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Fatty Acid Components of Rat-Tissue Lipids

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1. The lipids of rat heart, kidney, skeletal muscle and liver were separated by chromatography on silicic acid into cholesterol ester, triglyceride, free-fatty acid and phospholipid fractions. 2. The fatty acid compositions of these fractions were determined by gas–liquid chromatography. 3. Palmitic acid was always present in highest concentration in the cholesterol ester fraction; oleic acid was present in greatest percentage in the triglyceride fraction; arachidonic acid was in highest concentration in phospholipid, and in lowest concentration in triglyceride fractions. 4. The fatty acid compositions of the cholesterol ester fractions were broadly similar for all the extrahepatic tissues. 5. Some differences in fatty acid composition of the phospholipids were evident between the hepatic and extrahepatic fractions.

Although there is an extensive literature on the analysis of tissue fatty acids by gas–liquid chromatography, by far the greatest emphasis to date has been directed towards liver and plasma in monogastric animals (James, Lovelock, Webb & Trotter, 1957; Dole, James, Webb, Risack & Sturman, 1959; Lawrie, McAlpine, Pirrie & Rifkind, 1961; Okey, Shannon, Tinoco, Ostwald & Miljanick, 1961; Getz, Bartley, Stirpe, Notton & Renshaw, 1962; Grande, 1962). In contrast, there is little detailed information available on the fatty acids of extrahepatic tissues (Veerkamp, Mulder & van Deenen, 1962).

The purpose of the present investigation was to rectify this deficiency with respect to a common laboratory animal, and to extend previous studies of comparative lipid metabolism (Horgan & Masters, 1963; Masters, 1964*a*,*b*,*c*).

EXPERIMENTAL

Experimental animals. Rats were normal adult males of mixed laboratory strains, raised on a standard diet of rat cubes (Barnes Milling Ltd., South Brisbane, Australia) *ad libitum*. The cubes contained 4·0% of lipid (mainly triglyceride), with the following average fatty acid composition: lauric acid, 1%; myristic acid, 5%; palmitic acid, 21%; palmitoleic acid, 2%; stearic acid, 10%; oleic acid, 38%; linoleic acid, 20%; linolenic acid, 3%.

Extraction and fractionation of lipids. Rats were killed by stunning and decapitation. Heart, kidneys, liver and samples of lean skeletal muscle were excised. Perinephric and myocardial depot fat were removed and the tissues were stored in closed vessels at −10° in the dark until required. Lipids were extracted with boiling ethanol–ether (3:1, v/v) as described by Creasy, Hamkin & Handschumaker (1961). The extracts were evaporated at reduced pressure, dissolved in light petroleum, and separated into four fractions (cholesterol esters; triglycerides; free fatty acids, monoglycerides and diglycerides; phospholipid) by chromatography on silicic acid (Lis, Tinoco & Okey, 1961).

Preparation of methyl esters. Methyl esters of the fatty acids of lipid fractions were obtained by the inter-esterification and micro-sublimation method of Stoffel, Chu & Ahrens (1959), with sulphuric acid as catalyst. The methyl esters were stored in excess of light petroleum (b.p. 40–60°) at 4° until analysed by gas–liquid chromatography.

Chemicals. All solvents and chemicals were analytical-grade reagents. In handling the solutions and evaporating the solvents, nitrogen was used to prevent oxidation.

Gas–liquid chromatography. Analyses of the methyl esters were carried out on a Pye Argon chromatograph (Pye Ltd., Cambridge), with a gas flow of 40 ml./min. Polyethylene glycol adipate on 100-mesh Celite (10%, w/w) at 175° and Apiezon L at 200° were used as stationary phases. Calculation was based on the relative peak area, determined by the 'triangulation' procedure (James, 1960).

The peaks were identified by comparison with the retention times of pure standards, or with published values (Farquhar, Insull, Rosen, Stoffel & Ahrens, 1959; James, 1960; Woodford & van Ghent, 1960), and by observation of the effect of micro-bromination (James, 1960).

RESULTS

The relative percentage composition of the main lipid fractions in the heart, kidney, skeletal muscle and liver of the rat are given in Table 1. Phospholipid was the predominant fraction in all these tissues, with muscle containing the highest percentage of triglyceride, and kidney the most free fatty acid, monoglyceride and diglyceride.

In Tables 2 and 3 are listed the fatty acid compositions of heart and kidney, skeletal muscle and

liver respectively. The C_{12}–C_{22} fatty acids represented in general more than 90% of all the fatty acids present. In the phospholipid of heart, kidney and muscle, two unidentified fatty acids were present as minor components (A and B). The chromatographic data of these unsaturated acids (carbon numbers: on polyethylene glycol adipate, 15·6 and 17·6 at 175°; on Apiezon L, 16·6 and 18·6 at 200°) coincide with those of two ovine-tissue fatty acid components reported by Masters (1964c), and their identity is being investigated further. A comparison of the results in Tables 2 and 3 shows that the palmitic acid percentage varies considerably between different lipid fractions in the same tissue, but in all instances cholesterol ester yields the highest percentage of this component. With the C_{18} acids, stearic acid is always present in higher concentration than oleic acid in the cholesterol ester and phospholipid fractions of these tissues, whereas the reverse applies to all the triglyceride and free fatty acid fractions. In all cases oleic acid is present in highest concentration in triglyceride, and in lowest concentration in phospholipid. Two other features common to all these tissues are that the C_{18} dienoic acid was in lowest concentration in the cholesterol ester fraction, and that arachidonic

Table 1. *Fractionation of tissue lipids by chromatography on silicic acid*

Experimental details are given in the text. Values for each fraction are mean percentages by weight (three animals) of the total tissue lipids.

Fraction	Heart	Kidney	Muscle	Liver
Cholesterol esters	11	5	10	12
Triglycerides	14	7	18	9
Free fatty acids, monoglycerides and diglycerides	11	19	10	7
Phospholipids	64	69	62	72
Total lipid concn. (%, w/w, wet wt. of tissue)	3·7	3·5	3·0	4·7

Table 2. *Fatty acid composition of rat-heart and rat-kidney lipids*

Experimental details are given in the text. Values for each component are the percentage by weight of the total fatty acid methyl esters in each fraction. – indicates that the amount of fatty acid present was less than 0·5%. The fatty acids are designated $x:y$, where x is the no. of carbon atoms and y the no. of double bonds/molecule; (br.), singly methyl-branched chain.

	Heart				Kidney			
Fatty acid	Cholesterol esters	Triglycerides	Free fatty acids	Phospholipids	Cholesterol esters	Triglycerides	Free fatty acids	Phospholipids
12:0	–	–	–	–	1·0	–	–	–
13:0	–	–	–	–	0·6	–	–	–
14(br.)	–	–	–	–	0·9	–	–	–
14:0	1·6	1·2	1·4	–	3·2	1·1	0·7	–
14:1	–	–	–	–	2·1	–	–	–
15(br.)	–	–	–	–	–	–	–	–
15:0	0·6	–	–	–	0·9	–	–	–
15:1	0·4	–	–	–	–	–	–	–
Component A	0·8	–	–	1·0	1·6	–	–	1·0
16(br.)	–	–	–	–	–	–	–	–
16:0	26·5	15·5	15·4	14·9	30·2	27·1	15·9	8·2
16:1	3·5	5·2	3·0	1·4	6·1	4·2	2·5	1·1
17(br.)	–	–	–	–	–	–	–	–
17:0	1·0	0·9	0·6	0·6	1·2	0·6	0·9	–
16:2	–	–	–	–	–	–	0·6	–
Component B	–	–	–	–	0·5	–	–	1·1
18(br.)	–	–	–	–	–	–	–	–
18:0	23·8	15·5	16·6	24·7	19·2	11·3	11·9	18·6
18:1	18·9	37·9	24·5	13·1	14·9	33·1	17·9	15·6
18:2	5·5	16·1	21·1	30·5	2·3	12·9	13·5	13·7
18:2 (?)	–	–	–	–	–	–	–	–
18:3	0·6	0·6	1·3	0·7	–	0·7	1·8	–
20:0	1·3	–	1·2	–	1·6	1·8	1·7	–
20:1	0·6	0·7	–	–	–	1·9	–	–
20:2	0·9	–	–	–	–	–	–	–
21:0	3·0	0·5	2·0	–	2·8	2·4	2·0	–
20:4	4·9	1·2	10·2	11·3	3·5	1·1	28·0	37·2
22:0	2·9	–	–	–	6·5	–	1·8	–

Table 3. *Fatty acid composition of rat-skeletal-muscle and rat-liver lipids*

Experimental details are given in the text. Values for each component refer to the percentage by weight for the total fatty acid methyl esters in each fraction. – indicates that the amount of fatty acid present was less than 0·5%. The fatty acids are designated as described in Table 2.

	Skeletal muscle				Liver			
Fatty acid	Cholesterol esters	Tri-glycerides	Free fatty acids	Phospho-lipids	Cholesterol esters	Tri-glycerides	Free fatty acids	Phospho-lipids
12:0	1·3	–	–	–	–	–	–	–
13:0	–	–	–	–	–	–	–	–
14(br.)	0·6	–	–	–	–	–	–	–
14:0	2·1	0·6	1·5	0·6	1·0	0·9	0·5	–
14:1	1·5	–	–	–	–	–	–	–
15(br.)	–	–	–	–	–	–	–	–
15:0	0·8	–	0·7	–	–	–	–	–
15:1	–	–	–	–	–	–	–	–
Component A	–	–	–	1·9	0·8	–	–	–
16(br.)	1·1	–	–	–	–	–	–	–
16:0	23·9	16·9	15·5	17·8	24·2	17·1	15·2	16·9
16:1	2·9	3·5	5·2	3·1	2·9	3·8	2·7	1·1
17(br.)	0·8	–	–	–	–	–	–	–
17:0	1·3	0·5	0·5	–	0·8	0·5	–	0·9
16:2	0·8	–	–	–	–	–	0·6	–
Component B	–	–	–	1·0	0·5	–	–	–
18(br.)	1·1	–	–	–	–	–	–	–
18:0	20·0	7·1	9·5	23·1	19·7	14·2	11·4	37·6
18:1	19·1	43·4	32·9	14·4	15·2	30·3	14·3	9·6
18:2	4·9	24·7	20·4	21·1	16·1	24·2	24·7	15·2
18:2 (?)	0·6	–	–	–	–	–	0·7	–
18:3	1·1	0·8	1·0	–	–	–	1·4	–
20:0	3·4	–	1·0	–	0·5	–	–	–
20:1	5·1	–	0·7	–	0·8	0·6	–	–
20:2	–	–	–	–	–	–	–	–
21:0	2·6	–	1·2	–	1·2	0·7	1·1	–
20:4	2·4	0·6	6·1	15·3	13·7	5·5	24·1	16·7
22:0	2·4	–	0·9	–	0·8	–	–	–

acid was at its highest percentage content in phospholipid, and lowest in triglyceride.

Comparison of the individual lipid fractions shows that the concentration of the fatty acids in the different cholesterol ester fractions is similar for most of the major components. The linoleic acid and arachidonic acid content in liver, however, is considerably higher than that in the extrahepatic tissues. With the triglycerides, greater variations of the component percentages occur between tissues. The palmitic acid content of kidney triglycerides is markedly higher than that in other tissues, and arachidonic acid is present in liver triglyceride to a greater extent than in the extrahepatic tissues. The main component of all triglyceride fractions is oleic acid. In the free-fatty acid fractions, muscle possesses the highest ratio of unsaturated and saturated C_{18} acids, but the lowest percentage of arachidonic acid. The phospholipid fraction exhibits some differences between liver and extrahepatic tissues. The ratio of unsaturated and saturated C_{18} acids is very much less in liver than in heart, kidney or muscle, and the unusual unidentified fatty acids (A and B) are present in appreciable quantities only in extrahepatic phospholipid. Although all the phospholipid fractions display high arachidonic acid contents, kidney phospholipid is the richest source of this acid.

Of the two unidentified fatty acid components, the first (A) was usually present in greater quantity and was at its maximum concentration in muscle phospholipid. Trace quantities of these fatty acids appeared in other fractions and other tissues.

DISCUSSION

A number of workers have reported partial fractionation of the lipids from extrahepatic tissues (Bloor, 1943; Williams *et al.* 1945; Futter & Shorland, 1957; Kochen, Marinetti & Stotz, 1960). The present more complete fractionation constitutes

a more precise basis for metabolic studies than the examination of total lipid as one entity, or fractionation on the basis of acetone solubility (Lis *et al.* 1961; Masters, 1964*a*,*b*,*c*). Additional characterization of individual phospholipids and subcellular fractions would further enhance metabolic correlation.

The high percentages of phospholipid in the extrahepatic tissues are in agreement with investigations of other species (Bloor, 1943; Futter & Shorland, 1957), but the only directly comparable values for the other lipid fractions appear to be those for the sheep (Masters, 1964*c*), where values are of the same order as for the rat. In the liver, the rat possesses a considerably higher percentage of phospholipid, but lower content of triglyceride, than does the sheep (Horgan & Masters, 1963; Table 1).

There is little basis for interspecies comparison of fatty acid composition of the extrahepatic-tissue lipids. Whereas Futter & Shorland (1957) reported that the fatty acid compositions of rabbit kidney, liver and muscle broadly resembled one another, there is extensive dissimilarity between these tissues in the rat and the sheep (Horgan & Masters, 1963; Masters, 1964*c*). Futter & Shorland (1957) and Veerkamp *et al.* (1962) reported that the fatty acid patterns of neutral lipid in the different tissues of individual species resembled one another; but the present results show that a close similarity is restricted to the cholesterol ester fractions of rat extrahepatic-tissue lipids.

With regard to fatty acid composition, the most detailed data available for interspecies comparison is that for ovine extrahepatic-tissue lipids (Masters, 1964*c*). As a whole the ovine-tissue lipids are far more saturated than are the corresponding rat-tissue lipids. Wide differences appear in the phospholipid fraction that do not support the suggestion of Veerkamp *et al.* (1962) that tissue phospholipids possess similar fatty acid composition in the homologous tissues of different animals.

Liver has been the most extensively investigated of all these tissues in respect to fatty acid composition, and the results in Table 3 are in general agreement with comparable data from other workers (Okey *et al.* 1961; Getz *et al.* 1962; Veerkamp *et al.* 1962). In the triglyceride fraction, higher values have been obtained in the present investigation for stearic acid, but these differences are probably due to the higher content of this acid in the feed lipids of these rats.

Comparative data for the fatty acids of ovine liver (Horgan & Masters, 1963) show that the cholesterol esters and triglycerides of rat liver contain higher percentages of palmitic acid than does ovine liver, and a greater content of unsaturated C_{18} acids. Whereas the predominant unsaturated C_{18} acid in ovine-liver lipids is monoenoic rat-liver lipid displays a much higher C_{18} dienoic acid:C_{18} monoenoic acid ratio throughout. Also, ovine-liver triglycerides and phospholipids do not possess the high content of C_{20}–C_{22} polyunsaturated fatty acids that rat liver exhibits.

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