Gordon, A. H. (1957). *Biochem. J.* **66**, 255.
Gordon, A. H. (1960). *Biochem. biophys. Acta*, **42**, 23.
Gordon, A. H. & Humphrey, J. H. (1960). *Biochem. J.* **75**, 240.
Green, M. & Miller, L. L. (1960). *J. biol. Chem.* **235**, 3202.
Jacobs, S. (1956). *Analyst*, **81**, 502.
Jencks, W. P., Jetton, M. R. & Durrum, E. L. (1955). *Biochem. J.* **60**, 205.
McFarlane, A. S. (1958). *Nature, Lond.*, **182**, 53.
Oeff, K. (1954). *Z. ges. exp. Med.* **123**, 294, 309.
Porath, J. (1954). *Acta chem. scand.* **8**, 1813.
Smithies, O. (1955). *Biochem. J.* **61**, 629.
Yalow, R. S. & Berson, S. A. (1957). *J. clin. Invest.* **36**, 44.

Biochem. J. (1962) **82**, 540

The Lipid Composition of Phosphorylating 'Digitonin Particles' and Water- and Saline-extracted Mitochondria from Rat Liver

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(*Received* 10 *August* 1961)

Cooper & Lehninger (1956*a*) described the preparation of fragments of the mitochondrial membrane, by treatment with digitonin, that still retained the capacity for oxidative phosphorylation (see also Devlin & Lehninger, 1956; Cooper & Lehninger, 1956*b*). Devlin & Lehninger (1958) stated that 29 % of the digitonin particles was lipid and 95 % of this was phospholipid of undetermined nature. Remmert & Lehninger (1959) (see also Hulsmann, Elliott & Rudney, 1958; Elliott, Hulsman & Slater, 1959) have suggested that lipid factors may be concerned in the control of phosphorylation, and Pressman & Lardy (1956) have shown that oleic acid is a potent uncoupler of oxidative phosphorylation. Green (1959) has shown that lipid factors are concerned in the oxidation of succinate by electron-transport particles prepared from ox heart. The composition of the lipids from rat-liver mitochondria has been studied by Getz & Bartley (1961), Macfarlane, Gray & Wheeldon (1960), Paoletti & Grossi (1960), Clément, Clément & Le Breton (1956), Clément, Haimovici & Le Breton (1955), Collins & Shotlander (1961) and Getz, Bartley, Stirpe, Notton & Renshaw (1962), but no work has so far been published on the detailed lipid composition of digitonin particles prepared according to Devlin & Lehninger (1958). Among other methods for fractionating mitochondria are water extraction (Claude, 1946; Watson & Siekevitz, 1956) and saline extraction (Dallam, 1958). The present paper describes the fatty acid pattern of lipids extracted from digitonin particles and the insoluble mitochondrial residues remaining after extraction of these organelles with water or with saline.

EXPERIMENTAL

Chemicals. Digitonin was AnalaR grade (British Drug Houses Ltd.); it was used without further crystallization. All solvents were of analytical grade except ethanol, which was purchased as azeotropic.

Animals. White rats of the Wistar strain bred in the laboratory were used. They were fed on a standard diet of rat cubes supplied by Oxo Ltd. (Medical Department), Southwark Bridge Road, London, S.E. 1. The fatty acid analysis of the diet is given by Getz & Bartley (1961).

Preparation of the digitonin particles. The procedure described by Devlin & Lehninger (1958) was followed.

Extraction and fractionation of the lipids of digitonin particles. Digitonin particles (0·36 g. dry weight) were extracted with chloroform–methanol (2:1, v/v) according to Getz & Bartley (1961). The lipid extract was evaporated to dryness under nitrogen at 60° and chloroform was added to the residue. The mixture was warmed to 50° and filtered. The residue was extracted with warm chloroform several times (final vol. 80 ml.). The remaining precipitate and the filter paper were extracted with warm chloroform–methanol (2:1, v/v) (final vol. 50 ml.). Samples of each extract were estimated for their ester content. The chloroform extract contained 164 μmoles of ester and the subsequent chloroform–methanol extract 9·5 μmoles. After combining the two extracts they were evaporated to dryness and the solids were dissolved in chloroform. The extract (171 μmoles of lipid ester) was applied to an 18 g. silicic acid column (Hirsch & Ahrens, 1958) that had been exhaustively washed with chloroform. Neutral lipids were eluted from the column by 1 l. of chloroform. Tests on the last 16 ml. of chloroform passing through the column showed it to be free from lipid esters. The chloroform eluent containing the neutral lipids was free from phosphorus. Subsequent elution of the phospholipids was with the solvent sequence shown in Table 1. A fraction collector was used to collect 10 ml. samples of the eluent. These were analysed for phosphate by wet-ashing a 0·5 ml. sample and

estimating the liberated orthophosphate (see Berenblum & Chain, 1938; Bartley, 1953). The plot of phosphate eluted against quantity of solvent is given in Fig. 1. On the basis of the phosphate analysis, tubes were combined as indicated in Fig. 1 to give 12 peaks. A total of 139 μmoles of lipid ester, i.e. the acyl ester in the neutral-lipid fraction and the individual phospholipid peaks, was recovered from the silicic acid fractionation (128 μmoles of phospholipid). In

Table 1. *Elution sequence used to fractionate the phospholipids of digitonin particles on silicic acid*

Lipid ester (171 μmoles) was applied to a 18 g. silicic acid column. Main lipid expected is taken from Hanahan, Dittmer & Warashina (1957).

Solvent	Volume (ml.)	Main component expected
Chloroform	1000	Neutral lipids
Chloroform–methanol (11:1, v/v)	100	—
Chloroform–methanol (9:1, v/v)	100	—
Chloroform–methanol (7:1, v/v)	100	Mixed
Chloroform–methanol (4:1, v/v)	200	Ethanolamine-based phospholipid
Chloroform–methanol (3:2, v/v)	500	Phosphoinositides and lecithin
Chloroform–methanol (1:4, v/v)	200	Sphingolipids
Methanol	400	Residues

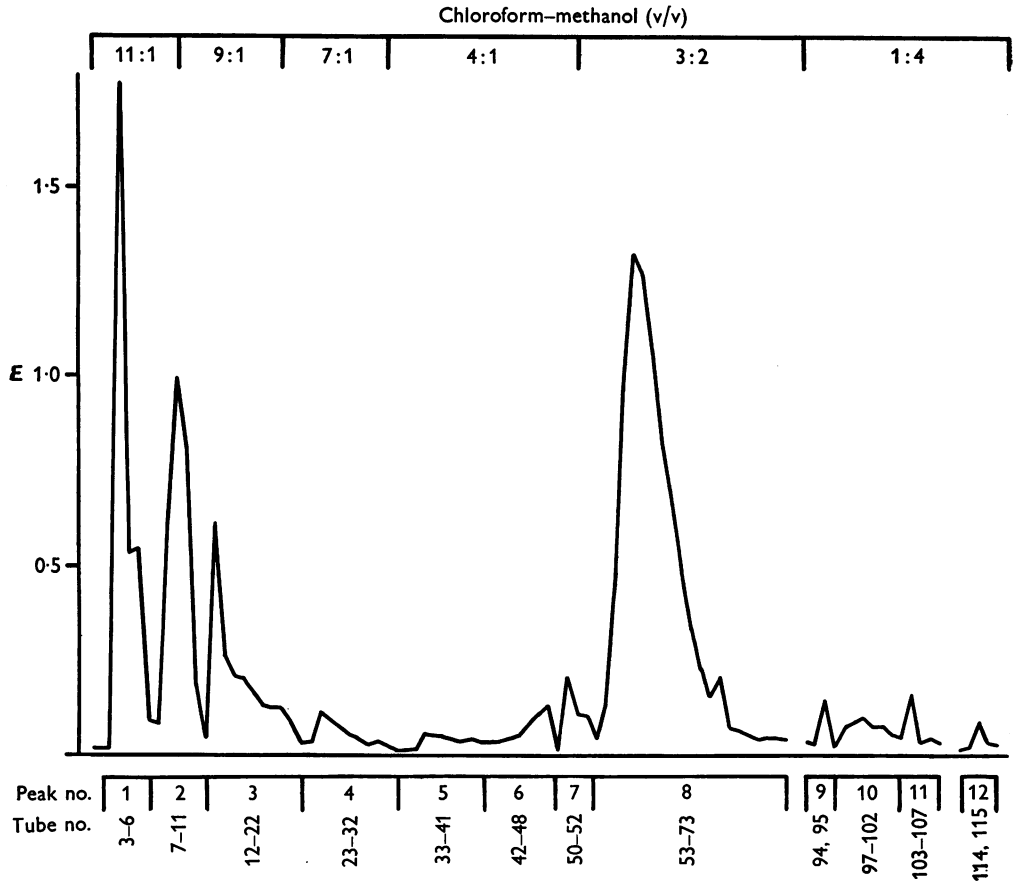

Fig. 1. Elution pattern of phospholipids of digitonin particles from silicic acid. The digitonin-particle lipids were loaded on the silicic acid column in chloroform and the neutral lipids were eluted with chloroform. The phospholipids were then eluted with the solvent sequence shown. Samples (0·5 ml.) of the 10 ml. fractions collected were analysed for their phosphate content. The ordinate is the extinction of phosphomolybdenum blue measured at 750 mμ as described by Berenblum & Chain (1938). Each peak was collected by combining the fractions indicated on the Figure.

peaks 9 and 12 there was insufficient material for a determination of acyl ester. Samples of each phospholipid peak were removed for the determination of amino nitrogen (Cocking & Yemm, 1954), and the remainder of each peak was evaporated to dryness under nitrogen. The subsequent saponification, extraction of the fatty acids and preparation of the methyl esters of the fatty acids by treatment with diazomethane were as described by Getz & Bartley (1961). The aqueous layer after removal of lipids was retained for estimation of choline (Wheeldon & Collins, 1958; Smits, 1957) and glycerol (Hanahan & Olley, 1958), and for identification of the amino acids of the various phospholipids. The quantities of methyl esters prepared from the fatty acids of each phospholipid peak were estimated by the method of Rapport & Alonzo (1955). Samples of the methyl esters were run on the Pye Argon chromatograph (Pye Ltd., Cambridge) to determine the proportions of the fatty acids present in each peak. Apiezon was used as the stationary phase for most chromatograms but some were separated on columns of polyadipate ester.

The neutral-lipid fraction eluted from the silicic acid column with chloroform was evaporated to 10 ml., and a sample removed for estimation of lipid esters. The remainder of the extract was fractionated on silicic acid according to the system of Barron & Hanahan (1958). A fraction collector was not employed but the conditions and quantities of solvents used by Barron & Hanahan (1958) were followed exactly. The solvent sequence and quantities of acyl ester eluted in each fraction are given in Table 2. The quantities of material were insufficient to estimate cholesterol and glycerol in each fraction, but the major fraction was eluted by the solvent shown by Barron & Hanahan (1958) to segregate the triglycerides. The neutral-lipid fractions were saponified and the methyl esters of the fatty acids prepared as described for the phospholipids. The sum of the methyl esters found in each fraction was greater than the amount of acyl glyceryl ester added to the column. The difference is assumed to be due to free fatty acid in the neutral lipids put on the silicic acid column. Samples of the methyl esters of the neutral lipids were subjected to gas–liquid chromatography as described for the fatty acid esters prepared from the phospholipids.

Fractionation of mitochondria by treatment with water or sodium chloride solution. Mitochondria were prepared from 129·7 g. of rat-liver pulp according to Werkheiser & Bartley (1957). The mitochondria were suspended in 0·25 M-sucrose solution to a final volume of 25 ml. The flow sheet of the subsequent treatment of the mitochondria is given in Fig. 2. The dried residue from the determination of dry weights was subsequently used for the determination of protein nitrogen by the Kjeldahl method. The 'direct saponification' and the extraction with ethanol and ether were as described by Getz & Bartley (1961). The proteins and lipids that were extracted by the treatment with water or saline were co-precipitated by adding aq. 50% (w/v) trichloroacetic acid to a final concentration of 5%. The precipitated material was well washed with water before being saponified or extracted with ethanol and ether.

Extraction and fractionation of the lipids of saline- and water-treated mitochondria. Lipids were extracted from the insoluble mitochondrial residues or the washed trichloroacetic acid precipitates by adding 10 vol. of ethanol and allowing to stand at room temperature for 3 hr., followed by two extractions with 10 vol. of ethanol–ether (3:1, v/v). The combined extracts from each fraction were evaporated to dryness under a stream of nitrogen and dissolved in 50 ml. of light petroleum (b.p. 40–60°). Samples of these extracts were used for the gravimetric determination of total lipid and for measurement of the acyl esters according to Rapport & Alonzo (1955). The remainder of the lipid extracts were chromatographed on 5 g. columns of silicic acid prepared according to Barron & Hanahan (1958). Elution of the lipids was with the series of solvents given in Table 3. The material eluted with each solvent was analysed for lipid ester, lipid phosphorus and amino nitrogen. Samples were used for the isolation of the fatty acids and for preparation of their methyl esters. The methyl esters were separated and identified by gas chromatography.

RESULTS

The digitonin particles

Phospholipids of digitonin particles. The analyses of the phospholipid fractions are given in Table 4. It is clear that the procedure used for the preparation of digitonin particles has produced extensive breakdown of the lipids. In all peaks there is a deficiency of ester and base for the amount of phosphorus found. Virtually all the choline occurs in peak 8, but this peak also contains appreciable amounts of amino nitrogen. Peak 1 contained virtually no base and analysis for glycerol showed that there was 1 g. atom of phosphorus/mole of glycerol.

Basford (1959) found in heart mitochondria that about one-half of the phospholipids derived from

Table 2. *Elution sequence used to fractionate the neutral lipids of digitonin particles on silicic acid*

Fatty acid ester (10·8 μmoles) was applied to a silicic acid column and eluted according to Barron & Hanahan (1958).

Fraction no.	Solvent	Volume (ml.)	Main component expected	Acyl ester content (μequiv.)
1	Hexane	150	Pigments; hydrocarbons	1·10
2	Benzene–hexane (3:17, v/v)	500	Sterol esters	0·40
3	Diethyl ether–hexane (1:19, v/v)	1300	Triglycerides	7·70
4	Diethyl ether–hexane (3:17, v/v)	1000	Free sterols	0·85
5	Diethyl ether–hexane (3:7, v/v)	600	Diglycerides	0·69
6	Diethyl ether–hexane (1:1, v/v)	600	Unknown	0·47
7	Diethyl ether	600	Monoglycerides	1·10

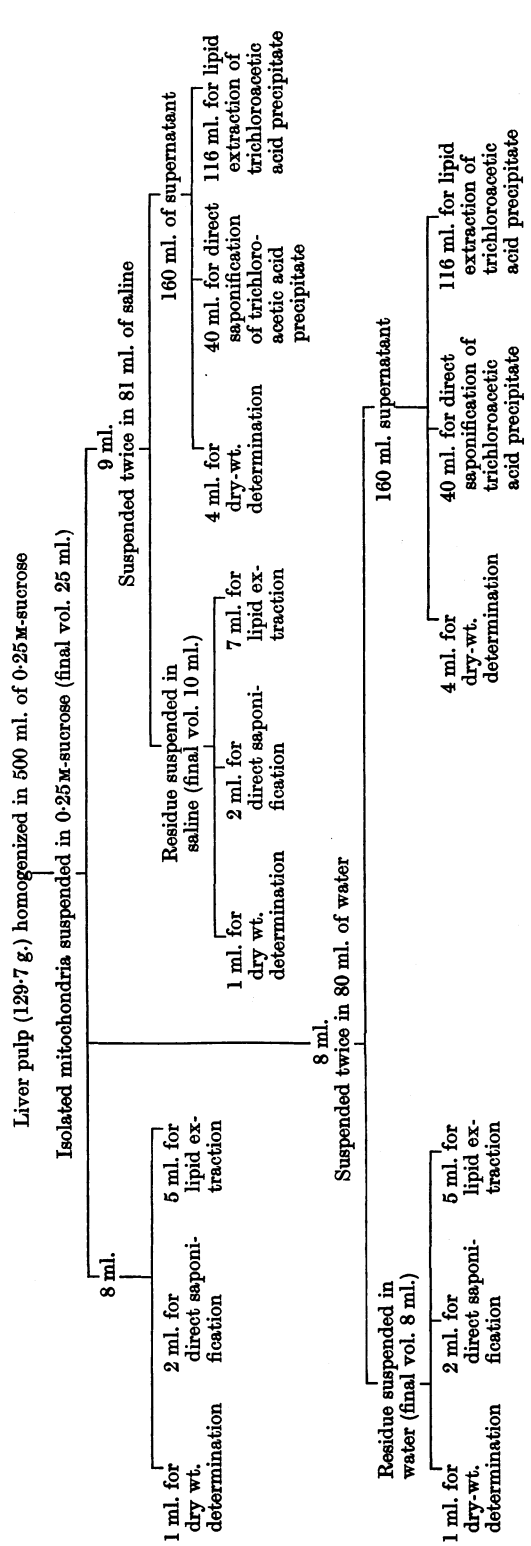

Fig. 2. Fractionation of rat-liver mitochondria by treatment with water or 0·9% sodium chloride solution.

the lipoproteins of the electron-transport chain is in the form of plasmalogens. Thus a ratio of fatty acid to phosphorus of 1·5:1 would be expected in this type of lipid. However, plasmalogens were not found in the lipids of the digitonin particles. The low acyl ester:phosphorus ratio cannot therefore be attributed to a concentration of plasmalogens in the digitonin particles.

Distribution of amino compounds in the peaks. The acidified aqueous layer remaining after removing the fatty acids from the saponified lipids was heated at 100° for 30 min. to liberate free amino compounds. Excess of barium carbonate was added and, after standing and filtering, the solution was desalted in an electrolytic desalter (Locarte Co.); samples of the solution were then freeze-dried and the solids redissolved in a small volume of water. Samples of these solutions were separated into the component amino compounds by high-voltage electrophoresis in 0·2 M-pyridine–acetic acid buffer, pH 4·5; the voltage gradient used was 155 v/cm. at a current of 42 mA for 10 min. Marker spots of serine and ethanolamine were run with each sample. The spots were detected by spraying with ninhydrin and heating, and the quantities were estimated visually from the size and colour of the spots. This assessment is given in Table 5. Thus peak 2 contains only ethanolamine phosphatides and peak 3 only a relatively small proportion of serine phosphatides. These two peaks contain the greater part of the amino nitrogen. Serine is much more widely distributed, the largest amount being associated with the choline-containing peak. No analysis was made for inositol but, in the normal elution sequence, inositides appear in the chloroform–methanol (3:2, v/v) fraction. However, it is possible that, in the separation of the lysophosphatides, inositides do not appear in this solvent, although the excess of phosphorus over base in peak 8 is consistent with their presence in this peak.

Distribution of fatty acids in the phospholipids of digitonin particles. Table 6 shows the distribution of fatty acids in the various phospholipids. Peak 1 contains the highest proportion of unsaturated acids (95·2% of the total) and shows a similar pattern of fatty acids to that described for the cardiolipin isolated by Gray & Macfarlane (1958) and Getz & Bartley (1959). The main fatty acid present is linoleic acid (82·8%) and the main saturated acid is palmitic acid (2·58%). The fatty acids removed by the digitonin treatment (some 50% of those present in the intact cardiolipin) must have had the same overall pattern as the acids remaining. The phosphorus:acyl ester:glycerol proportions (2:1:2) found in this peak are consistent with a structure: phosphate–glycerol–(fatty acid)–phosphate–glycerol.

Table 3. *Elution sequence used to fractionate the phospholipids of saline- and water-treated mitochondria on silicic acid*

Fraction no.	Solvent	Volume (ml.)	Main component expected
1	Diethyl ether	100	Neutral lipids
2	Chloroform–methanol (9:1, v/v)	25	Cardiolipin
3	Chloroform–methanol (4:1, v/v)	50	Amino phosphatides
4	Chloroform–methanol (3:2, v/v)	20	Inositides
5	Chloroform–methanol (3:2, v/v)	100	Lecithin
6	Chloroform–methanol (1:4, v/v)	50	Polar lipids

Table 4. *Analyses of the phospholipid fractions of digitonin particles from rat liver*

Lipid from digitonin particles (0·36 g. dry wt.) was extracted and fractionated on a silicic acid column (Hanahan *et al.* 1957).

Phospholipid fraction no.	Lipid-P content (μmoles/g. dry wt.)	Amino-N content (μmoles/g. dry wt.)	Choline content (μmoles/g. dry wt.)	Ester content (μequiv./g. dry wt.)	Ester: lipid P ratio	Base: lipid P ratio	Base: ester ratio
1	54·7	1·1	0	26·6	0·49	0·02	0·04
2	47·5	34·0	0	34·4	0·72	0·71	0·99
3	43·4	29·5	0	66·7	1·54	0·68	0·44
4	11·1	5·0	0	11·1	1·00	0·45	0·45
5	5·8	2·3	1·39	5·7	0·98	0·64	0·65
6	10·5	5·4	0·28	10·3	0·98	0·54	0·55
7	8·3	5·8	0·83	5·3	0·64	0·80	1·25
8	185·0	33·1	124·0	180·0	0·97	0·85	0·87
9	5·0	0·5	0·28	—	—	0·16	—
10	10·8	2·5	5·30	9·5	0·88	0·72	0·82
11	6·4	3·9	0·50	4·7	0·73	0·69	0·94
12	1·9	1·05	0·28	—	—	0·70	—
Total	390·4	124·15	132·86	354·3	—	—	—

Table 5. *Proportions of amino compounds found in phospholipid peaks*

The amino compounds were separated by high-voltage electrophoresis and detected by ninhydrin. The proportions given were arrived at by visual comparison of the size and intensity of colour of the spots.

Peak no.	Relative proportions of amino compounds on the electrophoretograms: Serine	Ethanolamine	Other amino compounds
1	—	—	—
2	—	Present	—
3	1	6	—
4	2	1	—
5	1	1	—
6	Present	—	—
7	—	—	Present
8	Present	—	—
9	Trace	—	—
10	Present	—	—
11	—	—	Present
12	—	—	Present

The percentage of unsaturated fatty acids is lower in peak 2 (56·3 % of the total) than in peak 1; the main component is arachidonic acid but the peak also contains a high proportion (13·4 %) of a C_{22} highly unsaturated acid; the main saturated acid is stearic acid. Since the only base found was ethanolamine, the lipid can be considered as 'lysokephalin', though there is an excess of phosphorus for the amount of base found. The percentage of unsaturated acids in peak 3 is slightly less than in peak 2, and arachidonic acid is again the main unsaturated acid, with palmitic acid as the chief component. This is the only peak where the molar ratio of fatty acid ester to phosphorus exceeds unity. Thus this peak is not entirely composed of 'lysolipids'. The base was chiefly ethanolamine but some serine was present (Table 5).

Only one peak (6) contained serine as the sole amino-nitrogen-containing compound. This peak had a much lower proportion of unsaturated fatty acids than peaks 1–5 (33·9 %). The main unsaturated acid was arachidonic acid (15·8 %) and stearic acid (42·9 %) was the predominant saturated acid.

The main choline-containing peak (8) accounted for 47 % of the lipid phosphorus and 94 % of the choline. The peak also contained serine (about one-quarter of the amount of choline); thus the fatty acids from this peak come from at least two phospholipids. The unsaturated acids constituted 47·7 % of the total but the main acid was palmitic acid (33·3 %); the main unsaturated acid was arachidonic acid (20·9 %).

Table 6. *Distribution of fatty acids in phospholipids from digitonin particles from rat liver determined by gas chromatography*

The value for each peak area is given as a percentage of the total area of all peaks on the chromatogram. The total quantity of material recovered in peak 9 was insufficient for accurate measurement of the fatty acid distribution.

Fatty acid. No. of C atoms and double bonds	Peak no. (see Fig. 1)										
	1	2	3	4	5	6	7	8	10	11	12
10–0	0·41	0·20	0·10	0·48	—	1·84	1·17	—	—	1·83	12·3
12–0	—	—	—	0·14	0·25	1·18	0·46	—	0·92	0·87	—
*12–hbr.	0·12	—	—	0·19	—	0·33	0·27	—	—	0·33	1·27
*13–0	—	—	—	—	—	—	—	—	—	0·21	0·42
14–0	0·41	—	0·18	0·43	1·20	1·84	0·91	0·27	1·31	2·66	3·96
15–0	—	—	0·18	0·33	0·84	1·18	0·32	0·50	0·46	1·20	—
16–0	2·58	19·4	27·3	29·2	20·4	15·8	44·3	33·3	37·3	42·8	39·3
16–1	3·32	0·42	0·73	1·14	1·89	2·88	1·19	1·46	2·17	3·24	4·24
17–0	—	0·97	0·64	0·64	0·77	1·05	1·23	0·50	0·85	0·66	—
*17–1 or 17–br.	—	—	0·46	1·28	—	0·26	—	—	—	2·24	—
18–0	1·38	23·2	20·3	23·7	27·7	42·9	32·2	17·6	29·8	21·2	20·5
18–1	4·60	3·72	2·73	1·71	2·13	10·0	8·30	3·03	12·1	10·3	11·2
18–2	82·8	9·25	14·7	14·1	10·4	3·30	1·64	17·7	8·02	8·06	6·79
*19–1 or 19–br.	—	—	—	0·83	—	—	—	—	—	0·79	—
*20–0	—	—	—	1·83	—	—	—	—	—	—	—
*20–1	—	—	0·66	—	3·67	—	—	—	—	—	—
20–2	1·28	—	—	—	—	—	—	—	—	—	—
20–3	1·69	—	0·64	0·71	1·72	1·31	0·87	—	—	—	—
20–4	1·41	28·2	25·5	19·9	29·0	15·8	7·02	20·9	7·10	3·61	—
*22–4 or 22–5	—	1·28	0·85	3·33	—	—	—	0·39	—	—	—
*22–5 or 22–6	—	13·4	4·91	—	—	—	—	4·21	—	—	—
Unsaturated fatty acids (% of total)	95·2	56·3	51·2	43·2	48·8	33·9	19·3	47·7	29·4	28·6	23·5

* Tentative identification only.

Peak 12 was unique in that it contained no arachidonic acid. The peaks which had the greatest degree of saturation were all particularly low in arachidonic acid and had palmitic acid in high proportion. Peaks 10–12 have a somewhat similar composition but the remainder of the peaks had a highly individual pattern of acids. In peak 12 there were no acids of chain length longer than C_{18}.

Distribution of fatty acids in the neutral lipids of digitonin particles. The fatty acid composition of the neutral-lipid fractions is given in Table 7. It should be noted that, whilst only 10·8 μmoles of fatty acid ester were put on the column, after saponification and methylation the total esters recovered were 12·31 μmoles (Table 2). It is assumed that the difference is due to the free fatty acid content of the original sample. According to Barron & Hanahan (1958), the free fatty acids are eluted in the same fraction as the triglycerides. Thus the total of 7·7 μmoles of fatty acid ester found in the triglyceride fraction after saponification and methylation is assumed to contain 1·51 μmoles derived from free fatty acids. The main neutral-lipid fraction was triglyceride (62 %), containing 71·5 % of unsaturated acids. Linoleic acid was the main acid (20·8 %), with palmitic acid as the main saturated acid. The supposed sterol esters are characterized by a very high proportion of an acid (37·9) which, from its retention time on the gas chromatograph with Apiezon, corresponded with a C_{13} branched acid. This acid occurred in substantial amounts in all fractions except the triglyceride, from which it was absent, and fraction 1, which contained only about 1 %. Arachidonic acid was present in substantial amounts only in the triglyceride fraction. The fatty acid patterns of the tri-, di- and mono-glycerides were quite different. The monoglyceride fraction contained the highest proportion of saturated fatty acids and contained no linoleic acid.

Water- and saline-extracted mitochondria

Recovery of lipid and protein in water- and saline-extracted mitochondria. Table 8 shows the recoveries of total lipid, dry weight, lipid esters, lipid phosphorus and protein in the mitochondria treated with water or saline.

The values show that in the residues of the extracted mitochondria the sum of protein and lipid recovered approximated to the measured dry weight. In the material extracted from the mitochondria only about 80 % of the dry weight was recovered in the sum of protein and lipid. Since about 90 % of both dry weight and protein expected from the amount of mitochondria extracted was recovered in the residue and supernatant fraction,

Table 7. *Distribution of fatty acids in neutral lipids from digitonin particles from rat liver*

Values are percentages of total acids (see Table 6).

Fatty acid. No. of C atoms and double bonds	Fraction eluted (see Table 2)						
	1	2	3	4	5	6	7
<10	—	0·95	—	—	—	—	—
10–0	—	1·09	—	—	0·94	1·55	—
11–0	—	3·41	—	1·09	1·10	—	1·15
*11–br.	—	0·61	—	—	—	—	—
12–0	0·98	3·28	0·58	1·09	1·89	1·06	—
*12–br.	—	0·89	—	0·66	1·26	1·50	—
*12–hbr.	1·25	0·82	—	—	—	—	—
13–0	2·40	4·44	—	—	2·36	—	2·12
*13–br.	1·25	37·9	—	15·1	32·6	29·8	13·9
*13–?	—	—	—	—	—	—	1·74
14–0	10·6	21·0	2·22	16·8	19·7	9·54	20·1
14–1	0·71	—	0·22	—	—	1·19	—
*14 unsaturated	—	—	—	1·97	—	—	—
15–0	1·87	—	1·15	2·46	—	1·19	—
*15–1 or 15–br.	1·60	—	0·88	—	—	0·80	—
*15 unsaturated	—	1·43	1·60	0·55	—	7·47	—
*15–?	—	—	—	—	—	1·59	—
16–0	26·7	12·3	18·8	20·0	15·6	13·7	14·5
16–1	11·7	3·28	5·10	9·62	7·87	6·81	4·83
*16 unsaturated	2·31	—	—	2·46	—	—	—
17–0	0·80	—	—	0·82	—	3·71	—
*17–1 or 17–br.	1·96	—	—	1·42	0·94	0·97	—
18–0	4·80	—	5·32	2·90	5·04	3·58	5·41
18–1	18·7	5·53	15·6	13·9	9·13	9·63	9·46
18–2	10·7	3·07	20·8	9·19	1·57	5·83	—
20–0	—	—	—	—	—	—	11·4
20–3	—	—	—	—	—	—	1·54
20–4	1·78	—	14·4	—	—	—	—
*22–4 or 22–5	—	—	1·77	—	—	—	1·83
*22–5 or 22–6	—	—	11·1	—	—	—	12·1
Unsaturated fatty acids (% of total)	52·0	54·5	71·5	54·9	53·4	65·6	45·4

* Tentative identification only.

it follows that the loss of material in the supernatant is largely due to loss of lipid. Getz & Bartley (1961) have shown that recoveries as low as 60% of the lipid may follow the use of trichloroacetic acid to co-precipitate proteins and lipids. Because of the poor recovery of the extracted lipids, the data on these are omitted.

Lipids of mitochondrial residues after water or saline extraction. Tables 9 and 10 show the analytical data on the lipids of water- and saline-treated mitochondria. With the exception of the neutral-lipid fraction, all the lipids are concentrated in the residues to about the same degree when compared with the lipids of the unfractionated mitochondria. Most of the phospholipids show a low ester:phosphorus ratio, suggesting considerable hydrolysis of lipid during the isolation of these lipid fractions; this is also suggested by the large amounts of free fatty acids found in the neutral-lipid fraction and the first phospholipid fraction in the elution sequence. Paper chromatography of the water-soluble products of mild alkaline hydrolysis according to Dawson (1960) showed that the lecithin fractions yielded about 80% of the lipid phosphorus as glycerylphosphorylcholine.

Fatty acids of mitochondrial residues after water or saline extraction. Table 11 presents the fatty acid analysis of the lipid fractions obtained from the water-fractionated mitochondria. The fatty acids of the unfractionated mitochondria are presented for comparison. The total fatty acids of the mitochondrial residue (column 3) resemble those of the mitochondria (column 2). Among the individual lipid classes the lecithin shows the same pattern of fatty acids as the corresponding lecithin in the untreated mitochondria. The patterns of fatty acids in the cardiolipin fractions also have many similarities. On the other hand, the neutral lipid contains less palmitate and much more polyunsaturated fatty acid than in the neutral-lipid fraction of the whole mitochondria. The neutral-lipid fraction contains the free fatty acids derived

Table 8. *Recoveries of total lipid, dry weight, lipid ester, lipid phosphorus and protein in the mitochondria treated with water or saline*

Protein figures were obtained from determinations on the trichloroacetic acid precipitates used for dry-weight measurement (N × 6·25). Direct saponification results are used for calculation of 'Esters (% of mitochondrial esters)'.

Fraction	Dry wt. (mg.)	Ester by direct saponification (μequiv.)	Ester in lipid extract (μequiv.)	Fats (% of dry wt.)	Protein (% of dry wt.)	% of dry wt. recovered	Total fat (% of mitochondrial total fat)	Esters (% of mitochondrial esters)	Dry wt. (% of mitochondrial dry wt.)	Protein (% of mitochondrial protein)	Phospholipids (% of mitochondrial phospholipids)
Whole mitochondria	751	—	—	37·3	—	—	100	100	100	100	100
Water residue	220	58·5	67·1	40·1	56·0	96·1	31·5	37·9	29·3	26·3	39·0
Water supernatant	444	60·0	31·4	8·5	68·2	76·7	13·6	29·3	59·2	64·7	15·6
Total water-treated mitochondria recovered	664	—	—	—	—	—	45·0	67·2	88·5	91·0	54·6
Saline residue	242	108	109	42·0	57·1	99·1	36·0	49·0	32·2	28·8	53·5
Saline supernatant	474	65·8	32·7	7·3	72·7	80·0	12·5	29·3	63·1	71·2	5·5
Total saline-treated mitochondria recovered	716	—	—	—	—	—	48·5	78·3	95·3	100	59·0

Table 9. *Analyses of water residue of mitochondria*

Results are expressed as μequiv./g. dry wt. of each fraction.

Fraction no. (see Table 3)	Lipid-P content	Amino-N content	Acyl ester content	Methyl ester content	Free acids	Acyl ester: lipid P ratio	Amino N: lipid P ratio
1	3·8	2·1	36·3	148	112	—	—
2	22·1	5·5	33·3	150	117	1·51	0·25
3	82·4	44·7	133	123	—	1·62	0·54
4	42·1	40·4	34·8	31·8	—	0·83	0·96
5	130	15·5	176	180	—	1·35	0·12
6	27·1	7·3	37·9	37·9	—	1·40	0·27
Total	307·5	115·5	451·3	670·7	—	—	—

Table 10. *Analyses of saline residue of mitochondria*

Results are expressed as μequiv./g. dry wt. of each fraction.

Fraction no. (see Table 3)	Lipid-P content	Amino-N content	Acyl ester content	Methyl ester content	Free acids	Acyl ester: lipid P ratio	Amino N: lipid P ratio
1	2·7	1·9	24·0	78·8	54·8	—	—
2	13·5	2·2	24·0	29·7	5·7	1·78	0·16
3	148	103	210	210	—	1·42	0·70
4	11·9	6·4	61·2	14·0	—	5·14	0·54
5	154	13·5	177	257	80·0	1·15	0·09
6	10·7	4·0	16·4	22	—	1·53	0·37
Total	340·8	131·0	512·6	611·5	—	—	—

from the hydrolysis of the phospholipids. These fatty acids may be derived from the kephalin fraction since these do not have their usual high content of C_{20}–C_{22} unsaturated fatty acids. However, the kephalins retain their normal high stearic acid:palmitic acid ratio.

The pattern of fatty acids of the saline-extracted mitochondrial residue (Table 12) differed from that of the mitochondria in having higher palmitic acid and lower C_{20}–C_{22} unsaturated fatty acid contents. The cardiolipin fatty acids closely resemble those of the whole mitochondrial cardiolipin. Both

Table 11. *Fatty acid composition of water-fractionated mitochondria*

Fraction numbers are those given in Table 3. Values are percentages of the total acids.

Fatty acid. No. of C atoms and double bonds	Total fatty acids of whole mitochondria	Total fatty acids of mitochondrial residue	Fraction no. of water-extracted residue					
			1	2	3	4	5	6
<12–0	—	—	—	0·2	—	—	—	—
12-0	—	—	0·2	0·1	—	—	—	—
14–0	0·3	0·3	1·6	0·4	2·0	0·6	0·3	—
14–1	—	—	0·2	0·1	—	—	—	—
*15–0 br.	—	—	0·4	0·1	—	—	0·1	—
15–0	0·5	0·3	0·6	0·2	3·8	1·0	0·9	—
*16–0 br.	—	—	—	0·1	—	—	0·3	—
16–0	16·5	21·5	11·6	8·1	32·6	40·0	28·5	41·9
16–1	2·0	1·1	3·2	2·4	5·8	1·2	1·8	5·7
17–0	0·7	0·6	—	0·3	3·4	1·3	0·8	—
*17–?	—	0·4	—	0·2	2·5	1·5	—	—
18–0	15·1	15·1	3·5	7·1	32·4	40·5	17·3	31·6
18–1	14·3	13·7	14·4	13·3	7·4	10·0	10·9	15·3
18–2	24·7	23·9	41·6	58·6	3·8	2·5	17·5	5·5
18–3	—	—	—	0·4	—	—	—	—
*19–?	0·4	0·3	—	—	1·6	—	0·3	—
*20–0	—	—	—	—	—	—	—	—
*20–1	—	0·5	—	—	—	—	—	—
20–2	—	0·5	—	0·5	2·2	—	—	—
20–3	1·5	1·5	1·4	1·0	—	—	1·6	—
20–4	16·2	14·7	13·0	4·7	2·5	1·3	16·6	—
*22–5	1·1	1·1	0·9	2·3	—	—	—	—
*22–6	6·2	4·4	7·4	—	—	—	3·0	—

* Tentative identification only.

Table 12. *Fatty acid composition of saline-fractionated mitochondria*

Values are percentages of total acids.

Fatty acid. No. of C atoms and double bonds	Total fatty acids of whole mitochondria	Total fatty acids of mitochondrial residue	Fraction no. of saline-extracted residue			
			1	2	3	5
<14–0	—	0·2	0·3	—	—	—
14–0	0·3	0·4	1·8	0·4	—	0·7
14–1	—	—	0·8	—	—	—
*15–0 br.	—	0·1	0·6	—	—	0·2
15–0	0·5	0·4	0·9	0·2	—	0·6
*16–0 br.	—	—	0·4	—	—	—
16–0	16·5	22·6	21·4	4·3	36·2	34·7
16–1	2·0	1·6	5·4	2·7	1·3	1·6
*17–?	—	0·4	0·3	—	0·4	0·3
17–0	0·7	0·7	0·6	—	2·2	0·8
*18–0 hbr.	0·4	0·1	0·5	—	—	—
18–0	15·1	14·4	7·4	1·5	31·4	18·8
18–1	14·3	12·8	19·9	14·1	13·2	15·3
18–2	24·7	25·8	24·4	73·7	12·4	17·0
*19–?	0·4	0·2	—	—	1·3	0·2
*20–1	—	0·4	—	—	—	—
20–2	—	0·5	—	0·3	—	—
20–3	1·5	1·3	—	0·8	—	1·1
20–4	16·2	13·9	8·8	2·1	1·5	8·6
*22–5	1·1	1·8	—	—	—	—
*22–6	6·2	3·8	6·3	—	—	0·2

* Tentative identification only.

lecithin and kephalin fractions, though rather low in C_{20}–C_{22} unsaturated fatty acids, resemble the corresponding fractions in the mitochondria. As in the residue from water extraction, the residue from saline extraction contains unusually large amounts of arachidonic and docosahexaenoic acids, presumably derived from the hydrolysis of some of the phospholipids.

DISCUSSION

Fatty acids of phospholipid fractions of digitonin particles. The ratio of the phospholipid fatty acids to the neutral-lipid fatty acids was almost exactly 10, whereas the data of Macfarlane *et al.* (1960) indicate that for whole mitochondria the ratio is about 5. Thus these values indicate that the digitonin treatment results in a loss of about 3·33 moles of phospholipid fatty acid ester/mole of neutral-lipid ester lost.

The fatty acid distribution in the lysocardiolipin fraction (peak 1) was similar to that of the cardiolipin fraction analysed by Gray & Macfarlane (1958) and Getz & Bartley (1959). The main difference is a somewhat higher proportion of linoleic acid and only half the amount of oleic acid. By contrast, the lysolecithin peak (peak 8) is very different in fatty acid composition from the mitochondrial lecithin analysed by Getz *et al.* (1962) and Macfarlane *et al.* (1960). Their values showed 58·8 and 61·7% of unsaturated acids respectively, whereas the lyso compound contained only 47·7% of unsaturated acids. The kephalins of the digitonin particles were separated into many more fractions than was the case with the mitochondrial kephalin analysed by Getz *et al.* (1962). To overcome this difficulty, the weighted means of the fatty acid compositions of the main two kephalin peaks of mitochondrial lipids and of the several kephalin peaks of the digitonin particles (peaks 2–7) have been calculated and are compared in Table 13. Digitonin particles contain relatively more palmitate, stearate and arachidonate and relatively less oleate and linoleate.

Hydrolysis of mitochondrial phospholipids during the formation of digitonin particles. The existence in the mitochondria of lyso compounds which are selectively retained during the preparation of digitonin particles cannot be conclusively excluded. Table 14 summarizes the lipid composition of the digitonin particles and compares it with that of the whole mitochondria. These data show that, while there was only about 3% of lysophosphatide in the whole mitochondria, all the phosphatide of the digitionin particles was in the form of lysophosphatide. This suggests that the detergent action of digitonin may activate phospholipases present in the mitochondria to bring about the extensive lipid breakdown observed (see, for example, Bangham & Dawson, 1959; Dawson, 1958; Wills, 1955; Kates, 1957; Simon, 1958). However, the fatty acid specificity of such phospholipases is unusual

Table 13. *Comparative fatty acid composition of major phospholipids of mitochondria and digitonin particles*

Values are percentages of total fatty acids.

Fatty acid. No. of C atoms and double bonds	Weighted mean of kephalin of whole mitochondria	Weighted mean of kephalin of digitonin particles	Lecithin of whole mitochondria	Lecithin of digitonin particles
10–0	—	0·34	—	—
12–0	—	0·14	Trace	—
*12–0 br.	—	0·05	0·1	—
13–0	0·04	—	Trace	—
14–0	0·23	0·36	0·5	0·3
15–0	0·41	0·26	0·5	0·5
16–0	18·7	25·0	19·7	33·3
16–1	0·81	0·93	1·5	1·5
17–0	0·98	0·78	0·8	0·5
*17–0 br.	0·53	0·36	0·3	—
18–0	21·4	24·5	18·7	17·6
18–1	9·73	3·68	12·2	3·0
18–2	16·1	11·7	20·0	17·7
*19–0 br.	0·16	0·07	0·2	—
*20–0	0·07	0·16	—	—
*20–1	—	0·49	0·5	—
20–2	0·18	—	0·3	—
20–3	0·88	0·59	0·8	—
20–4	20·9	24·3	19·5	20·9
*22–5	0·98	1·02	0·4	0·4
*22–6	7·67	5·81	3·4	4·2

* Tentative identification only.

Table 14. *Composition of lipids of digitonin particles**

Fraction	Lipid content of: Whole mitochondria (μmoles/g. dry wt.)†	Lipid content of: Digitonin particles of mitochondria (μmoles/g. dry wt.)	Lipid: lipid P percentage ratio of: Whole mitochondria (moles/ 100 moles of lipid P)†	Lipid: lipid P percentage ratio of: Digitonin particles of mitochondria (moles/ 100 moles of lipid P)	Enrichment factor: lipid content of digitonin particles / lipid content of whole mitochondria
Acyl glyceryl ester	360	354	237	90·8	0·98
Ethanolamine and serine phosphatides	63·8	124	42	31·8	1·94
Polyglycerolphosphatide	12·8	53·6	8·4	13·8	4·19
Lecithin	58·7	124	38·6	31·8	2·11
Polar lipid fraction	3·2	24·1	2·1	6·2	7·53
'Lysophosphatides'	—	—	2·95	109	37·0

* The total lipid-P content of whole mitochondria was 152 μmoles/g. dry wt., and that of digitonin particles 390 μmoles/g. dry wt., giving an enrichment factor of 2·57.

† Data from Getz *et al.* (1962) (mean of two experiments).

since the loss of fatty acids resulting from their action is comparatively unselective. The lack of effect of snake-venom phospholipase observed by Aravindakshan & Braganca (1961) is hardly surprising since virtually all the phospholipid of the digitonin particles is already lysophosphatide.

Phospholipids of the digitonin particles. Table 14 shows that all the lipid fractions of the digitonin particles contained at least twice as much lipid as the mitochondria when calculated on the basis of lipid phosphorus. Thus 152 μmoles of lipid phosphorus were found/g. dry wt. in the whole mitochondria, but 390 μmoles of lipid phosphorus/g. were present in the digitonin particles. No correction is made here for the digitonin content of the particles. Calculations from the value of 29% lipid (95% phospholipid) given by Devlin & Lehninger (1958) indicate that their digitonin particles contained either 550 μmoles of lipid phosphorus/g. dry wt., assuming lysophospholipids similar to those described in this paper, or 354 μmoles of lipid phosphorus/g. dry wt., if the phospholipid were unhydrolysed. Whichever value is assumed, it is clear that lipid phosphorus is preferentially retained, compared with protein, during the formation of digitonin particles from mitochondria.

This retention of phospholipid is not evenly distributed throughout the various phospholipid classes. Thus, in terms of composition/100 moles of lipid phosphorus, the digitonin particles have a lower proportion of acyl ester, ethanolamine phosphatide, serine phosphatide and lecithin than the whole mitochondria, but a larger proportion of polyglycerolphosphatide and polar lipid fraction. The composition of the last fraction is uncertain. If hydrolysis and removal of the bases from the phospholipids occurred during the formation of the digitonin particles, this value would be in error.

About 1% of mitochondrial phospholipid is serine phosphatide (G. S. Getz, D. Lurie & W. Bartley, unpublished work). As seen from Tables 4 and 5, the digitonin particles contain a much larger proportion of serine phosphatide.

The ratio of the quantity of phospholipid/unit dry wt. of digitonin particles to the quantity of the same phospholipid/unit dry wt. of mitochondria (enrichment factor) for each phospholipid class is given in Table 14. Thus, although the total lipid phosphorus is enriched about 2·5 times in the digitonin particle, there is no enrichment of neutral lipids. The ethanolamine and serine phosphatides and lecithin are both enriched twofold in the digitonin particles, the 1:1 ratio found in the mitochondria by Getz *et al.* (1962) being thus maintained. There is a much greater enrichment of the polyglycerolphosphatide and polar lipid fractions. The enrichment of the polyglycerolphosphatide is particularly interesting in view of its almost exclusive localization in the mitochondria (Marinetti, Erbland & Stotz, 1958; Strickland & Benson, 1960, Macfarlane *et al.* 1960; Getz *et al.* 1962), its slightly higher concentration in the purified cytochrome oxidase and cytochrome *b* and *c* preparations of Marinetti, Erbland, Kochen & Stotz (1958) than that in the parent mitochondria, and its considerably higher concentration in the cytochrome oxidase isolated from ox-heart mitochondria (Fleischer, Brierley & Klouwen, 1961). Even though this compound does not appear to function as an intermediate in the synthesis of adenosine triphosphate (Conover, Marinetti, Witter & Stotz, 1960), the above observations, together with the high rate of labelling of the polyglycerolphosphatide with radioactive phosphorus in rat-liver mitochondria *in vitro* (Marinetti, Erbland, Albrecht & Stotz, 1957), strongly suggest its close association with some aspect of the oxidative phosphorylation mechanism.

Fatty acids of neutral lipids of digitonin particles. Table 14 shows that digitonin particles contain about the same amount of neutral-lipid fatty acid/g. dry wt. as do the whole mitochondria. Since the phospholipid is considerably enriched in the digitonin particles it follows that neutral lipid is lost more readily than phospholipid in the preparation of the particles. As with the phospholipid fatty acids there is considerable retention of arachidonic acid at the expense of other fatty acids. A similar retention also occurs with docosahexaenoic acid. For example, the triglyceride fraction of whole mitochondria contains 7·2 and 3·5% of C_{20}–C_{24} and C_{22}–C_{26} acids respectively, though the values for the corresponding fraction of the digitonin particles are 14·4 and 11·1% respectively. It is possible that most of these acids found in this fraction are in the form of free fatty acids. In whole mitochondria more arachidonic acid was found in the free fatty acids associated with the triglycerides than in the triglycerides themselves (Getz *et al.* 1962). These acids are likely to arise by hydrolysis of phospholipids.

Another striking difference is the presence in relatively high amounts of an acid behaving as a C_{13} acid on the gas chromatograph with Apiezon as the stationary phase. This is present in all the neutral-lipid fractions with the exception of the triglyceride fraction. It is present in the neutral lipid of the particles to the extent of 1 μmole/g. dry wt. This acid is presumably revealed because of selective retention in the particles.

The analysis of digitonin particles shows that the amounts of phospholipid associated with particles of very high specific activity with respect to oxidative phosphorylation (Devlin & Lehninger, 1958) are considerably larger than the amounts associated with whole mitochondria. The concentration of polyglycerolphosphatide is one striking example. An almost similar degree of enrichment of phospholipid was found by Siekevitz & Watson (1956) in deoxycholate preparations of mitochondrial membranes which also show a considerably increased specific activity of succinoxidase and cytochrome oxidase compared with that of untreated mitochondria. This is in accord with the importance ascribed to lipids in relation to mitochondrial function by Green (1959). However, there is no support for the idea that one single phospholipid is necessary for oxidative phosphorylation or electron transport with β-hydroxybutyrate as substrate. Maintenance of the integrity of lecithin does not appear necessary for succinoxidase activity as it does in the mitochondria (Macfarlane, 1950; Nygaard & Sumner, 1953; Macfarlane & Datta, 1954). The free fatty acids found in digitonin particles do not apparently uncouple oxidation from phosphorylation. Free fatty acids are potent uncouplers in mitochondrial preparations (see, for example, Pressman & Lardy, 1956). The selective retention of certain fatty acids (especially arachidonic acid) in the digitonin particles may mean that they have a special function in the integrated oxidative mechanisms of the mitochondria.

Fatty acids of the lipids of water- and saline-fractionated mitochondria. The pattern of total fatty acids of the mitochondrial fractions was very similar to that of the unfractionated mitochondria. In contrast with the digitonin particles, there is no evidence of selective retention of unsaturated fatty acids in the water- and saline-insoluble portions of the mitochondria. The fatty acids of the 'cardiolipin' and lecithins are similar to those of the fractions in the whole mitochondrial lipid (Getz *et al.* 1962). The fatty acids of the kephalin fraction are much more saturated than those of the whole mitochondria. They have little arachidonic acid and virtually no docosahexaenoic acid. This deficiency could be partly explained by the selective removal of these acids during hydrolysis of these phospholipids. The neutral-lipid fatty acids which contained the highest proportion of free fatty acids had substantially higher docosahexaenoic and arachidonic acids than the whole mitochondria. Fatty acids hydrolysed from phospholipids would be segregated with the neutral-lipid fraction on silicic acid chromatography.

Lipids of water and saline residues of mitochondria. The composition of the lipids of the mitochondrial residues remaining after water or saline extraction are shown in Table 15 together with the composition of the parent mitochondria. Both residues contain a higher proportion of lipid than the mitochondria. In contrast with the digitonin particles, the polyglycerolphosphatide fraction is least enriched in the residues. The relative distribution of phospholipids on the whole resembles that in the whole mitochondria, but there is a slightly lower proportion of polyglycerolphosphatide and a higher proportion of polar lipid fraction. Between one-half and two-thirds of the phospholipid is 'lysophosphatide', whereas no 'lysophosphatide' was seen in the parent mitochondria. Much of the fatty acid liberated in the formation of these lysophosphatides appears in the neutral-lipid fraction and, for the water residue, in the polyglycerolphosphatide. This probably accounts for the deviation of its fatty acid composition from that of the mitochondrial 'cardiolipin' previously reported (Getz & Bartley, 1959).

The last two columns of Table 15 show the enrichment of individual lipids in the two mitochondrial residues. The enrichment of all the phospholipids with the exception of polyglycerolphosphatides is similar in both mitochondrial residues (cf. digitonin particles). As with the digitonin

Table 15. *Composition of lipids of water and saline residues*

Fraction	Lipid content of			Lipid: lipid P percentage ratio of			Enrichment factors	
	Whole mitochondria (μmoles/g. dry wt.)	Water residue of mitochondria (μmoles/g. dry wt.)	Saline residue of mitochondria (μmoles/g. dry wt.)	Whole mitochondria (moles/100 moles of lipid P)	Water residue of mitochondria (moles/100 moles of lipid P)	Saline residue of mitochondria (moles/100 moles of lipid P)	Lipid content of water residue of mitochondria / Lipid content of whole mitochondria	Lipid content of saline residue of mitochondria / Lipid content of whole mitochondria
Acyl glyceryl ester	377	451	512	256	147	150	1·20	1·36
Ethanolamine and serine phosphatides	60·8	116	131	41	38	38	1·90	2·15
Lecithin	59·7	115	140	40·7	37·2	41·2	1·92	2·35
Polyglycerolphosphatide	10·0	16·6	11·3	6·8	5·4	3·3	1·66	1·13
Polar lipid fraction	—	27·1	10·7	—	8·8	3·1	—	—
'Lysophosphatides'	—	—	—	0	65·0	56·6	—	—

particles, there is no enrichment of neutral lipid, but a large relative loss from the saline residues. It appears that the neutral lipids are not particularly associated with the insoluble portions of the mitochondria. However, some neutral lipids have been shown to be of importance for the succinoxidase activity of acetone-extracted particles from ox-heart mitochondria (Crane, Fechner & Ambe, 1959; Crane & Ehrlich, 1960).

The digitonin particles derived from rat-liver mitochondria have lost most of the capacity to oxidize tricarboxylic acid cycle substrates, but have a much increased capacity to carry out oxidative phosphorylation with β-hydroxybutyrate as substrate (Devlin & Lehninger, 1958). Thus the digitonin particle seems to be a specific fragment of the mitochondrial membrane. On the other hand, water-treated mitochondria retain the capacity to oxidize succinate with only twice the specific activity of the untreated mitochondria (Siekevitz & Watson, 1956). Water treatment of the organelles caused considerable swelling and high-speed blending in water ruptured the membrane, but some of the matrix was retained attached to the membrane (Watson & Siekevitz, 1956). Similarly, saline-extracted mitochondria retained most of their activity for the oxidation of the acids of the tricarboxylic acid cycle and the saline-insoluble residue was seen, by phase-contrast microscopy, to consist of swollen but morphologically intact mitochondria. The electron microscope showed a separation of the inner and outer membranes (Dallam, 1958). The morphological changes brought about by saline or water treatment of mitochondria are similar. This similarity is reflected in the lipids that the mitochondrial residues retained after extraction.

The major difference between the lipids of the phosphorylating digitonin particles and the mitochondrial residues remaining after water or saline extraction is the retention by the former particles of polyglycerolphosphatide and certain fatty acids of which arachidonic acid is the most notable.

SUMMARY

1. The phospholipid and fatty acid composition of particles of rat-liver mitochondria prepared by the action of digitonin is described.

2. The phospholipid fractions separated by silicic acid chromatography were all 'lyso' compounds, the ratio of fatty acid to phosphorus approaching unity. Of the lipid phosphorus, 47 % appeared in the choline-containing fraction and 14 % in the polyglycerolphospholipid fraction. Of the fatty acids, 51 % appeared in the choline-containing fraction and only 7·5 % in the polyglycerolphospholipid fraction.

3. A sample of digitonin particles contained 355 μmoles of phospholipid fatty acid/g. dry wt. and 34 μmoles of neutral-lipid fatty acid/g. dry wt.

4. Arachidonic acid and linoleic acids were selectively retained by the digitonin particles, both in the neutral-lipid and phospholipid fractions.

5. The insoluble residue of mitochondria remaining after treatment with water or saline contained a higher proportion of lipid than the original mitochondria. Free fatty acids were liberated from the mitochondria by the saline or water treatment.

6. The relationship of mitochondrial lipids to the functions of the mitochondria is discussed.

We wish to thank Professor Sir Hans Krebs, F.R.S., for his interest in the work. The work was aided by the Rockefeller Foundation and the following National Institutes of Health, United States Public Health Service grants: no. A. 3369 to the Department of Biochemistry, University of Oxford, and no. A. 3991 to G.S.G. at the University of the Witwatersrand. G.S.G. also wishes to acknowledge help from the Wellcome Trust.

REFERENCES

Aravindakshan, I. & Braganca, B. M. (1961). *Biochem. J.* **79**, 84.

Bangham, A. D. & Dawson, R. M. C. (1959). *Biochem. J.* **72**, 486.

Barron, E. J. & Hanahan, D. J. (1958). *J. biol. Chem.* **231**, 493.

Bartley, W. (1953). *Biochem. J.* **54**, 677.

Basford, R. E. (1959). *Biochim. biophys. Acta*, **33**, 195.

Berenblum, I. & Chain, E. (1938). *Biochem. J.* **32**, 295.

Claude, A. (1946). *J. exp. Med.* **84**, 61.

Clément, G., Clément, J. & Le Breton, E. (1956). In *Biochemical Problems of Lipids*, p. 385. Ed. by Popják, G. & Le Breton, E. London: Butterworths Scientific Publications.

Clément, G., Haimovici, D. & Le Breton, E. (1955). *C.R. Acad. Sci., Paris*, **240**, 1827.

Cocking, E. & Yemm, E. W. (1954). *Biochem. J.* **58**, xii.

Collins, F. D. & Shotlander, V. L. (1961). *Biochem. J.* **79**, 321.

Conover, T. E., Marinetti, G. V., Witter, F. R. & Stotz, E. (1960). *Biochim. biophys. Acta*, **41**, 264.

Cooper, C. & Lehninger, A. L. (1956*a*). *J. biol. Chem.* **219**, 489.

Cooper, C. & Lehninger, A. L. (1956*b*). *J. biol. Chem.* **219**, 519.

Crane, F. L. & Ehrlich, B. (1960). *Arch. Biochem. Biophys.* **89**, 134.

Crane, F. L., Fechner, W. & Ambe, K. S. (1959). *Arch. Biochem. Biophys.* **81**, 277.

Dallam, R. D. (1958). *Arch. Biochem. Biophys.* **77**, 395.

Dawson, R. M. C. (1958). *Biochem. J.* **70**, 559.

Dawson, R. M. C. (1960). *Biochem. J.* **75**, 45.

Devlin, T. M. & Lehninger, A. L. (1956). *J. biol. Chem.* **219**, 507.

Devlin, T. M. & Lehninger, A. L. (1958). *J. biol. Chem.* **233**, 1586.

Elliott, W. B., Hulsmann, W. C. & Slater, E. C. (1959). *Biochim. biophys. Acta*, **33**, 509.

Fleischer, S., Brierley, G. & Klouwen, H. (1961). *Fed. Proc.* **20**, 45.

Getz, G. S. & Bartley, W. (1959). *Nature, Lond.*, **184**, 1229.

Getz, G. S. & Bartley, W. (1961). *Biochem. J.* **78**, 307.

Getz, G. S., Bartley, W., Stirpe, F., Notton, B. M. & Renshaw, A. (1962). *Biochem. J.* (In the Press.)

Gray, G. M. & Macfarlane, M. G. (1958). *Biochem. J.* **70**, 409.

Green, D. E. (1959). *Advanc. Enzymol.* **21**, 73.

Hanahan, D. J., Dittmer, J. C. & Warashina, E. (1957). *J. biol. Chem.* **228**, 685.

Hanahan, D. J. & Olley, J. N. (1958). *J. biol. Chem.* **231**, 813.

Hirsch, J. & Ahrens, E. H., jun. (1958). *J. biol. Chem.* **233**, 311.

Hulsmann, W. C., Elliott, W. B. & Rudney, H. (1958). *Biochim. biophys. Acta*, **27**, 663.

Kates, M. (1957). *Canad. J. Biochem. Physiol.* **35**, 127.

Macfarlane, M. G. (1950). *Biochem. J.* **47**, xxix.

Macfarlane, M. G. & Datta, N. (1954). *Brit. J. exp. Path.* **35**, 191.

Macfarlane, M. G., Gray, G. M. & Wheeldon, L. W. (1960). *Biochem. J.* **77**, 626.

Marinetti, G. V., Erbland, J., Albrecht, M. & Stotz, E. (1957). *Biochem. biophys. Acta*, **26**, 130.

Marinetti, G. V., Erbland, J., Kochen, J. & Stotz, E. (1958). *J. biol. Chem.* **233**, 740.

Marinetti, G. V., Erbland, J. & Stotz, E. (1958). *J. biol. Chem.* **233**, 562.

Nygaard, A. P. & Sumner, J. B. (1953). *J. biol. Chem.* **200**, 723.

Paoletti, P. & Grossi, E. (1960). *Ric. sci.* **30**, 726.

Pressman, B. C. & Lardy, H. A. (1956). *Biochim. biophys. Acta*, **21**, 458.

Rapport, M. M. & Alonzo, N. (1955). *J. biol. Chem.* **217**, 193.

Remmert, L. F. & Lehninger, A. L. (1959). *Proc. nat. Acad. Sci., Wash.*, **45**, 1.

Siekevitz, P. & Watson, M. L. (1956). *J. biophys. biochem. Cytol.* **2**, 653.

Simon, E. W. (1958). *Biochem. J.* **69**, 67.

Smits, G. (1957). *Biochim. biophys. Acta*, **26**, 434.

Strickland, E. H. & Benson, A. A. (1960). *Arch. Biochem. Biophys.* **88**, 344.

Watson, M. L. & Siekevitz, P. (1956). *J. biophys. biochem. Cytol.* **2**, 639.

Werkheiser, W. C. & Bartley, W. (1957). *Biochem. J.* **66**, 79.

Wheeldon, F. W. & Collins, F. D. (1958). *Biochem. J.* **70**, 43.

Wills, E. D. (1955). *Biochem. J.* **60**, 429.