tissues and found to be present in amounts from 0.5 to 150 $\mu\text{mg./g.}$ dry wt.

3. The metal was also determined in nine samples of human blood and found to be present in amounts from 0.5 to 2.3 $\mu\text{mg./g.}$ of whole blood, with a mean of 1.1 $\mu\text{mg./g.}$ (0.10 $\mu\text{g./100 ml.}$).

4. The limited data on tungsten/molybdenum ratios indicate that tungsten, though much less common than molybdenum, is accumulated to an approximately equal extent from the environment by living organisms.

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The Phospholipids of Ox Spleen with Special Reference to the Fatty Acid and Fatty Aldehyde Compositions of the Lecithin and Kephalin Fractions

By G. M. GRAY*

The Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W. 1

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The fatty acid compositions of lecithin and kephalin fractions from different animal tissues have not yet been studied in a systematic manner, though several excellent papers have been published by Klenk and his co-workers (Klenk & Bohm, 1951; Klenk, Debuch & Daun, 1953; Klenk & Krickau, 1957) on the whole glycerophosphatide fraction and some purified phospholipids from a number of animal tissues. From their results it is apparent that different tissues contain lecithin and kephalin fractions which differ widely in their fatty acid composition.

The plasmalogens differ from the classical ester phosphatides in having only one fatty acid esterified with the glycerol moiety instead of two.

Instead of the other fatty acid there is a carbon chain of similar length attached to the glycerol by an $\alpha\beta$ -unsaturated ether linkage. This linkage is easily ruptured by weak acid to give a long-chain fatty aldehyde and a lysophosphatide. It has been shown that in ox brain (Debuch, 1958) and ox heart (Debuch, 1958; Gray, 1958) the fatty acids of the choline and ethanolamine plasmalogens are mostly unsaturated. Is this a characteristic difference between the plasmalogens and ester phosphatides which is common to all tissues?

Much less is known of the fatty aldehyde composition of plasmalogens from different tissues. Stearaldehyde and palmitaldehyde are the main components of aldehyde mixtures isolated from ox-heart muscle (Klenk, Stoffel & Eggers, 1952; Gray, 1958) and 50% of the aldehydes isolated from

* Beit Memorial Fellow.

ox-brain tissue have been identified as unsaturated compounds, mostly with one double bond (Debuch, 1958). Aldehydes from the plasmalogens shown to be present in many other tissues have not been identified. A systematic study of the distribution patterns of fatty acids and fatty aldehydes of the lecithin and kephalin fractions separated quantitatively from selected tissues has been started. In this paper those isolated from ox spleen are compared with those of ox heart, ox liver, pig heart and ram semen.

The term kephalin is used for mixtures of phosphatidylethanolamine, phosphatidylserine and the corresponding plasmalogens (referred to separately as kephalin plasmalogen).

EXPERIMENTAL AND RESULTS

Analytical methods

The estimations of P, total N, amino N, ethanolamine and serine, choline, aldehyde, inositol, fatty acid ester groups and iodine value were carried out as described by Gray & Macfarlane (1958).

Extraction and fractionation of the phospholipids from ox spleen

The extraction was based on that of Folch, Ascoli, Lees, Meath & LeBaron (1951). Ox spleen (365 g. wet wt.), obtained 2–3 hr. after death, was cut up and blended for 30 sec. in an MSE blender at room temperature with 5 vol. of chloroform-methanol (1:1, v/v) and kept for 15–20 min. in a large jar with occasional stirring. The extract was filtered on a Büchner funnel and the residue re-extracted twice with 1 vol. of chloroform-methanol (2:1, v/v). The extracts were combined and poured into a 5 l. jar (solvent volume

approx. 2.7 l.). The solution was washed by stirring with 1 vol. of tap water; after 1–2 hr. the water was siphoned off and kept. A red-brown interfacial solid was removed from the surface of the chloroform and two more washings were carried out with 0.5 vol. (approx. 1 l.) of water; these washings were combined with the first one. The chloroform solution was kept at -10° overnight to freeze out the remaining water, filtered at 2° and the solution dried over anhydrous Na_2SO_4 . The solvent was removed *in vacuo* at 35° and the lipid dissolved in light petroleum (b.p. $40-60^{\circ}$). Non-P lipid (e.g. neutral fat, cholesterol, sterol esters) was removed from the phospholipids by the dialysis method of van Beers, de Jongh & Boldingh (1958). This method is most useful for removing non-P lipid with the advantage of quantitatively retaining all the phospholipids. After dialysis the light-petroleum solution of ox-spleen phospholipids was evaporated to dryness at 35° *in vacuo* and redissolved in chloroform. The analysis of this solution is given in Table 1. No inositide was found in the chloroform extract. The aqueous washings, saved from the extraction procedure, were evaporated to dryness at 35° *in vacuo* and the residue was extracted with ether and chloroform-methanol (2:1, v/v). The solvents were evaporated off and the residue was dissolved in a known volume of chloroform. Analysis gave total P, 1.2 mg.; inositol was not detected. The presence of small amounts of phosphatidylinositol cannot be excluded as it is possible that the substance was not extracted by organic solvents from the large amount of inorganic salts, nucleotides, amino acids etc., present in the residue owing to occlusion or the formation of a complex.

Fractionation of phospholipids on silicic acid. The phospholipids as a 10% solution in chloroform-methanol (49:1, v/v) were chromatographed on a column (diameter, 31 mm.) containing 160 g. of silicic acid in chloroform-methanol (49:1, v/v). Elution was started with the same solvent. A fast-running yellow-coloured fraction containing no P was quickly eluted from the column and found to be mainly free aldehydes, which indicated the probable breakdown of some plasmalogen. This fraction was closely followed by another small P-free fraction which was probably a trace of neutral fat not removed during the previous dialysis procedure. The concentration of methanol in chloroform was gradually increased as the different phospholipid fractions were eluted from the column. Fig. 1 illustrates the separation curve obtained based on analysis of the fractions for P, amino N and fatty acid ester groups. Approximately 90% (193 mg.) of the total P loaded on to the column was recovered. An attempt to recover the remainder by increasing the methanol concentration in chloroform to 90% was unsuccessful so the solvent was changed to chloroform-methanol-water (1:4:1, by vol.) and finally to chloroform-methanol-aq. 2N-NH_3 soln. (1:4:1, by vol.). The last solvent eluted a further fraction (H) containing 8 mg. of P. Table 2 shows the analysis of the bulked fractions A–H.

Table 1. *Analysis of total phospholipid extract of ox spleen*

Values given are those of 365 g. (wet wt.) of ox spleen. The percentage of P (on dry-weight basis) was 3.98.

	Amount present (mg.)	Molar ratio (P = 1)
Total P	228.0	1
Total N	124.0	1.19
Amino N*	46.5	0.45
Serine N†	21.6	0.21
Ethanolamine N‡	27.5	0.27
Choline N	51.2	0.50
Aldehyde	406.0	0.23
Ester P†	200.6	0.88
Fatty acid ester groups	—	1.73

* Estimated by the ninhydrin method of Lea & Rhodes (1954).

† Estimated by the method of Axelrod, Reichenthal & Brodie (1953).

‡ Estimated by the method of Schmidt, Benotti, Hershman & Thannhauser (1946).

*Nature of the phospholipids
in the various fractions*

Acidic phospholipids. A small fast-moving band (fraction A), low in amino N and very unsaturated, was eluted with chloroform-methanol (19:1, v/v). It was presumably substantially cardiolipin (Gray & Macfarlane, 1958).

Diacyl phosphatides and plasmalogens. Fractions B, C and D were mixtures of phosphatidylethanolamine, ethanolamine plasmalogen, phosphatidylserine and serine plasmalogen. Fraction B was eluted as a very sharp band with chloroform-methanol (9:1, v/v) and contained 88% of serine phosphatides and only 4% of ethanolamine phos-

phatides. The plasmalogens accounted for 7% of the total P in the fraction. Fraction C also contained approx. 10% of non-amino-N-containing material, which was most probably more cardiolipin trailing from fraction A. The separation of a fast-running serine fraction is unusual and may have been due to a relatively high acidity, either of the original phospholipid solution or of the silicic acid column, and the presence of some phosphatidylserine in the free acid form. Fraction D was a small band containing a mixture of diacyl phosphatides, plasmalogens and probably the remainder of the cardiolipin. Fraction E contained approx. 80% of ethanolamine phosphatides and 20% of serine phosphatides. Plasmalogens account

Fig. 1. Fractionation of the phospholipid extract from ox spleen on silicic acid. O, Total P; ▲, P present in N-free phospholipid, calculated from P and amino N values; □, amino N; ●, ester P, calculated from fatty acid ester group:P ratio.

Table 2. *Distribution and analysis of phospholipids separated by silicic acid chromatography*

The total P on the column was 215 mg. and the total P recovered was 201 mg.

Fraction (see Fig. 1)	Tubes	P content (% of total lipid P)	Molar ratio (P = 100)					Double bonds
			Amino N*	Ethanol- amine N†	Serine N†	Aldehyde	Fatty acid ester group	
A	8-15	0.6	6	—	—	Nil	210	320
B	16-20	7.1	89	4	88	7	206	99
C	21-30	5.8	64	—	—	21	190	163
D	31-83	17.2	107	80	23	32	179	166
E	84-95	11.2	85	63	16	9	100	102
F	97-151	36.8	2	Nil	Nil	11	190	214
G	152-252	11.0	Nil	Nil	Nil	4	20	95
H	253-	3.7	98	38	21	Nil	145	296

* By the ninhydrin method of Lea & Rhodes (1954).

† By the method of Axelrod *et al.* (1953).

for 32% of the total P. Fraction F was a mixture of phosphatidylcholine (89%) and choline plasmalogen (11%).

Lysophosphatides. Fraction E was mainly lyso-phosphatidyl-ethanolamine and -serine from the breakdown of the ethanolamine and serine plasmalogens. It was slightly contaminated with lecithin from fraction F.

Sphingomyelin. Fraction G was substantially sphingomyelin. The molar ratio P:N of 1:1.91 (theory 1:2) and the presence of 10% of ester P, estimated by the method of Schmidt, Benotti, Hershman & Thannhauser (1946), showed that it was contaminated with lecithin from the previous fraction F (Fig. 1).

Other components. Fraction H, eluted with some difficulty from the column, was not examined in detail. It had a P:N ratio of 1:1.7; P, 2.6% on a dry wt. basis. Apparently some ethanolamine and serine (Table 2) were present, but since the total amino N was much higher than the sum of these two compounds it is possible that the method used (Axelrod, Reichenthal & Brodie, 1953) was not estimating either, but some other amino N compound. Contamination of the material with ammonia from the eluting solvent was ruled out because the fraction had been evaporated to dryness at 35° *in vacuo*, dissolved in ether and extracted with 2N-HCl before analysis. The presence of unsaturated fatty acids was indicated by the high ester value and iodine value (double bonds, Table 2). It is not known whether the fraction was

homogeneous or a mixture of fragments which had been very strongly adsorbed on the silicic acid.

Composition of ox-spleen phospholipid. From the analyses in Table 2 and the results in Table 3 the distribution of phospholipids was computed (as percentage of the total lipid P) as: cardiolipin (and similar compounds), 3.0; phosphatidylethanolamine, 9; phosphatidylserine, 14; ethanolamine plasmalogen, 10; serine plasmalogen, 5; phosphatidylcholine, 35; choline plasmalogen, 4; sphingomyelin, 10; unidentified, 4; not recovered, 6%.

Fatty acids and fatty aldehydes in the kephalin and lecithin fractions

The fatty acids and aldehydes were isolated from the kephalin, kephalin plasmalogen, lecithin and choline plasmalogen of ox spleen and from the following phospholipid fractions of other tissues: the kephalin and lecithin fractions of ox heart, pig heart (Gray & Macfarlane, 1958) and ox liver (prepared by Dr M. G. Macfarlane) and the lecithin fraction of ram semen (Gray, 1960a). The procedures used for the ox-spleen fractions are applicable to the kephalin and lecithin fractions of any tissue.

The isolation of the fatty acids from phosphatidylethanolamine, phosphatidylserine and phosphatidylcholine and the fatty acids and aldehydes from the ethanolamine, serine and choline plasmalogens is carried out by first treating the mixed kephalin and mixed lecithin fractions with 90% acetic acid at 38° for 18 hr. (Gray, 1958). Under these conditions the aldehydes split off from the

Table 3. Separation of the products of 90%-acetic acid hydrolysis of the kephalin fraction on silicic acid

Fraction	Eluting solvent CHCl ₃ -methanol (ratio, v/v)	Tubes	Total P (mg.)	Molar ratio		Comment
				P:fatty acid ester groups	Ethanolamine:serine	
(a)	49:1	1-10	—	—	—	Free aldehydes
(b)	19:1	11-20	4.8	1:2	—	Contained only 18% of amino N
(c)	9:1	21-30	19.7	1:2	1:3	Diacyl phosphatide
(d)	9:1	31-40	1.96	1:1.2	—	Lysophosphatidic acid
(e)	4:1	41-65	12.6	1:2	1:0.69	Diacyl phosphatide
(f)	4:1	66-80	2.4	1:1.9		
(g)	11:9	81-100	19.5	1:1	1:0.51	Lysophosphatide
(h)	11:9	101-140	3.3	1:1		

Table 4. Separation of the products of 90%-acetic acid hydrolysis of the lecithin fraction on silicic acid

Fraction	Eluting solvent CHCl ₃ -methanol (ratio, v/v)	Tubes	Total P (mg.)	Molar ratio		Comment
				(P:fatty acid ester groups)		
(i)	4:1	1-7	—	—	—	Free aldehydes
(ii)	1:1	8-50	65.0	1:1.98	—	Phosphatidylcholine
(iii)	1:1	51-73	1.17	1:1.63	—	—
(iv)	1:1	74-91	0.45	1:1.64	—	—
(v)	3:7	92-140	5.55	1:1.07	—	Lysophosphatidylcholine

Table 5. *Fatty acid composition of different phospholipids from various tissues*

The shortened designation (column 2) was evolved by Ahrens *et al.* (1959); e.g. br 16:1 represents a branched mono-unsaturated C₁₆ compound; hbr, probably highly branched; un, unknown; tr, trace.

Familiar name of acid	Shortened designation	Ox spleen		Ox liver		Ox heart		Pig heart	
		Lecithin	Choline plasmalogen	Lecithin	Choline plasmalogen	Lecithin	Choline plasmalogen	Lecithin	Choline plasmalogen
Capric	10:0	—	—	—	—	—	—	—	—
Undecanoic	11:0	tr	—	—	tr	—	—	—	—
	br 12:0	—	—	tr	0.1	—	—	—	—
Lauric	12:0	tr	0.3	tr	0.2	—	—	—	—
Tridecanoic	13:0	—	—	—	—	—	—	—	—
Myristic	14:0	0.6	0.3	tr	0.2	0.4	tr	0.1	tr
Myristoleic	14:1	—	—	—	—	—	—	—	—
	hbr 15:0	0.3	—	—	—	—	—	—	—
Pentadecanoic	br 15:0	tr	—	0.2	tr	—	—	—	—
	15:0	1.2	0.9	0.2	tr	0.5	tr	0.1	—
Palmitic	br 16:0	1.0	0.3	—	—	0.4	tr	0.2	0.7
	16:0	45.0	37.8	12.7	14.0	36.7	6.9	26.4	8.3
Palmitoleic	16:1	1.6	1.7	0.6	0.9	4.2	2.3	1.5	0.6
	un	—	—	—	—	—	—	—	—
Heptadecanoic	hbr 17:0	0.9	1.1	—	—	—	—	—	—
	br 17:0	2.4	2.3	0.6	0.3	1.4	—	—	—
	17:0	1.4	1.5	0.7	0.8	1.0	—	0.4	—
	17:1	—	—	—	0.2	1.5	1.0	—	—
	17:2	—	—	—	—	—	—	—	—
	br 18:0	—	—	—	—	tr	2.0	—	—
Stearic	18:0	16.3	14.0	35.0	21.9	8.7	2.5	9.3	1.7
Oleic	18:1	19.1	17.3	14.3	12.1	30.0	36.4	25.8	26.6
isoOleic	iso 18:1	—	tr	1.6	3.0	—	—	—	—
Linoleic	18:2	6.1	7.1	7.7	4.7	14.5	42.4	25.1	53.6
Linolenic	18:3	—	tr	tr	0.7	1.3	2.5	tr	0.9
	un	—	—	—	—	—	—	—	—
	un	—	—	—	tr	—	—	—	—
	20:3	0.9	0.6	3.5	3.0	—	1.8	—	—
Arachidonic	20:4	3.5	13.6	7.8	8.5	—	2.4	3.0	7.9
	20:5	—	—	2.6	3.9	—	tr	—	tr
	un	—	—	0.6	1.2	—	—	—	—
	un	—	—	1.2	—	—	—	—	—
	22:5	—	—	9.6	18.3	—	—	—	—
	22:6	—	—	1.5	5.9	—	—	—	—

Table 5 (cont.)

Familiar name of acid	Shortened designation	Ram semen		Ox spleen		Ox liver		Ox heart		Pig heart kephalin
		Lecithin	Choline plasmalogen	Kephalin	Kephalin plasmalogen	Kephalin	Kephalin plasmalogen	Kephalin	Kephalin plasmalogen	
Capric	10:0	—	—	—	tr	—	—	tr	0.5	—
Undecanoic	11:0	0.3	0.6	—	—	—	—	tr	0.2	—
	br 12:0	—	—	—	—	—	—	tr	0.7	—
Lauric	12:0	0.2	0.8	0.2	tr	0.1	0.2	tr	0.9	tr
Tridecanoic	13:0	—	—	—	tr	—	—	—	—	—
Myristic	14:0	5.0	5.8	0.2	0.2	tr	0.2	0.2	0.3	0.2
Myristoleic	14:1	0.4	1.0	—	—	—	—	—	—	—
	hbr 15:0	0.8	1.2	—	—	0.1	tr	tr	tr	—
Pentadecanoic	15:0	1.9	2.3	—	0.2	0.1	0.1	tr	tr	0.2
	br 16:0	0.5	2.2	—	—	0.1	tr	—	2.1	1.4
Palmitic	16:0	37.9	32.0	5.8	6.0	12.3	15.2	3.5	7.8	11.1
Palmitoleic	16:1	3.1	5.4	0.4	0.4	0.3	0.5	1.2	0.8	0.6
	un	2.8	tr	—	—	—	—	—	—	—
hbr 17:0	br 17:0	—	—	—	—	—	—	—	—	—
	un	—	—	—	—	—	—	—	—	—
Heptadecanoic	17:0	0.9	1.6	0.6	1.3	1.0	0.7	0.8	2.1	—
	17:1	16.2	10.1	0.6	1.3	1.2	1.2	1.0	—	0.8
17:2	br 18:0	4.3	2.2	—	—	—	—	—	—	—
	un	—	—	—	—	—	—	—	—	—
Stearic	18:0	8.2	11.7	63.0	43.0	39.4	39.5	58.0	12.1	0.5
Oleic	18:1	6.5	9.5	21.4	12.7	13.2	13.9	6.5	15.8	29.0
isoOleic	iso 18:1	tr	tr	—	—	6.5	8.5	2.9	tr	11.9
Linoleic	18:2	0.9	tr	4.3	2.8	6.8	4.35	13.6	40.7	25.2
Linolenic	18:3	—	—	—	—			0.9	3.3	tr
	un	4.2	2.9	—	—	tr	0.4	—	—	—
un	un	4.2	—	—	—	—	—	—	—	—
	20:3	tr	—	tr	1.9	1.3	1.5	tr	4.9	tr
Arachidonic	20:4	1.9	11.5	6.9	29.2	11.1	9.4	11.6	7.1	18.6
	20:5	—	—	tr	1.5			tr	0.8	—
un	un	—	—	—	—	—	—	—	—	—
un	un	—	—	—	—	—	—	—	—	—
22:5	22:5	—	—	—	—	5.1	3.8	—	—	—
22:6	22:6	—	—	—	—	1.1	0.6	—	—	—

plasmalogen with the formation of the lysophosphatide, but the ester phosphatides are not attacked. The free aldehydes, the diacyl phosphatide and the lysophosphatide are easily separated by chromatography on silicic acid and the fatty acids are obtained from the phosphatide fractions by mild alkaline hydrolysis.

Kephalin fraction. Fractions B, C, D and E (Table 2) were bulked and a volume containing 70 mg. of P was evaporated to dryness. The residue was dissolved in 7 ml. of 90% (v/v) acetic acid and incubated at 38° for 18 hr. The solution was cooled, neutralized with NaOH and then shaken with an equal volume of chloroform-methanol (2:1, v/v). The phases were separated by centrifuging and the upper aqueous methanol layer was removed. The chloroform layer was washed three times with 0.2 vol. of 0.5M-NaCl. The presence of the NaCl was necessary to keep emulsion formation to a minimum. The chloroform solution was dried over anhydrous Na_2SO_4 , filtered and evaporated at 35° *in vacuo*; the residue was redissolved in a known volume of chloroform-methanol (49:1, v/v). The total P in the chloroform solution was 66.5 mg. and the water-soluble P (in aqueous methanol extract) was 3.2 mg.

The phospholipid extract was chromatographed on 65 g. of silicic acid in chloroform-methanol (49:1, v/v) and the results are shown in Table 3. The aldehydes in fraction (a) were added to those collected from the original separation after breakdown of some of the kephalin plasmalogens (as described previously), and the combined aldehydes were converted into their dimethylacetal derivatives with the usual precautions (Gray, 1960b). Fraction (b) was probably a mixture of cardiolipin [present in fractions B and C (Table 2)] and some phosphatidic acid, formed from the ester phosphatide by a slight loss of base during the 90% acetic acid hydrolysis. Fractions (c), (e) and (f) contained the diacyl phosphatides, (c) being rich in phosphatidylserine (cf. fraction C, Table 2). Fractions (g) and (h) contained the lysophosphatidylethanolamine and lysophosphatidylserine.

Fractions (c), (e) and (f) were combined and the solvent was removed by evaporation at 35° *in vacuo*. The residue (34 mg. of P) was dispersed in 50 ml. of aqueous N-NaOH and saponified in a stoppered flask under N_2 at room temperature overnight. Fractions (g) and (h) were combined (22.5 mg. of P) and saponified in 30 ml. of aqueous N-NaOH overnight. The mixtures were acidified with 5N-HCl and the fatty acids were extracted with ether. The ethereal solutions were washed with water and dried over anhydrous Na_2SO_4 . The ether was evaporated off and the mixtures of acids were refluxed for 3 hr. with 5-10 vol. of anhydrous methanolic 0.8N-HCl and quantitatively converted

into their methyl esters for analysis by gas-liquid chromatography.

Lecithin fraction. A major portion (74 mg. of P) of fraction F, the lecithin fraction containing 11% of choline plasmalogen, was treated with 7 ml. of 90% acetic acid as described previously. Water-soluble P, found after hydrolysis, was 1.23 mg. and chloroform-soluble P was 72.2 mg. The chloroform solution of phospholipids and free aldehydes was chromatographed on 60 g. of silicic acid in chloroform-methanol (4:1, v/v) and the results are shown in Table 4.

Fraction (i) containing the aldehydes was dried and the aldehydes were converted into the dimethylacetals. Portions of the phosphatidylcholine [30 mg. from fraction (ii)] and of the lysophosphatidylcholine [fraction (v), P, 5.5 mg.] were saponified and the acids were isolated and converted into their methyl esters.

Gas-liquid chromatography. The chromatographic apparatus was a laboratory-constructed model, with the argon β -ray-ionization detector designed by Lovelock, James & Piper (1959). All columns were of glass, 4 ft. long and with internal diameter of 4 mm. The stationary phases used were Apiezone L grease (1.8 g./7 g. of Celite) and Reoplex 400 (2.1 g./7 g. of Celite). The Celite (100-120 mesh) was prepared by the method of James & Martin (1952) and for use with Apiezon L grease was pretreated with ethanolic alkali (James, Martin & Smith, 1952).

Major acids were identified by comparison with retention volumes of pure standards. The presence of unsaturated components was confirmed by bromination and their degree of unsaturation assessed by the method of James (1959). The separation and identification of the fatty aldehydes has been described in detail elsewhere (Gray, 1960b). The fatty acid composition of the ester phosphatides and the fatty acid and aldehyde composition of the plasmalogens of ox spleen are shown in Tables 5 and 6 and are compared with similar components from ox heart, ox liver, pig heart and ram semen.

DISCUSSION

The results obtained from previous investigations (Debuch, 1958; Gray, 1958) suggested that most of the fatty acids in plasmalogens would be unsaturated. The results obtained from the study of five different tissues (Tables 5 and 7) do not, however, support this idea. The acids from the plasmalogens from ox and pig hearts are mostly unsaturated but the plasmalogens of ox spleen, ox liver and ram semen have widely varying proportions of saturated and unsaturated acids. The major unsaturated acids in all the phosphatide fractions with the exception of those from ox liver are oleic

acid, linoleic acid and arachidonic acid, higher proportions of the last being found in the kephalin and kephalin plasmalogen fractions. Linolenic acid occurs only in very small amounts. The ox-liver phosphatides also contain substantial amounts of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid (cf. Klenk & Montag, 1957; Klenk & Tomuschat, 1957). Fatty acids with odd-numbered carbon chains and branched-chain acids only occur in small or trace amounts in all fractions except those from ram semen, which apparently contain substantial amounts of heptadecanoic acids with one and two double bonds. It is necessary to examine ram-semen phosphatides from a number of different sources before accepting these acids as normal components. With the exception of ox liver the major saturated acid in the lecithins and in the choline plasmalogens is palmitic acid (C_{16}) whereas the major saturated acid in the kephalins and in the kephalin plasmalogens is stearic acid (C_{18}). This distribution of saturated C_{16} and C_{18} acids may be a characteristic pattern in phosphatides from the majority of tissues.

The distribution patterns of the aldehydes (Table 6) are very complicated. Debuch (1958) found that 50% of the total aldehydes from the ethanolamine plasmalogen of ox brain were unsaturated, but the aldehydes from the plasmalogens studied in this work contain only small proportions of mono-unsaturated components. Surprising features are the large proportions of branched-chain aldehydes in ox spleen and ox liver. The

most common aldehyde of this type appears to be a branched-chain C_{15} compound; it is a major component of the aldehydes from ox-spleen choline plasmalogen and the major component of the aldehydes from ox-liver plasmalogens. The chemical structures of these compounds are not yet known, but a structural similarity to the branched-chain acids (Table 5) is to be expected.

The distribution of C_{16} and C_{18} fatty acids, in different phosphatide fractions, is paralleled by the distribution of C_{16} and C_{18} aldehydes. In all cases (ox liver excepted) the proportion of palmitaldehyde (C_{16}) in choline plasmalogen is much greater than that in kephalin plasmalogen from the same tissue. In the same way the proportion of stearaldehyde (C_{18}) in the kephalin plasmalogen is much greater than that in the choline plasmalogen. If the patterns in these few tissues are representative of most tissues they suggest that in the biosynthesis of lecithin/choline plasmalogen and kephalin/kephalin plasmalogen there is an enzymic preference for saturated fatty acids and aldehydes of 16 and 18 carbon atoms respectively. Whether or not this is true might be verified by using the enzyme systems described by Kennedy (1957) for the biosynthesis of lecithin and kephalin starting with model diglycerides containing the appropriate fatty acids.

SUMMARY

1. Phospholipids equivalent to about 600 mg. of phosphorus/kg. of fresh tissue were extracted from ox spleen with chloroform-methanol mixtures and were fractionated on silicic acid columns.

2. The percentage composition of the phospholipid was: cardiolipin, 3; phosphatidylethanolamine, 9; phosphatidylserine, 14; ethanolamine plasmalogen, 10; serine plasmalogen, 5; phosphatidylcholine, 35; choline plasmalogen, 4; sphingomyelin, 10; unidentified, 4; not recovered, 6.

3. The fatty acids of the ester phosphatides and the fatty acids and aldehydes of the corresponding plasmalogens were isolated after treatment of the mixed phosphatide fractions with 90% acetic acid at 38° and separation of the products on silicic acid.

4. The composition of the various fatty acid and fatty aldehyde mixtures from the phosphatide fractions of ox spleen were determined by gas-liquid chromatography and the distribution patterns were compared with those of similar fractions from ox heart, ox liver, pig heart and ram semen.

5. Large amounts (approx. 50%) of branched-chain aldehydes are present in ox-spleen choline plasmalogen and ox-liver plasmalogens. A branched-chain C_{15} compound is a major component of the aldehydes from both these tissues.

Table 7. *Approximate amounts of unsaturated fatty acids present in different phospholipid fractions*

	Fraction as percentage of total phospholipid in tissue	Unsaturated acids in fraction (%)
1. Lecithin		
Ox spleen	35	31
Ox heart	18	51
Ox liver	58	50
Pig heart	27	55
Ram semen	36	33
2. Choline plasmalogen		
Ox spleen	4	40
Ox heart	24	90
Ox liver	6	72
Pig heart	23	89
Ram semen	36	43
3. Kephalin		
Ox spleen	23	34
Ox heart	25	38
Ox liver	26	45
Pig heart	27	56
4. Kephalin plasmalogen		
Ox spleen	15	50
Ox heart	18	73
Ox liver	6	43

6. The results suggest a selective incorporation of saturated fatty acids and aldehydes of 16 carbon atoms into lecithin and choline plasmalogen and of 18 carbon atoms into kephalin and kephalin plasmalogen.

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Studies in the Biosynthesis of Fungal Metabolites

THE BIOSYNTHESIS OF PALITANTIN

BY P. CHAPLEN AND R. THOMAS*

Department of Biochemistry, London School of Hygiene and Tropical Medicine, University of London

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Palitantin, $C_{14}H_{22}O_4$, a metabolite of *Penicillium palitans* Westling and other species, was first isolated and characterized by Birkinshaw & Raistrick (1936). It was further investigated by Birkinshaw (1952) and by Bowden, Lythgoe & Marsden (1959), who proposed the structure (I) (Fig. 1).

The biosynthesis of this C_{14} -alicyclic compound is of interest in its relation to auroglaucon (II) and flavoglaucon, phenolic products of *Aspergillus glaucus* (Gould & Raistrick, 1934; Quilico, Cardani & Lucattelli, 1953), in that they each contain a C_1 and a linear C_7 side chain attached to adjacent carbon atoms of a C_6 ring. Also auroglaucon has been shown by Birch, Schofield & Smith (1958) to be derived by the condensation of acetate units. Since the formation of aromatic rings by this mechanism may well involve alicyclic intermediates

the possible relevance of palitantin biosynthesis is readily apparent.

Although acetate coupling is the pathway most readily visualized (Bowden *et al.* 1959), the presence of three vicinal oxygen substituents is reminiscent of 5-dehydroshikimic acid (III), which is a known precursor of the aromatic amino acids and consequently an alternative source of benzenoid rings. Although the necessary additional modifications render this latter mechanism less probable, the possible operation of such a pathway added interest to the problem of palitantin biosynthesis.

The approach used in this investigation was based on the incorporation of labelled precursors into palitantin and subsequent determinations of the resulting distribution of radioactive carbon atoms. Thus a palitantin-yielding strain of *Penicillium cyclopium* Westling was grown in surface culture on Czapek-Dox medium and its rate of production determined (Fig. 2). The peak yield occurred at about 16 days and an attempt was

* Present address: Department of Microbiology, Squibb Institute for Medical Research, New Brunswick, N.J., U.S.A.