Peel, D. & Quayle, J. R. (1961). *Biochem. J.* **81**, 465.
Quayle, J. R. (1961). *Annu. Rev. Microbiol.* (in the Press).
Quayle, J. R. & Keech, D. B. (1959*a*). *Biochem. J.* **72**, 623.
Quayle, J. R. & Keech, D. B. (1959*b*). *Biochem. J.* **72**, 631.
Quayle, J. R. & Keech, D. B. (1960). *Biochem. J.* **75**, 515.
Rabinowitz, J. C. (1960). In *The Enzymes*, vol. 2, p. 185. Ed. by Boyer, P. D., Lardy, H. & Myrbäck, K. New York: Academic Press Inc.
Sakami, W. (1955*a*). *Handbook of Isotope Tracer Methods*, p. 1. Cleveland, Ohio: Department of Biochemistry, Western Reserve University.
Sakami, W. (1955*b*). *Handbook of Isotope Tracer Methods*, p. 5. Cleveland, Ohio: Department of Biochemistry, Western Reserve University.
Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.
van Niel, C. B. (1954). *Annu. Rev. Microbiol.* **8**, 105.
Vishniac, W., Horecker, B. L. & Ochoa, S. (1957). *Advanc. Enzymol.* **19**, 1.

Biochem. J. (1961) **81**, 480

Composition of Phospholipids of Rabbit, Pigeon and Trout Muscle and Various Pig Tissues

By G. M. GRAY* and MARJORIE G. MACFARLANE
Lister Institute of Preventive Medicine, London, S.W. 1

(*Received* 17 *April* 1961)

Studies on the distribution and structure of the phospholipids in certain mammalian tissues and subcellular components have been reported previously (Gray & Macfarlane, 1958; Gray, 1960*a*, *b*; Macfarlane, Gray & Wheeldon, 1960; Macfarlane, 1961*a*). The object of the present work was to obtain a general picture of the pattern of fatty components in phospholipids in other normal tissues, and to examine further the distribution of choline plasmalogen. This compound is less than 1% of the total phospholipid in brain (Klenk, Debuch & Daun, 1953; Webster, 1960) and a minor component in liver and spleen; but it is a major component (36%) in ram semen and in ox and pig heart (Lovern, Olley, Hartree & Mann, 1957; Gray, 1960*a*; Klenk & Debuch, 1955; Marinetti & Erbland, 1957; Gray & Macfarlane, 1958). It appeared possible that choline plasmalogen is associated directly with a contractile process, though the small amount found in cod muscle (Garcia, Lovern & Olley, 1956) indicated a considerable species difference; Hartree & Mann (1959), on the other hand, suggested that fatty acids derived from plasmalogen participate in the aerobic endogenous metabolism of sperm.

METHODS

The estimation of P, total N, amino N, choline, aldehyde, inositol, fatty acid ester groups, alkali-labile P and phosphomonoester were carried out as in Gray & Macfarlane (1958). The method for the estimation of ethanolamine and serine (Axelrod, Reichental & Brodie, 1953) was modified slightly by substituting an acid hydrolysis and chloroform–methanol-extraction procedure used by Dr C. Long (personal communication) for the usual alkaline hydrolysis. Chromatography of phospholipids on silicic acid-impregnated paper was done in di*iso*butyl ketone–acetic acid–water (40 : 20 : 3, by vol.) at 2° and of glycerides in light petroleum (b.p. 60–80°)–di*iso*butyl ketone (96 : 6, v/v) at room temperature (Marinetti, Erbland & Kochen, 1957). Chromatography on Whatman no. 1 acid-washed paper in butan-1-ol–water–conc. NH_3 (100 : 15 : 2, by vol.; Coulon-Morelec, Faure & Maréchal, 1960) was also used (R_F cardiolipin 0·74; phosphatidic acid 0·48). Chromatography of water-soluble esters obtained by mild hydrolysis was done according to Dawson (1954).

Myofibrils were prepared from rabbit muscle by the method of Perry & Grey (1956); the lipid was compared with that extracted from a portion of the whole muscle from the same animal. Muscle from rainbow trout and pigeons was extracted within 1 hr. after death, and pig tissue obtained from a slaughterhouse within 2 hr.

The extraction of lipid from the tissues was based on the method of Folch, Lees & Sloane-Stanley (1957) and Gray (1960*b*). In general the tissue was blended mechanically with 5 vol. of cold chloroform–methanol (1 : 1, v/v) and re-extracted twice with 1 vol. of chloroform–methanol (2 : 1, v/v). The combined extracts were washed two or three times with 0·2 vol. of salt solution (0·04 M-$MgCl_2$ or 0·05 N-NaCl), and the chloroform solution was dried and evaporated. The residue was dissolved in light petroleum and dialysed according to van Beers, de Iongh & Boldingh (1958) to separate most of the non-P lipid (neutral lipid fraction) from the phospholipid.

The phospholipid was fractionated on silicic acid as in Gray (1960*b*) and neutral lipid by the system of Hirsch & Ahrens (1958). The isolation of the fatty acids from phosphatidylethanolamine, phosphatidylserine and phosphatidylcholine and of the fatty acids and aldehydes from the ethanolamine, serine and choline plasmalogen was carried out as described by Gray (1960*b*).

* Beit Memorial Fellow.

Table 1. *Yield of total lipid and phospholipid from various tissues*

Weights are calculated for 100 g. of fresh tissue.

Tissue	Total lipid (g.)	Total cholesterol (g.)	Lipid P (mg.)	Phospholipid* (g.)	Phospholipid* (% of total lipid)
Rabbit					
Skeletal muscle 1	2·26	—	20·4	0·51	23
2	—	—	18·0	0·45	—
Pigeon					
Heart muscle	3·86	0·17	102·0	2·55	66
Breast muscle	5·16	0·09	89·0	2·22	43
Trout					
Muscle 1	5·31	0·05	25·4	0·63	12
2	3·72	—	27·8	0·70	19
Pig					
Spleen	2·45	—	49·5	1·24	50
Lung	2·00	0·14	63·6	1·59	79
Kidney	2·90	0·28	93·5	2·34	81
Ram					
Semen	1·37	—	24·8	0·62	45

* Calculated from P content ×25.

The fatty acids as methyl esters and the aldehydes as dimethyl acetals were identified by gas chromatography. The analysis of aldehydes from the plasmalogens of different tissues by gas chromatography described by Gray (1960*c*) made possible the identification of a number of hitherto unknown, naturally occurring aldehydes with branched carbon chains. Further work (Gray, 1961) has shown that these aldehydes belong to either the *iso*- or *anteiso*-series of compounds analogous to the branched-chain acids found in animal tissues, and can be positively identified by their gas-chromatographic behaviour on polar and non-polar stationary phases.

EXPERIMENTAL AND RESULTS

In the fractionation of phospholipid mixtures on silicic acid columns the volume of any one solvent mixture necessary to separate a component varies with the proportion of the component and the nature of the mixture. After passage of chloroform to elute the remaining non-P lipid, the stepwise increase in methanol concentration was not made at stages preset by the volume of solvent to be passed, but at stages determined for individual columns by the separation curve, based on analysis of eluent fractions for P and for amino N, fatty acid esters etc. as appropriate. In general, elution was begun with 2% (v/v) methanol in chloroform, increased in steps of 2% until the cardiolipin fraction was eluted, and then in larger steps, e.g. 10, 15, 20, 25, 35, 50 and 70% (v/v) methanol in chloroform. The usual type of separation curve has been illustrated previously (e.g. Gray, 1960*b*). Eluent fractions were pooled on the basis of the separation curve, supplemented by paper chromatography, and the larger fractions further characterized by analysis for ethanolamine, serine, aldehyde, inositol, alkali-stable P etc. and by paper chromatography. The procedure permitted in general the substantial separation of (1) cardiolipin, (2) phosphatidylinositol, (3) phosphatidylserine or phosphatidylethanolamine, or both, and corresponding plasmalogens, (4) phosphatidylcholine and choline plasmalogen, (5) sphingomyelin. The methods of analysis for various groups and structures present in a phospholipid molecule are, however, not so accurate that the presence of small amounts of other compounds of similar general structure can be excluded. For example, the analyses have not indicated the possible presence in the kephalin and lecithin fractions of the corresponding glycerol ether compound (Carter, Smith & Jones, 1958), but during some recent investigations on the hydrolysis of plasmalogens (Pietruszko & Gray, 1960) evidence was obtained that glycerol ethers may normally occur in small amounts in these fractions. However, it is thought that the computation from the analysis gives a fair picture of the distribution of phosphorus amongst the main components, and the separation curves and detailed analyses for each tissue have been omitted.

The yield of total lipid and lipid P from various tissues is given in Table 1; the composition of the phospholipid in Table 2 and the fatty acid composition in Tables 3–5. Comments on the individual tissues are made below.

Rabbit myofibrils. Choline plasmalogen was present in the myofibrils in slightly higher proportion than in the whole muscle; about 50% of the kephalin was plasmalogen in both whole muscle and myofibrils.

Pigeon-breast muscle. The lipid content was high. About 43% of the total was phospholipid, 2%

cholesterol and the remainder almost entirely triglycerides containing a high proportion of oleic acid. Phosphatidic acid was not detected in the cardiolipin fraction by paper chromatography in butanol–water–conc. ammonia. Choline plasmalogen was present in amounts similar to rabbit skeletal muscle, and was highly unsaturated, about 65 % of the fatty acids being C_{20}–C_{22} polyenoic acids, compared with 7 % of these acids in the phosphatidylcholine fraction (Table 4).

Trout muscle. The total lipid was much higher than that reported for cod and haddock flesh (cf. Garcia *et al.* 1956), mainly owing to an increase in triglyceride content. On fractionation of the neutral lipid on silicic acid, 83 % was recovered in the triglyceride fraction, and 0·9 % as free cholesterol, with only a trace in the cholesterol ester fraction.

The small cardiolipin fraction of the phospholipid was eluted rapidly with 2–4 % methanol in chloroform, and contained some cholesterol and other material. After precipitation with acetone, the product had a ratio P:fatty acid ester:amino N of 1:2·1:0·05, and was still low in P (3 %), but gave a single spot corresponding to cardiolipin on paper. The ethanol-insoluble potassium salt obtained by saponification in ethanolic 0·2 N-potassium hydroxide at room temperature, however, contained about 10 % of phosphomonoester by enzymic assay; the content of glycerophosphate P in the salt by Burmaster's (1946) method, after hydrolysis in N-hydrochloric acid at 100° for 10 min., was only 85 % of the total P against 92 % found for authentic cardiolipin ester, although the apparent α-glycerophosphate (42 %) after oxidation with periodate at room temperature was similar to that of cardiolipin (see Gray & Macfarlane, 1958). Some unidentified phospholipid was possibly present. The fatty acid composition of the cardiolipin fraction was significantly different from that of pigeon and ox in the low content of linoleic acid (6 %) and high content (35 %) of saturated acids (Table 3).

Table 2. *Distribution of phospholipids in various tissues*

Values are given as percentage of total lipid P.

	Rabbit muscle		Pigeon muscle			Pig		
Phospholipid	Whole	Myofibrils	Heart	Breast	Trout muscle	Spleen	Lung	Kidney
Cardiolipin	4	6	8	9	4	1	2	4
Phosphatidylinositol	4	4	8	2	2	2	5	3
Phosphatidylethanolamine	24	20	29	31	21	14	12	17
Ethanolamine plasmalogen						13	8	14
Phosphatidylserine				5	4	13	8	7
Serine plasmalogen						3	3	3
Phosphatidylcholine	54	51	43	33	66	33	50	36
Choline plasmalogen	11	14	6	16	< 1·0	2	1	3
Sphingomyelin	—	—	5	3	2	19	12	14
Recovery from column (%)	—	—	95	94	98	98	98	98

Table 3. *Fatty acid composition of triglycerides and cardiolipin*

Values are given as percentage of total fatty acid methyl esters.

		Triglycerides		Cardiolipin			
Fatty acid	Designation	Pigeon breast	Trout muscle	Pigeon breast	Trout muscle	Pig lung	Pig kidney
Myristic	14 : 0	0·3	2·8	Trace	2·0	0·4	—
Palmitic	16 : 0	19·0	20·0	0·4	19·1	5·9	5·8
Stearic	18 : 0	8·4	4·9	2·2	13·5	4·9	1·5
Total normal saturated	—	27·7	27·8	2·6	34·8	11·2	7·3
Palmitoleic	16 : 1	7·0	10·1	0·7	4·1	2·1	3·1
Oleic	18 : 1	52·8	42·3	12·8	44·6	13·8	15·6
Total monoenoic	—	59·8	52·4	13·5	48·7	15·9	18·7
Linoleic*	18 : 2	12·4	3·3	84·0	6·5	67·1	74·0
Eicosatrienoic	20 : 3	—	3·6	—	—	1·5	—
Arachidonic	20 : 4	Trace	1·3	—	—	2·5	—
Eicosapentanoic	20 : 5	—	3·3	—	—		—
Docosapentaenoic	22 : 5	—	2·6	—	1·7	—	—
Docosahexaenoic	22 : 6	—	3·2	—	—	—	—

* Includes any linolenic acid. Small amounts of branched C_{14}, C_{15}, branched C_{17} and C_{19} acids in some fractions have been omitted.

Table 4. *Fatty acid composition of phospholipids of pigeon and trout muscles*

Values are expressed as percentage of total methyl esters.

		Pigeon-breast muscle			Trout-muscle lecithin			
Fatty acid	Designation	Kephalin	Lecithin	Choline plasmalogen	I	II	III	Mean*
Myristic	14 : 0	—	Trace	Trace	—	0·4	2·6	1·6
Palmitic	16 : 0	4·2	24·4	9·2	6·6	29·6	33·2	26·2
Stearic	18 : 0	50·0	17·9	6·5	5·5	3·0	1·7	2·7
Total normal saturated	—	54·2	42·3	15·7	12·1	33·0	37·5	31·5
Palmitoleic	16 : 1	0·2	1·8	0·5	0·9	—	4·6	2·9
Oleic	18 : 1	4·2	22·6	8·7	8·2	8·7	12·8	11·0
Total monoenoic	—	4·4	24·4	9·2	9·1	8·7	17·4	13·9
Linoleic	18 : 2	7·6	24·1	9·7	—	0·3	4·6	1·6
Eicosatrienoic	20 : 3	5·5	1·6	7·9	2·0	—	—	0·4
Arachidonic	20 : 4	26·9	3·8	25·6	12·0	2·4	2·9	10·9
Eicosapentaenoic	20 : 5	1·2				6·7	8·2	
Total C_{20} polyenoic	—	33·6	5·4	33·5	14·0	9·1	11·1	11·3
Docosapentaenoic	22 : 5	—	2·0	24·4	3·9	2·2	3·0	3·0
Docosahexaenoic	22 : 6	—	0·5	7·5	61·0	43·4	29·3	38·5
Total C_{22} polyenoic	—	Nil	2·5	31·9	64·9	45·6	32·3	41·5

* Weighted mean. Includes any linolenic acid. Small amounts of branched C_{16}, branched C_{17} and C_{17} acids in some fractions have been omitted.

The kephalin fraction contained about 11 % of plasmalogen and was highly unsaturated (double bonds/molecule of P, 5·6); possibly in consequence of this, considerable decomposition occurred in this fraction on storage before saponification, and analyses for fatty acids were therefore not done.

The lecithin fraction contained less than 1 % of choline plasmalogen and was very highly unsaturated. Three successive fractions I, II and III from the silicic acid column, containing 19, 20 and 60 % of the lecithin P, had a P content 3·8–4·0 % and a ratio N:P of 0·96–1·08:1; these fractions gave a single spot on paper chromatography, and 96 % of the P was labile to mild alkali and acid. On saponification by refluxing for 2 hr. in 0·5N-potassium hydroxide in 95 % ethanol, 93 % of the P became water-soluble, all of which was present as glycerophosphate P by Burmaster's (1946) method. The three lecithin fractions were analysed separately for fatty acids (Table 4); nearly 40 % of the acids were C_{22} hexaenoic acids, and less than 2 % linoleic acid.

Pig spleen, lung and kidney. Approximately 80 % of the total lipid in lung and kidney and 50 % in spleen was phospholipid. The three tissues were generally similar in composition with no particular phospholipid conspicuous by its absence (Table 2).

Compared with that in heart tissue the amount of cardiolipin found was low, especially in spleen. A fatty acid analysis was not carried out on the cardiolipin from this source because the small fraction obtained was contaminated with too much phosphatidylethanolamine and phosphatidylserine. The ratio of phosphatidylethanolamine to phosphatidylserine varied from approximately 1:1 in spleen to 3:2 in lung and 5:2 in kidney. The spleen both from pig and ox (Gray, 1960*b*) appears to be one of the richest natural sources of phosphatidylserine. Ethanolamine plasmalogen was the major aldehydogenic compound in all three tissues with serine plasmalogen (3 %) and choline plasmalogen (3–1 %) present as minor components. Lecithin was as usual the major tissue phospholipid and sphingomyelin also occurred in substantial amounts (12–19 %). Phosphatidylinositol was present in minor quantities, though in lung its proportion (5 %) was greater than that of cardiolipin, serine plasmalogen or choline plasmalogen.

The fatty acid distribution in the lecithin, kephalin, choline plasmalogen and kephalin plasmalogen of the three tissues differed in a number of ways (Table 5). Spleen had the highest degree of unsaturation (expressed as double bonds per molecule of fatty acid) and lung the lowest. A large proportion of the acids from the kephalin plasmalogen fractions were C_{20} and C_{22} polyenoic compounds, that in spleen kephalin plasmalogen being exceptionally high (75·6 % of total acids). The kephalin plasmalogen and choline plasmalogen fatty acids were more unsaturated than those of the corresponding kephalin and lecithin, with the exception of lung choline plasmalogen, which contained 71 % of saturated acids and only 24 % of a monounsaturated acid.

The aldehydes (Table 6) from the choline plasmalogen and kephalin plasmalogen of lung and the

Table 5. *Fatty acid composition of different phospholipids from pig spleen, lung and kidney*

Values are expressed as percentage of total methyl esters

Acid	Designation	Kephalin			Kephalin plasmalogen			Lecithin			Choline plasmalogen		
		Spleen	Lung	Kidney	Spleen	Lung	Kidney	Spleen	Lung	Kidney	Spleen	Lung	Kidney
Myristic	14 : 0	0·1	0·2	Trace	Trace	—	0·1	0·4	1·3	0·2	0·4	2·0	0·1
Palmitic	16 : 0	3·8	9·1	11·0	3·3	4·3	4·9	30·0	47·1	29·0	27·2	64·3	18·8
Stearic	18 : 0	38·8	26·8	45·0	13·4	9·1	17·3	14·7	8·5	16·0	7·9	5·1	8·1
Total normal saturated		42·7	36·3	56·0	26·7	13·4	22·3	45·1	56·9	45·2	35·5	71·4	27·0
Total odd-numbered saturated		0·4	1·1	0·6	0·8	0·7	0·2	1·0	1·0	0·5	0·6	1·2	0·1
Total branched-chain saturated		0·4	0·6	0·2	Trace	0·6	Trace	0·8	0·7	0·5	0·4	0·7	0·1
Palmitoleic	16 : 1	0·4	0·9	0·3	0·3	0·9	0·2	0·8	2·8	0·7	2·0	3·6	0·3
Oleic	18 : 1	18·2	41·1	28·5	8·9	21·5	9·9	24·8	26·2	26·3	12·9	20·4	26·2
Total monoenoic		18·6	42·0	28·8	9·2	22·4	10·1	25·6	29·0	27·0	14·9	24·0	26·5
Linoleic	18 : 2	3·1	4·8	7·0	3·1	4·1	6·3	9·5	6·3	18·0	7·6	2·6	18·9
Linolenic	18 : 3	Trace	Trace	—	—	—	0·3	Trace	0·5	Trace	—	Trace	0·8
Total C_{18} di- and tri-enoic		3·1	4·8	7·0	3·1	4·1	6·6	9·5	6·8	18·0	7·6	2·6	19·7
Eicosaenoic	20 : 1	—	0·4	—	—	Trace	—	—	—	—	—	—	—
Eicosadienoic	20 : 3	7·3	2·4	} 5·7	11·4	—	5·7	3·9	0·9	1·6	10·2	—	4·4
Arachidonic	20 : 4	16·8	9·3		43·9	39·4	46·5	} 12·3	4·9	6·9	23·8	0·3	16·3
Eicosapentaenoic	20 : 5	2·2	Trace	1·1	5·0	2·5	3·0		—	Trace	3·1	—	2·9
Total C_{20} polyenoic		26·3	12·1	6·8	60·3	41·9	54·2	16·2	5·8	8·5	37·1	0·3	23·6
Docosatetraenoic	22 : 4	3·2	—	—	6·0	4·7	—	—	—	—	1·7	—	—
Docosapentaenoic	22 : 5	2·5	2·4	Trace	6·4	5·4	2·6	0·7	—	0·6	1·8	—	1·8
Docosahexaenoic	22 : 6	1·5	0·5	—	2·9	1·1	2·9	—	—	—	0·4	—	0·7
Total C_{22} polyenoic		7·2	2·9	Trace	15·3	11·2	5·5	0·7	—	0·6	3·9	—	2·5
Average no. of double bonds per fatty acid molecule		1·6	1·1	0·7	3·2	2·5	2·7	1·1	0·65	0·98	1·9	0·3	1·7

Table 6. *Aldehyde composition of plasmalogens from pig, pigeon and trout*

Values are expressed as percentage of total dimethyl acetals

Aldehyde	Designation	Pig spleen		Pig lung		Pig kidney		Pigeon muscle		Trout muscle
		Choline plasmalogen	Kephalin plasmalogen	Choline plasmalogen	Kephalin plasmalogen	Choline plasmalogen	Kephalin plasmalogen	Choline plasmalogen	Kephalin plasmalogen	Kephalin plasmalogen
Lauraldehyde	12 : 0	—	—	—	—	—	—	—	1·7	—
Myristaldehyde	14 : 0	Trace	Trace	0·5	0·4	3·0	Trace	—	0·3	—
12-Methyltridecanal / 11-Methyltridecanal	br. 14 : 0	Trace	0·1	Trace	Trace	—	Trace	0·1	—	—
Pentadecanal	15 : 0	0·9	0·3	1·8	1·3	3·9	3·0	1·0	2·7	11·0
13-Methyltetradecanal	*iso*-br. 15 : 0	Trace	—	Trace	0·8	3·8	3·4	—	—	0·5
12-Methyltetradecanal	*anteiso*-br. 15 : 0	Trace	0·1	0·8	0·3	Trace	1·4	0·5	0·2	—
Palmitaldehyde	16 : 0	66·6	41·3	81·5	80·0	81·5	50·0	81·5	43·0	66·5
Palmitoleylaldehyde	16 : 1	1·0	0·6	—	—	—	—	—	2·4	—
14-Methylpentadecanal / 13-Methylpentadecanal	br. 16 : 0	0·3	0·7	0·2	0·2	0·4	8·2	—	0·9	—
Heptadecanal	17 : 0	2·4	3·0	1·9	2·1	—	5·8	0·7	2·6	Trace
	17 : 1	4·4	1·7	—	—	—	—	—	—	—
16-Methylheptadecanal	*iso*-br. 17 : 0	0·8	0·4	0·4	0·7	0·5	Trace	—	—	4·3
15-Methylheptadecanal	*anteiso*-br. 17 : 0	1·7	0·9	0·8	1·1	1·6	1·2	—	Trace	—
Stearaldehyde	18 : 0	12·2	39·6	5·4	6·4	3·1	11·6	10·5	36·7	3·7
Oleylaldehyde	18 : 1	6·7	12·2	5·1	6·3	1·6	4·5	4·6	9·9	10·7
iso-Oleylaldehyde	*iso* 18 : 1	2·7	5·5	1·2	Trace	Trace	0·8	1·3	—	—
Linoleylaldehyde	18 : 2	0·4	1·0	0·4	0·6	0·4	—	—	—	—
17-Methyloctadecanal	*iso*-br. 19 : 0	—	—	—	—	—	—	—	—	—
16-Methyloctadecanal	*anteiso*-br. 19 : 0	—	—	—	—	—	10·3	—	—	3·4
Eicosanal	20 : 0	—	—	—	—	—	—	—	0·13	Trace

choline plasmalogen of kidney had a high proportion (80%) of palmitaldehyde. The spleen aldehydes contained a higher proportion of unsaturated aldehydes, mainly oleylaldehyde and linoleylaldehyde but including some monounsaturated heptadecanal, than lung or kidney. The kidney aldehydes on the other hand had a higher proportion of branched-chain compounds. The aldehydes from the kidney kephalin plasmalogen were peculiar in the amounts of branched C_{19} and branched C_{16} (8%) compounds present.

DISCUSSION

It might be expected that differences in family, species and diets would be reflected in variation in the nature and proportion of the different phospholipids and in their fatty acid composition. The analyses reported here for various tissues indicate little difference between beast, bird and fish in the nature of the phospholipids in the same kind of tissue, except for the virtual absence of choline plasmalogen from trout muscle. Moreover there is comparatively little difference in the fatty acid composition of the same phospholipid from the same tissue of different species in the proportions of saturated and polyenoic acids: the most notable exception to this is that cardiolipin from trout muscle contained little linoleic acid and a high proportion of saturated acids, and the lecithin contained a high proportion of docosahexaenoic acid.

The most substantial differences in fatty acid composition in these tissues appear to lie between the different kinds of phospholipid irrespective of tissue or species. The results of the present and previous work are presented graphically in Fig. 1 to show the C_{16} and C_{18} saturated acids, the mono- and di-enoic acids, and the C_{20} and C_{22} polyenoic acids in the triglycerides, lecithin and kephalin fractions. The preponderance of stearic acid as the saturated acid in kephalin and kephalin plasmalogen and of palmitic acid in lecithin and choline plasmalogen, noted previously (Gray, 1960*b*), holds except for liver lecithin, which both in the ox and the rat contains more stearic than palmitic acid. The distribution patterns of other fatty acids were far less predictable, though certain trends were indicated; the proportions of C_{20} polyenoic acids, especially arachidonic acid, and in general the C_{22} polyenoic acids in the kephalins were higher than in the corresponding lecithins and the lecithins contained a higher proportion of linoleic acid. Similar differences in the distribution of the unsaturated acids occur between the kephalin plasmalogens and the choline plasmalogens.

The tissue triglycerides analysed, though few in number, are remarkably similar to each other and to those reported for normal aorta (Böttcher, Boelsma-van Houte, Romeny-Wachter, Woodford & van Gent, 1960) and differ substantially from the kephalins in that palmitic acid is the preponderant saturated acid, and from both kephalins and lecithins in the low content of linoleic and polyenoic acid. It is clear that there is a considerable selectivity towards the fatty components in these compounds, as well as in cardiolipin and phosphatidylinositol, which should be borne in mind in considering the general validity of routes for the interconversion of tri- and di-glycerides and phospholipids—for example, in the biosynthesis of lecithin by transmethylation of phosphatidylethanolamine.

Saturated fatty acids with an odd number of carbon atoms and branched-chain fatty acids with both even and odd carbon numbers were minor components of all the tissues. The most commonly occurring, and very often the only members of these series, were the *anteiso* C_{15} and C_{17} acids, 12-methyltetradecanoic and 14-methylhexadecanoic acid. It seems possible that these branched fatty acids are of bacterial origin, for it is now known that they are the predominant or major kind of

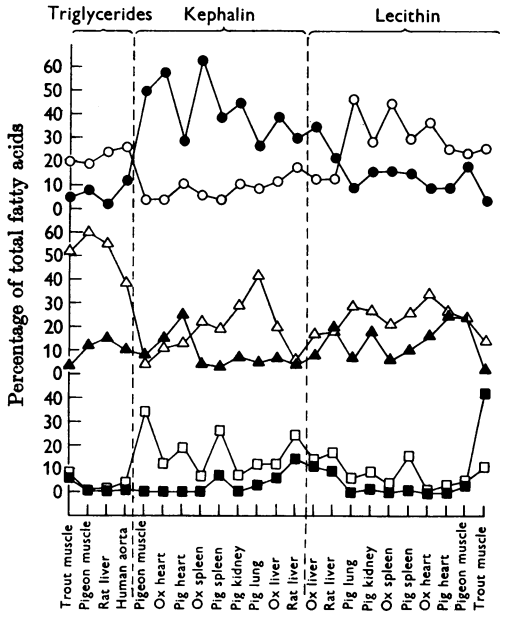

Fig. 1. Relative proportions of certain fatty acids occurring in the triglyceride, kephalin and lecithin fractions from different tissues. ○, Palmitic acid; ●, stearic acid; △, monoenoic C_{16} and C_{18} acids; ▲, dienoic C_{18} (including traces of trienoic C_{18} acid); □, polyenoic C_{20} acids; ■, polyenoic C_{22} acids. Values for triglycerides of human aorta are from Böttcher, Boelsma-van Houte, Romeny-Wachter, Woodford & van Gent (1960).

fatty acid in certain bacteria such as *Sarcina*, *Bacillus subtilis* and *Micrococcus lysodeikticus* (Akashi & Saito, 1960; Macfarlane, 1961*b*). James, Webb & Kellock (1961) note the occurrence of heptadecanoic acid as well as 10-hydroxystearic acid in the faecal lipids of subjects with steatorrhoea and discuss the possibility of synthesis by micro-organisms.

The distribution of palmitaldehyde (C_{16}) and stearaldehyde (C_{18}) in the plasmalogens of different tissues was similar to that of palmitic acid and stearic acid in the corresponding diacyl compounds (Gray, 1960*c*). The main difference was that, although there was more stearaldehyde in kephalin plasmalogen than in choline plasmalogen, palmitaldehyde was a large and usually the major normal saturated component in both. Saturated aldehydes with an odd number of carbon atoms and branched-chain aldehydes were minor components of all the tissues examined in this recent work. The C_{15} and C_{17} normal and branched-chain (both *iso* and *anteiso*) compounds occurred most frequently though small amounts of branched C_{14} and C_{16} aldehydes were often present. The aldehydes from pig-kidney and trout-muscle kephalin plasmalogen also included a branched-chain C_{19} aldehyde which was probably 16-methyloctadecanal.

In general aldehydes isolated from the plasmalogens of ox tissues (Gray, 1960*c*) had a far greater proportion of branched-chain compounds than those isolated from pig tissues (Table 7). In ox-spleen choline plasmalogen half of the total aldehydes were branched compounds, mainly 13-methylpentadecanal, but in pig spleen only 3% of the total aldehydes were branched compounds. The proportion of aldehydes with odd-numbered carbon chains was small (2–11%) varying only slightly from tissue to tissue and species to species. The proportions of unsaturated aldehydes varied more in pig tissues than in ox tissue.

The data so far obtained on the fatty acid and aldehyde composition of tissue phospholipids of different species suggests further limitations (Gray, 1960*b*) to the enzyme systems which are involved in the biosynthesis and metabolism of the plasmalogens. In all the sources so far examined the range of aldehydes (C_{14}–C_{18}) present is narrower than that of the fatty acids (C_{14}–C_{22}). Presumably the enzyme responsible for producing the typical $\alpha\beta$-unsaturated ether linkage in the plasmalogen molecule will not in general act on compounds with a carbon chain longer than 18 carbon atoms in the α-position of the glycerol moiety. Of course it is also possible that the high degree of unsaturation normally present in the C_{20} and C_{22} carbon chains and not the chain length prevents the enzyme from functioning.

SUMMARY

1. The lipid was extracted from pig spleen, lung and kidney, pigeon, trout and rabbit muscle, and the phospholipid fractionated quantitatively on silicic acid columns.

2. The distribution of the lipid phosphorus amongst cardiolipin, phosphatidylinositol, ethanolamine-, serine- and choline-containing diacyl

Table 7. *Amounts of branched-chain aldehydes, normal saturated odd-numbered carbon chain aldehydes and unsaturated aldehydes present in plasmalogens from different tissues*

Values for each class of aldehyde are expressed as a percentage of the total aldehydes.

	Branched-chain	Normal saturated odd-numbered carbon chain	Unsaturated
Choline plasmalogen			
Ox spleen	50·4	8·3	1·2
Ox heart	15·9	4·9	1·7
Ox liver	56·5	6·1	10·2
Pig heart	2·9	3·3	4·8
Pig spleen	2·9	3·3	15·2
Pig lung	2·2	2·0	6·8
Pig kidney	6·3	3·9	2·1
Pigeon muscle	0·6	1·7	5·9
Ram semen	17·7	8·9	2·4
Kephalin plasmalogen			
Ox spleen	9·1	6·5	8·7
Ox heart	7·9	7·1	6·9
Ox liver	52·4	8·6	5·0
Pig heart	4·4	4·7	10·5
Pig spleen	2·2	3·3	20·9
Pig lung	3·1	3·4	6·9
Pig kidney	23·3	8·8	5·2
Pigeon muscle	1·1	5·3	12·3
Trout muscle	8·2	11·0	10·7

and plasmalogen compounds and sphingomyelin was computed from the analysis of the fractions.

3. Choline plasmalogen was present in similar proportions in the whole muscle and myofibrils of rabbit muscle, but was virtually absent from trout muscle.

4. The constituent fatty acids and aldehydes from the separated phospholipids and some triglyceride fractions were identified and estimated by gas chromatography.

5. In general the results showed a preponderance of C_{18} saturated acids or aldehydes, and of polyenoic acids, in the kephalins, and of the C_{16} saturated compounds in phosphatidylcholine and choline plasmalogen, with the exception of liver and trout-muscle lecithins, which contained large amounts of C_{22} acids.

6. The fatty aldehydes fall in a narrower range (C_{14}–C_{18}) than the fatty acids (C_{14}–C_{22}), and included both unsaturated and branched-chain aldehydes, which varied considerably in proportion in different species.

7. The different fatty acid composition of lecithin, kephalin and triglyceride components of various tissues should be considered in possible routes of biosynthesis.

The support of this work by the British Empire Cancer Campaign is gratefully acknowledged. Mr M. J. Shaw assisted in the work on rabbit muscle.

REFERENCES

Akashi, S. & Saito, K. (1960). *J. Biochem., Tokyo*, **47**, 222.
Axelrod, J., Reichental, J. & Brodie, B. B. (1953). *J. biol. Chem.* **204**, 903.
Böttcher, C. J. F., Boelsma-van Houte, E., Romeny-Wachter, C. Ch.ter.Haar., Woodford, F. P. & van Gent, C. M. (1960). *Lancet*, ii, 1162.
Burmaster, C. F. (1946). *J. biol. Chem.* **164**, 233.
Carter, H. E., Smith, D. B. & Jones, D. N. (1958). *J. biol. Chem.* **232**, 681.
Coulon-Morelec, M. J., Faure, M. & Maréchal, J. (1960). *Bull. Soc. Chim. biol., Paris*, **42**, 867.
Dawson, R. M. C. (1954). *Biochim. biophys. Acta*, **14**, 374.
Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
Garcia, M. D., Lovern, J. A. & Olley, J. (1956). *Biochem. J.* **62**, 99.
Gray, G. M. (1960*a*). *Biochem. J.* **74**, 1P.
Gray, G. M. (1960*b*). *Biochem. J.* **77**, 82.
Gray, G. M. (1960*c*). *J. Chromat.* **4**, 52.
Gray, G. M. (1961). *J. Chromat.* (in the Press).
Gray, G. M. & Macfarlane, M. G. (1958). *Biochem. J.* **70**, 409.
Hartree, E. F. & Mann, T. (1959). *Biochem. J.* **71**, 423.
Hirsch, J. & Ahrens, E. H. (1958). *J. biol. Chem.* **233**, 311.
James, A. T., Webb, J. P. W. & Kellock, T. D. (1961). *Biochem. J.* **78**, 333.
Klenk, E. & Debuch, H. (1955). *Hoppe-Seyl. Z.* **299**, 66.
Klenk, E., Debuch, H. & Daun, H. (1953). *Hoppe-Seyl. Z.* **292**, 241.
Lovern, J. A., Olley, J., Hartree, E. F. & Mann, T. (1957). *Biochem. J.* **67**, 630.
Macfarlane, M. G. (1961*a*). *Biochem. J.* **78**, 44.
Macfarlane, M. G. (1961*b*). *Biochem. J.* **80**, 45P.
Macfarlane, M. G., Gray, G. M. & Wheeldon, L. W. (1960). *Biochem. J.* **77**, 626.
Marinetti, G. V. & Erbland, J. (1957). *Biochim. biophys. Acta*, **26**, 429.
Marinetti, G. V., Erbland, J. & Kochen, J. (1957). *Fed. Proc.* **16**, 837.
Perry, S. V. & Grey, T. C. (1956). *Biochem. J.* **64**, 184.
Pietruszko, R. & Gray, G. M. (1960). *Biochim. biophys. Acta*, **44**, 197.
van Beers, G. J., de Iongh, H. & Boldingh, G. (1958). In *Essential Fatty Acids*, p. 43. Ed. by Sinclair, H. M. London: Butterworths Scientific Publications.
Webster, G. R. (1960). *Biochim. biophys. Acta*, **44**, 109.

Biochem. J. (1961) **81**, 488

Alternative Pathways of Carbohydrate Metabolism in Foetal and Adult Tissues

BY C. A. VILLEE AND J. M. LORING
Department of Biological Chemistry, Harvard Medical School, and Research Laboratories of the Boston Lying-in Hospital, Boston, Mass., U.S.A.

(*Received* 3 *March* 1961)

The recognition of pathways of carbohydrate metabolism other than the classical Embden–Meyerhof glycolytic sequence has stimulated efforts to clarify the reaction mechanisms involved and to evaluate the relative importance of these alternative pathways in different tissues. Evidence about the fractions of glucose metabolized via the glycolytic sequence and via the hexose monophosphate pathway has been obtained from experiments in which the differential utilization of specifically labelled glucose or other carbohydrate intermediates has been measured. There is as yet