	...	...	1	2	3	4	5	6
Olive oil (% w/w)	...	...	...	...	...	0	0	1	2	4	16
Dietary linoleate as a percentage of the total calories						0.07	0.07	0.30	0.52	0.97	3.67
Total fatty acids						190	182	173	193	185	211
Total cholesterol						101	110	107	117	121	125
Total phospholipid						125	150	138	130	148	150
Non-esterified fatty acids						3.6	3.8	4.4	2.2	4.4	4.6
Cholesterol esters						149.6	159.3	144.0	167.0	171.6	172.4
Unesterified cholesterol						15.8	17.6	18.8	16.8	17.8	20.7
Triglycerides						39.2	26.4	60.8	30.8	26.8	67.1

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

In this and subsequent Tables percentage composition is calculated as (peak area of fatty acid/total peak area) × 100. Some minor fatty acids have been omitted for clarity.

Carbon no.* on poly(ethylene glycol adipate)	Designation†	Description or trivial name of acid	Pig no.					
			1	2	3	4	5	6
12:0	12:0	Lauric	0.6	0.2	0.2	0.2	0.3	0.8
14:0	14:0	Myristic	0.8	0.6	0.7	0.6	0.5	0.9
16:0	16:0	Palmitic	13.7	16.1	15.3	16.2	14.5	13.2
16:3	16:1	Palmitoleic	4.9	5.1	4.1	5.0	2.8	3.3
17:0	17:0	—	0.6	0.5	0.7	0.5	0.4	0.4
17:3	17:1	—	0.8	0.8	0.6	0.8	0.3	0.2
18:0	18:0	Stearic	13.7	12.4	11.7	13.1	12.3	10.0
18:3	18:1	Oleic	44.1	45.1	43.7	37.5	40.6	43.2
18:9	18:2	Octadecadienoic (linoleic)	9.1	8.9	13.6	15.0	18.3	19.6
19:6	18:3	Linolenic	0.6	0.7	0.4	0.9	0.5	0.4
20:0	20:0	Arachidic	0.2	0.2	0.1	0.1	0.1	0.2
20:6	20:2?	—	0.8	0.7	0.5	0.6	0.5	0.3
21:0	20:3	Eicosatrienoic	6.0	5.3	3.8	3.3	2.1	0.8
21:2	?	—	0.4	0.4	0.5	0.7	0.6	0.7
21:5	20:4	Eicosatetraenoic (arachidonic)	2.6	2.1	3.1	3.9	5.2	4.7
	20:3	—	2.3	2.5	1.2	0.9	0.4	0.2
	20:4							
Total unsaturated acids			70.2	69.8	71.1	69.1	71.8	74.3

* Woodford & van Gent (1960).

† Number of carbon atoms:number of double bonds.

In the total fatty acids of serum (Table 2) there was a fall in octadecadienoic acid from 19.6 to 9.0% and in eicosatetraenoic acid from 4.7 to 2.4% when dietary linoleate was reduced from 3.7 to 0.1% of the calories. This decrease was balanced to some extent by a rise in eicosatrienoic acid from 0.8 to 5.7% and in palmitoleic acid from 3.3 to

5.0%. These alterations in fatty acid patterns were more marked in some lipid fractions than in others, the major changes being shown below.

Sterol esters (Table 3 and Fig. 1). Octadecadienoic acid fell from 32.6 to 15.2%, palmitoleic acid rose from 1.8 to 6.6% and oleic acid from 51.5 to 58.4%. Eicosatetraenoic acid fell from 3.2 to

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

See also Table 2. tr., Trace.

Carbon no. on poly(ethylene glycol adipate)	Designation	Pig no.					
		1	2	3	4	5	6
12.0	12:0	tr.	tr.	tr.	tr.	tr.	tr.
14.0	14:0	0.7	0.7	0.6	0.5	0.4	0.2
16.0	16:0	7.8	8.1	9.9	8.4	8.4	6.5
16.3	16:1	6.1	7.1	4.5	3.8	2.7	1.8
17.0	17:0	0.2	0.3	0.2	0.2	0.1	0.2
17.3	17:1	1.1	1.0	0.7	0.9	0.7	0.6
18.0	18:0	2.3	3.1	3.1	2.5	1.8	1.5
18.3	18:1	58.3	58.5	54.2	51.8	50.0	51.5
18.9	18:2	15.6	14.7	21.2	25.3	29.9	32.6
19.6	18:3	0.8	0.7	0.6	0.5	0.6	0.5
20.0	20:0	0.4	0.1	0.2	0.1	tr.	tr.
20.6	20:2?	0.4	0.2	0.3	0.2	0.1	0.3
21.0	20:3	2.5	2.2	1.4	1.3	0.5	0.2
21.2	?	0.3	tr.	0.3	0.4	0.2	tr.
21.5	20:4	1.8	1.5	1.8	2.6	3.5	3.2
22.3	22:1	0.3	0.5	0.1	0.2	tr.	0.3
	20:3	1.4	1.5	0.8	0.5	0.2	0.1
	20:4						
Total unsaturated acids		88.5	87.6	85.8	88.1	89.1	91.5

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

See also Table 2. tr., Trace.

Carbon no. on poly(ethylene glycol adipate)	Designation	Pig no.					
		1	2	3	4	5	6
12.0	12:0	tr.	0.3	tr.	tr.	0.2	0.2
14.0	14:0	0.3	0.3	0.3	0.3	0.2	0.4
16.0	16:0	19.8	23.6	20.3	19.8	21.7	19.2
16.3	16:1	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	22.5	23.4	19.4	21.3	19.7	18.7
18.3	18:1	27.5	29.1	26.0	25.4	25.7	27.2
18.9	18:2	6.1	4.2	7.9	8.2	8.3	11.3
19.6	18:3	0.4	0.3	0.4	0.3	0.3	0.4
20.0	20:0	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	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	2.6	2.0	3.9	5.8	7.5	7.4
22.0	22:0	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	3.0	3.1	1.4	0.9	0.4	0.2
	20:4						
Total unsaturated acids		54.9	49.6	57.2	55.3	55.6	57.8

1.7% and eicosatrienoic acid rose from 0.2 to 2.4%. The most notable feature of this fraction was that nearly 90% of the fatty acids were unsaturated.

Phospholipids (Table 4 and Fig. 1). Octadecadienoic acid fell from 11.3 to 5.3% and eicosatetraenoic acid from 7.4 to 2.3%, and eicosatrienoic acid rose from 1.4 to 7.5%. The phospholipid fatty acids were about 50% unsaturated and were characterized by the presence of C₂₂ and C₂₄ acids.

Triglycerides (Table 5). Octadecadienoic acid fell from 8.5 to 2.5% and palmitoleic acid rose from 3.1 to 5.0%. The fatty acids were about 75% unsaturated and were characterized by the presence of 2-3% of lauric acid and myristic acid.

Non-esterified fatty acids (Table 6). Octadecadienoic acid fell from 10.8 to 7.0% and palmitoleic acid rose from 3.1 to 6.0%. About 60% of the fatty acids were unsaturated.

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

		See also Table 2. tr., Trace.					
Carbon no. on poly(ethylene glycol adipate)	Designation	Pig no.					
		1	2	3	4	5	6
12.0	12:0	2.3	1.5	2.3	1.3	1.3	2.6
14.0	14:0	2.5	1.6	2.4	1.7	1.5	2.7
16.0	16:0	14.4	16.1	14.7	14.2	15.9	14.6
16.3	16:1	4.5	5.5	5.0	4.5	3.2	3.1
17.0	17:0	0.5	0.6	0.8	0.6	0.5	0.8
17.3	17:1	0.9	1.0	0.6	0.6	0.3	0.4
18.0	18:0	5.6	4.8	4.9	6.0	6.1	6.1
18.3	18:1	57.8	59.2	58.4	61.4	58.1	56.7
18.9	18:2	2.6	2.3	4.8	4.5	6.2	8.5
19.6	18:3	0.5	0.5	0.3	0.2	0.3	0.4
20.0	20:0	0.2	0.1	0.1	0.2	0.2	0.2
20.6	20:2?	1.2	1.1	0.9	0.6	0.7	0.3
21.0	20:3	1.9	2.4	2.1	2.2	1.7	0.7
21.2	?	0.1	tr.	0.1	tr.	0.2	0.2
21.5	20:4	1.2	0.6	0.7	0.5	0.6	0.7
22.0	22:0	0.8	0.6	0.2	—	0.4	0.4
22.3	22:1	0.6	0.2	0.1	—	0.1	0.4
	20:3	1.6	4.0	3.0	4.4	2.8	1.0
	20:4						
Total unsaturated acids		73.4	74.6	74.4	75.8	72.8	72.4

Table 6. Percentage composition of the non-esterified fatty acids of serum from pigs given different amounts of linoleic acid

		See also Table 2. tr., Trace.					
Carbon no. on poly(ethylene glycol adipate)	Designation	Pig no.					
		1	2	3	4	5	6
12.0	12:0	0.8	1.3	0.2	0.4	0.8	0.3
14.0	14:0	3.0	2.3	1.0	2.0	1.6	1.6
16.0	16:0	24.3	18.8	25.5	21.3	26.2	26.9
16.3	16:1	4.6	7.4	4.5	4.0	3.7	3.1
17.0	17:0	0.4	0.7	0.6	0.4	0.9	0.9
17.3	17:1	0.6	1.0	0.4	0.5	0.4	0.3
18.0	18:0	13.3	8.1	15.6	10.7	13.5	15.9
18.3	18:1	36.3	39.6	34.9	50.2	35.8	31.9
18.9	18:2	6.3	7.7	6.5	5.5	7.2	10.8
19.6	18:3	0.4	0.7	0.3	0.5	0.3	0.3
20.0	20:0	0.2	0.3	0.2	0.1	0.5	0.3
20.6	20:2?	1.1	0.5	0.6	0.3	0.3	0.3
21.0	20:3	2.1	1.7	2.9	0.8	0.8	0.5
21.2	?	tr.	0.2	0.4	0.2	0.3	0.4
21.5	20:4	1.6	0.8	2.1	1.0	2.1	2.5
22.0	22:0	0.6	0.8	1.5	0.3	1.2	0.7
22.3	22:1	0.4	2.1	0.6	—	0.7	0.5
	20:3	1.3	2.1	1.4	0.8	0.4	0.2
	20:4						
Total unsaturated acids		56.4	66.5	54.8	64.6	54.2	52.4

Table 7. *Percentage composition of the fatty acids of the outer fat from the backs of pigs given different amounts of linoleic acid as olive oil*

Carbon no. on poly(ethylene glycol adipate)	Designation	Pig no.						Olive oil
		1	2	3	4	5	6	
12:0	12:0	0.1	0.1	0.2	0.1	0.2	0.1	—
14:0	14:0	1.2	1.7	1.5	1.8	1.9	1.1	—
16:0	16:0	25.0	24.4	23.7	20.3	23.2	12.5	11.1
16:3	16:1	4.2	4.2	3.8	3.2	3.6	2.4	1.1
17:0	17:0	0.2	0.1	0.3	0.2	0.2	0.2	tr.
17:3	17:1	0.3	0.3	0.4	0.3	0.2	0.2	tr.
18:0	18:0	14.6	13.4	11.6	10.9	10.1	4.0	2.3
18:3	18:1	51.5	53.1	55.7	60.0	55.7	69.0	74.7
18:9	18:2	0.6	0.5	1.3	1.5	3.2	7.6	10.4
19:7	18:3	0.2	0.1	0.1	0.1	0.2	0.4	0.4
20:0	20:0	0.2	0.1	0.1	0.1	0.1	0.1	—
20:7	20:2?	0.1	0.1	tr.	0.1	0.1	0.6	—
21:0	21:0?	0.1	0.1	0.1	0.1	0.1	0.4	—
21:5	20:4	—	—	—	—	—	0.3	—
Total unsaturated acids		58.3	59.6	62.3	66.3	64.0	81.5	86.6

Fig. 1. Major changes in the fatty acid composition of the serum phospholipids (a) and sterol esters (b) of pigs given various amounts of linoleic acid. ○, Eicosatrienoic acid; ●, arachidonic acid; △, oleic acid; ▲, linoleic acid; □, palmitoleic acid.

Fig. 2. Contribution of phospholipids (○), sterol esters (△) and triglycerides (▲) to the content of arachidonic acid (a) and eicosatrienoic acid (b) in the total fatty acids of serum (●).

Depot fat (Table 7). Octadecadienoic acid fell from 7.6 to 0.6 % and palmitoleic acid rose from 2.4 to 4.2 %. The fatty acids were characterized by an almost complete absence of eicosatrienoic acid and eicosatetraenoic acid.

The contribution of each lipid fraction to the total content of eicosatrienoic acid and eicosatetraenoic acid in serum is shown in Fig. 2. From this it is seen that from 70 to 75 % of these acids is confined to the phospholipid fraction at all dietary concentrations of linoleate.

DISCUSSION

Although the small number of animals used in this experiment does not warrant definite conclusions being drawn it would appear that the pig, in showing a lower concentration of serum cholesterol

when diets low in fat are given, is similar to man and the rat (Keys, Anderson & Grande, 1957).

The presence of trienoic acid in tissue lipids when diets low in fat are fed has been used by Holman (1960) in an attempt to evaluate the degree of essential-fatty acid deficiency in chemical terms. He has shown in rats that a triene:tetraene ratio greater than 0.4 indicates a deficiency of essential fatty acids. On this basis, with the triene:tetraene ratio in the total fatty acids of serum used as an index, the minimum dietary requirement of the pig for linoleic acid is about 1 % of the total calories (Leat, 1962*b*). The triene:tetraene ratio in the phospholipid fatty acids gives a similar value but the ratio in the sterol ester fatty acids suggests a linoleate requirement of nearer 0.6 % of the calories. The triene:tetraene ratio in triglyceride fatty acids gives no indication of nutritional status for essential fatty acids. Thus the triene:tetraene ratio in the phospholipids appears to be a more reliable measure

of requirement especially in the presence of large amounts of triglycerides and sterol esters.

Composition of total fatty acids. Below a dietary concentration at which linoleate supplied 1% of the calories, marked changes occurred in the composition of the total fatty acids of serum. The amounts of octadecadienoic acid and eicosatetraenoic acid were lower ($P < 0.05$) and those of eicosatrienoic acid and palmitoleic acid higher ($P < 0.05$ and $P < 0.01$ respectively) when dietary linoleate supplied less than 0.95% of the calories than when it supplied more than 0.95%. Increasing dietary linoleate above 1% of the calories had little further effect on the fatty acid composition of serum lipids. That this is not peculiar to the particular diet used in this experiment is indicated by similar observations on the lipids of the liver and heart of miniature pigs given diets of a completely different composition (Hill, Warmanen, Silbernack & Holman, 1961). It is of interest to compare these results with those of Dole, James, Webb, Rizack & Sturman (1959), who found that the fatty acid composition of the non-esterified fatty acids, phospholipids and cholesterol esters plus triglycerides of the plasma of various species was relatively constant although dietary habits varied considerably.

Although, in my experiment, large amounts of oleic acid were being ingested concomitantly with the linoleic acid, no change was noted in the oleic acid content of the total fatty acids of serum although that of depot fat was increased. This would indicate that changes in the fatty acid composition of serum lipids were related more to the amount of dietary linoleate than to that of dietary oleate.

Fatty acids of the sterol esters. The major fatty acids of the sterol esters were oleic acid and linoleic acid, which together accounted for 80% of the fatty acids. The percentage of linoleic acid was much higher in the sterol esters than in any other lipid fraction or in the dietary fat. Linoleic acid thus appears to be preferentially esterified in the sterol esters, in which was located 60–70% of the total serum linoleate. The amounts of octadecadienoic acid and eicosatetraenoic acid were lower ($P < 0.05$ and $P < 0.02$) and those of palmitoleic acid and eicosatrienoic acid higher ($P < 0.05$) when dietary linoleate supplied less than 0.95% of the calories than when it supplied more than 0.95%. The amounts of oleic acid and a C_{17} monoenoic acid also rose when dietary linoleate was low. Thus a deficiency of linoleic acid in the sterol esters is compensated by an elevation in the amounts of various monoenoic acids, namely, oleic acid, palmitoleic acid and a C_{17} monoenoic acid. Some of these changes have been noticed in the plasma-cholesterol esters of some atherosclerotic humans, as compared with healthy controls (Schrade, Böhle & Biegler, 1960). The arachidonic acid

content of the sterol esters was low (3–4%) and similar to that of man. Swell, Field & Treadwell (1960) have shown that the arachidonic acid content of sterol esters of various species is inversely related to the susceptibility to atherosclerosis. The pig may therefore prove to be a valuable experimental animal for investigation of this disease. Saturated acids accounted for only 8–14% of the fatty acids of sterol esters, and this amount remained unchanged although the amount of dietary linoleate varied considerably. Although large amounts of oleic acid were being ingested with the higher amounts of linoleic acid, there was no rise in the oleic acid content of the sterol esters. In fact the oleic acid content of the sterol esters from pigs nos. 5 and 6, which were ingesting respectively 7.0 and 26.5% of the calories as oleic acid, was significantly lower ($P < 0.01$) than that found in pigs nos. 1 and 2, which were ingesting very little oleic acid.

Fatty acids of the phospholipids. The fatty acid composition of the phospholipids was different from that of the sterol esters. There was less linoleic acid and oleic acid and more eicosatrienoic acid and eicosatetraenoic acid in the phospholipids, with saturated acids accounting for 45–50% of the total. Eicosatrienoic acid was higher ($P < 0.02$) and eicosatetraenoic acid lower ($P < 0.05$) when the diet contained less than 0.95% of the calories as linoleic acid than when it contained more than 0.95%. Of the total eicosatrienoic acid and eicosatetraenoic acid in serum 70–75% was located in the phospholipids. In contrast with the sterol esters, the content of oleic acid and palmitoleic acid in the phospholipids was unaffected by changes in dietary linoleate. Thus in the phospholipids essential-fatty acid deficiency is characterized by a decrease in linoleic acid and arachidonic acid and a rise in eicosatrienoic acid. The percentage of an eicosadienoic acid having a carbon number of 20.6 was also found to increase when dietary linoleate was restricted, which is in agreement with observations on essential-fatty acid-deficient rats (Fulco & Mead, 1959; Enser & Bartley, 1962). This acid may be an intermediate in the formation of eicosatrienoic acid. A similar rise was also noted in the triglyceride fraction. The most notable feature of the phospholipid fatty acids was the presence of large amounts of C_{22} and C_{24} fatty acids, which were absent from the other lipid fractions. These fatty acids were derived from the sphingomyelin in the serum phospholipids and were absent from the total fatty acids of serum because the method of isolation involving alkaline hydrolysis did not hydrolyse the fatty acid linkage in sphingomyelin.

Fatty acids of the triglycerides. The major fatty acid of the serum triglycerides was oleic acid, which accounted for 60% of the total. The fatty acid

composition of the triglycerides was affected to a lesser degree by changes in dietary linoleate than were those of the sterol esters and phospholipids. The content of octadecadienoic acid was lower ($P < 0.05$) and that of palmitoleic acid higher ($P < 0.01$) in pigs given less than 0.95 % of the calories as linoleic acid compared with those given greater amounts. The amounts of eicosatrienoic acid and eicosatetraenoic acid were low and variable. Appreciable amounts of lauric acid and myristic acid were present, which probably reflected their presence in the diet. Comparison of the composition of the triglycerides of serum with those of depot fat shows that the latter were more saturated, contained more stearic acid and palmitic acid and less linoleic acid. Eicosatrienoic acid and eicosatetraenoic acid were almost completely absent from depot fat although present in small amounts in serum triglycerides. Another notable difference between the two sources of triglycerides was that whereas the fatty acid composition of serum triglycerides was relatively unaffected by large amounts of dietary fat, that of depot fat was markedly altered. The feeding of 16 % of olive oil to pig no. 6 altered the fatty acid composition of the depot fat to such an extent that it resembled closely that of the fed fat, although the composition of the serum triglycerides was unchanged.

Non-esterified fatty acids. The non-esterified fatty acids were more saturated than the serum triglycerides, with oleic acid, palmitic acid and stearic acid predominating. The composition of the non-esterified fatty acids was quite distinct from that of the other serum lipids and was little affected by changes in dietary linoleate. Gordon & Cherkes (1956) have shown that in the fasting rat the fat depots are the main source of the non-esterified fatty acids in plasma. If this is so in the pig the difference between the composition of the non-esterified fatty acids of serum and that of depot fat would indicate a preferential release or metabolism of certain fatty acids. This is particularly well illustrated by pig no. 6, where there was a considerable difference between the composition of the depot fat and that of the non-esterified fatty acids in serum. Thus in essential-fatty acid deficiency linoleic acid and its derivative, arachidonic acid, are replaced by oleic acid and its derivative eicosatrienoic acid and by palmitoleic acid. The lipid fractions of serum have characteristic fatty acid patterns which are affected differently by essential-fatty acid deficiency. The fatty acids of the sterol esters are highly unsaturated and a decrease in linoleic acid is compensated by a rise in oleic acid and palmitoleic acid. The phospholipid fatty acids are more saturated than those of sterol esters and a decrease in linoleic acid is accompanied by a fall in arachidonic acid and a rise in eicosatrienoic acid. Tri-

glycerides and non-esterified fatty acids are least affected. These major changes occur when dietary linoleate accounts for less than 1 % of the calories. Increasing dietary linoleate above this amount results in little further change in the fatty acid composition of serum lipids, although the composition of adipose tissue is greatly affected. Although the percentage composition of the individual unsaturated fatty acids of the serum lipids varies with dietary linoleate, the total percentage of unsaturated acids remains constant.

SUMMARY

1. The fatty acid compositions of the serum lipids of six pigs fed with olive oil to supply from 0.1 to 3.7 % of the calories as linoleic acid have been examined.

2. The major changes in fatty acid composition occurred when dietary linoleate supplied less than 1 % of the calories.

3. In the phospholipid fraction decreasing dietary linoleate resulted in a decrease in the percentage of linoleic acid and arachidonic acid and an increase in that of eicosatrienoic acid. Of the total eicosatrienoic acid and arachidonic acid in serum 70–75 % was associated with the phospholipids.

4. In the sterol esters a decrease in dietary linoleate resulted in a fall in the percentage of linoleic acid, which was compensated by a rise in that of oleic acid and palmitoleic acid. Of the total linoleic acid in serum 60–70 % was associated with the sterol esters.

5. The fatty acid composition of the triglycerides and non-esterified fatty acids were least affected by changes in dietary linoleate.

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Urinary Steroids of Cats

1. ABSENCE OF 17-OXO STEROIDS

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Not much information is available on steroid metabolism in the cat. Borrell (1958) reported that cats do not excrete corticoid glucosiduronates via the urine, though small amounts of Porter-Silber chromogens were present as the free compounds. Taylor & Scratcherd (1960, 1962) have shown that metabolites of administered [4-¹⁴C]progesterone and [4-¹⁴C]corticosterone are excreted mainly in the bile, with only a small amount of radioactivity appearing in the urine. Setekleiv, Skang & Kaada (1961) showed an increase in the plasma 17-hydroxy corticosteroids after cerebral-cortical and amigdaloid stimulation.

To the best of the author's knowledge, the presence of 17-oxo steroids has not been reported in the urine of cats, although such compounds have been studied in the urines of dogs (Glenn & Heftmann, 1951; Tewell & Freeman, 1954), rats (Richter 1962; Munday & Blane, 1960; Paroli & Melchiorri, 1961), rabbits (Davis, Slater & Krichesky, 1949), cows (Klyne & Wright, 1959), goats (Klyne & Wright, 1957), guinea pigs (Brooks, Clayton & Hammant, 1960) and pigs (Huis in't Veld, Louwerens & Reilingh, 1961).

The study of steroid metabolism in the cat is desirable for more than the obtaining of knowledge of comparative endocrinology. Since the cat appears to be deficient in its ability to conjugate materials with glucuronic acid (Borrell, 1958; Hartiala, 1955; Dutton & Greig, 1957; Dutton, 1959; Robinson & Williams, 1958; Stevenson & Dutton, 1962), it is possible that a study of steroid metabolism in the cat may reveal other pathways for the conjugation of steroids.

In the present paper the results of studies of the urinary excretion of 17-oxo steroids by the cat are reported.

METHODS

Animals. Adult male and female cats weighing 2.5–3 kg. were used. To obtain complete collections of urine the bladders were placed between the skin and the ventral musculature by using the operative procedure of Arteta & Borrell (1958). The animals were not used until at least 2 weeks after the operation.

Treatment of urine. No preservatives were added to the urine. Hydrolysis and extraction were usually performed on the day of collection, but in a few experiments urines were kept at 4° for 2 days.

Urine samples were hydrolysed and extracted by one of the four procedures following:

Method A. The urinary steroids were acid-hydrolysed and extracted with ether, and the acidic components removed by washing the ether with 2N-sodium hydroxide, as described by Pincus (1945). The washed extracts were dried on a steam bath.

Method B. After acidification, the urine was extracted with ether continuously for 48 hr. The ether extracts were treated as in method A.

Method C. The urine was acid-hydrolysed and extracted continuously with carbon tetrachloride according to the procedure of Dorfman & Shipley (1956). The final carbon tetrachloride extracts were evaporated to dryness under reduced pressure. The residues were dissolved in ether and the ether extracts were treated as in method A.

Method D. The sample of urine was brought to boiling under reflux. Then 0.1 vol. of conc. hydrochloric acid was added and the boiling was continued for 10 min. After cooling under running water, 0.33 vol. of carbon tetrachloride was added and the mixture was refluxed again for 10 min. This first extract was removed, and a second portion