CLXXXVII. THE COMPOSITION OF THE LIVER FATS OF SOME NEW ZEALAND FARM ANIMALS

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It is now generally recognized that the liver fats of mammals normally include phosphatides, glycerides and free fatty acids, together with minor proportions of free cholesterol and cholesteryl esters. Apart from an investigation of ox liver lipoids by Klenk & Schoenebeck [1932] and the recent studies of Channon *et al.* [1937] on the deposition of fat in the liver and carcass of rat, however, quantitative data for the component fatty acids of the phosphatide and glyceride constituents are lacking. Other investigators have confined themselves to a study of total liver fatty acids [e.g. Irving & Smith, 1935] or to the identification of fatty acids in purified lecithin [e.g. Levene & Simms, 1921; 1922]. In this connexion may be mentioned also the earlier work of Hartley [1909], who detected in the phosphatide fatty acids of pig liver, palmitic, stearic, linoleic and an isomeric ($\Delta^{12:13}$ octadecenoic) oleic acid, the existence of which later workers failed to prove either in pig liver [Channon *et al.* 1934] or in sheep liver fatty acids which were qualitatively examined by Turner [1930].

Until quite recently, the known fatty acids of phosphatides included palmitic, stearic, C_{18} unsaturated acids and arachidonic acid.[1] By applying the method of ester fractionation to the study of animal phosphatides, however, Klenk and co-workers [1930 *et seq.*] have indicated, in addition to myristic, palmitic and stearic acids, C_{14}, C_{16}, C_{18}, C_{20} and C_{22} unsaturated acids with 2–6 double bonds, affording a range and complexity of acids equalled, perhaps, only in the case of aquatic fats.

Leathes [1909] expressed the view that a function of the liver was to desaturate depot and food fats for subsequent oxidation in other tissues. Many workers have since studied this problem, relying solely upon mean mol. wt. and I.V. determinations of the respective depot and liver fats for the interpretation of results. Some workers have even based arguments on the I.V. of the total liver fatty acids regardless of their origin as phosphatide or glyceride. Klenk & Schoenebeck [1932] have drawn attention to the inadequacy of a simple desaturation hypothesis; for although liver glyceride somewhat resembles depot fat as regards the proportions of major component fatty acids, the higher I.V. of the liver fatty acids is due almost entirely to the presence of C_{20} and C_{22} highly unsaturated acids. These are certainly not the result of desaturation of food and depot fatty acids which belong almost exclusively to the C_{16} and the C_{18} series. These investigators have also shown that as compared with the corresponding liver glycerides, the phosphatides contain greater proportions of stearic as well as of C_{20} and C_{22} highly unsaturated acids.

The object of this investigation has been to determine the composition of the component fatty acids present as glycerides and phosphatides respectively, in the livers of the ox, cow, pig and sheep and to compare the data thus obtained

[1] For a full bibliography, see MacLean & MacLean [1927].

with those available for the corresponding depot fats. A detailed study of this nature, it was hoped, would contribute towards the elucidation of the complex problem of animal-fat metabolism.

The experimental material was supplied through the courtesy of Messrs R. and W. Hellaby, Ltd., Auckland, New Zealand. In this connexion we wish to express our sincere thanks to Dr J. C. Andrews who selected suitable livers and prepared ether extracts therefrom and, in addition, provided the detailed information summarized in Table I.

The livers were certified free from disease and with the exception of the cow liver (which exhibited fatty degeneration) they were normal. The samples were sliced and steam-cooked within 3 hr. of killing. The drained material was minced and then exhausted with ether in a continuous extractor. After drying the extract with anhydrous sodium sulphate, all but traces of solvent were removed and the "fat" was bottled for transport.

Table I. *Particulars of samples used in this investigation*

Animal	No.	Liver average wt. lb.	oz.	% ether extract (calc. on wt. of raw liver)	Remarks
Ox	98	17	8	6·2	Selected from prime beasts
Cow	1	16	0	13·4	Aged Jersey cow, rather poor condition, putty-coloured liver showing fatty degeneration
Pig	11	3	9	3·4	Selected from 500 prime baconers (av. dressed wt. 143 lb.)
	10	3	13	2·9	Selected from 237 prime baconers (av. dressed wt. 139 lb.)
Sheep	22	1	12	5·4	Selected from 200 prime cross-bred wethers (av. dressed wt. 53 lb.). The extract was green and more solid than either that of the ox, cow or pig livers

Experimental

After a preliminary resolution of these fats into phosphatide and "glyceride"[1] by means of acetone, the separated constituents were analysed according to the usual methods adopted in this laboratory [cf. Hilditch, 1934]. The simple acetone separation of phosphatide and glyceride is admittedly incomplete but may be improved by the addition of a neutral salt of a bivalent metal [Nerking, 1908; MacLean, 1914] which precipitates almost all the so-called acetone-soluble phosphatide. "Phosphatide" prepared in this way from an ethereal extract of animal tissue, however, may contain neutral fat, cholesterol and inorganic P [cf. Le Breton, 1921] as well as nitrogenous substances [MacLean & MacLean, 1927], the last traces of such impurities being extremely difficult to remove. The separation is also vitiated to some extent by the fact that di- and tri-saturated glycerides, such as oleopalmitostearins and palmitodistearins, typically present in animal fats, are only slightly soluble in acetone. These glycerides are thus likely to accumulate in the "phosphatide" fraction when a concentrated ethereal solution of lipoid is precipitated by excess acetone according to the usual procedure. Such a possibility does not seem to have been emphasized by previous workers. In this work therefore some allowance has

[1] "Glyceride" or, more strictly, glyceride, free fatty acid and unsaponifiable matter, i.e. non-phosphatide constituents.

been made for imperfections in the phosphatide-glyceride separation by use of a third or intermediate fraction. We believe that with these precautions the data for the "phosphatide" component fatty acids of cow, sheep and pig respectively, except in the case of the highly unsaturated C_{20} and C_{22} unsaturated acids (where the pitch-black residues obtained during ester fractionation have not permitted a precise determination of saponification equivalent), are accurate almost to within the usual limits of the ester fractionation method [cf. Hilditch, 1934]. The data for the "glyceride" fraction may not be so reliable owing to the presence of free fatty acid and, to a minor extent, of cholesteryl esters of uncertain composition. A high free fatty acid content in animal liver "glycerides" is apparently normal. Bloor & Snider [1930] report that approximately one-third of the acetone-soluble fraction ("glyceride") of beef liver lipoid is free fatty acid. The present results indicate a free fatty acid content of 9·5, 23·4, 40·1 and 55·9% for the acetone-soluble fractions of the cow, pig, ox and sheep liver lipoids respectively (cf. Table II). Although several months necessarily elapsed between the preparation of the sample and the analysis, it is not unreasonable to suppose that the observed free fatty acids may have been present as such in the living animal, and are not the result of subsequent enzyme or bacterial action. This view is supported not only by the similar observations of Bloor & Snider [1930] but also by the fact that, although the samples were prepared under similar conditions, the free fatty acid content varied enormously; moreover, there was no odour suggestive of the onset of rancidity, which often accompanies the production of free fatty acids by enzymes or bacteria.

In the case of the ox liver lipoid a simple acetone separation was employed, the fat being extracted with 4 vol. boiling acetone for 15 min. After cooling in the ice-chest overnight the clear supernatant liquid was decanted from the insoluble "phosphatide". This extraction was repeated four times and the extracts were united to give the "glyceride" fraction. For the remaining lipoids the method was modified as follows. After digesting the lipoid with 4 vol. of boiling acetone for at least 15 min. the solution was kept overnight in the ice-chest, when the supernatant liquid was poured into another flask labelled "crude glyceride". The "phosphatide", which appeared as a hard cake covered by a layer of crystalline material, was washed with cold acetone and the liquid decanted free from suspended matter into the "crude glyceride" flask. The crystalline material was then agitated with acetone and the resulting suspension transferred to another flask labelled "mixed phosphatide-glyceride". This process was repeated until the crystalline material had been satisfactorily separated from the phosphatide cake. The phosphatide was then re-extracted at least three times according to the above process. After adjusting the concentration of solvent in the solution of "crude glyceride" to 9 vol. the flask was kept 24 hr. in the ice-chest, where some insoluble material ("insoluble glyceride") settled out. The supernatant liquid was then removed by decanting and the insoluble material transferred to the mixed glyceride-phosphatide fraction. The last traces of phosphatide were removed from the acetone-soluble material by means of a solution of anhydrous calcium chloride in methyl alcohol [cf. Bloor & Snider, 1930]. The calcium chloride precipitate was combined with the mixed phosphatide-glyceride fraction and the whole thoroughly washed by shaking with 9 vol. of ice-cold acetone. After repeating this process several times the combined washings were added to the "glyceride" fraction. In order to obtain more detailed information with regard to the acetone method, the three constituents of the sheep liver mixed phosphatide-glyceride fraction, viz. "crystalline fraction", "insoluble glyceride" and "calcium chloride precipitate" were not

combined but were analysed separately by a semi-micro-fractionation method. The general characteristics of these fractions are collected in Table II.

Table II. *General characteristics of the constituent lipoid fractions*

Ox:	Ether extract %	P %	N %	Sap. equiv.	I.V.	Non-sap. %	Free fatty acid %	Cholesterol Free %	Cholesterol Combined %	Ratio* phosphatide-glyceride
"Phosphatide"	57·4	2·83	1·39	268·1	73·2	3·7	—	—	—	0·92
"Glyceride"	42·6	0·42	0·21	310·1	101·7	10·7	40·1	5·8	1·7	
Cow:										
Phosphatide	12·2	3·63	1·70	207·5	53·4	2·5	—	—	—	0·23
Mixed fraction	11·1	0·92	0·40	267·0	32·7	4·6	—	—	—	
"Glyceride"	76·7	0·18	—	286·5	69·8	4·9	9·5	2·3	0·8	
Pig:										
Phosphatide	59·2	3·62	2·06	206·6	—	3·7	—	—	—	1·92
Mixed fraction	10·6	1·78	0·93	340·2	62·8	29·4	—	14·3	3·7	
"Glyceride"	30·2	0·15	0·07	349·5	106·6	17·1	23·4	9·2	4·1	
Sheep:										
Phosphatide	44·7	4·14	1·84	208·0	83·1	2·3	—	—	—	1·19
"$CaCl_2$ precipitate"	4·7	3·61	—	238·8	108·2	7·4	—	—	—	
"Insoluble glyceride"	3·6	3·25	—	286·4	100·4	13·5	—	4·1	3·0	
"Crystalline fraction"	1·5	2·88	1·37	312·5	81·8	19·1	—	—	—	
"Glyceride"	45·5	0·13	—	314·6	112·9	13·8	55·9	6·7	2·8	

* Determined from consideration of phosphorus content of each fraction.

After saponification with alcoholic KOH the unsaponifiable matter was extracted with ether and the acids submitted to a lead salt separation according to the usual practice in this laboratory. In the case of the "phosphatide" fractions certain difficulties were encountered owing to the appearance of ether-insoluble impurities which were not apparent in the original material.[1] These impurities tended to remain at the interface of the ether and water during extraction of the fatty acids and were removed either by filtration or by running the water layer and the lower portion of the ether layer into another funnel for re-extraction with ether. This process was repeated until the impurities were satisfactorily separated from the fat. After removal of ether, the fatty acids were dissolved in 9 vol. of acetone and kept overnight in the ice-chest. Usually the amount of acetone-insoluble material thus obtained was small but in the case of the sheep liver phosphatide (201·0 g.) 4·1 g. of brown powder were isolated. This material (P 3·45%, N 2·47%) became sticky when heated on the water-bath and may have represented unhydrolysed phosphatide. The fatty acids after purification generally gave the expected yields of methyl esters. The yields from the "solid" (S) and "liquid" (L) acids of pig liver phosphatide were respectively about 11 and 6% lower than theoretical. Part of this loss (=1% of "solid" acids) was traced to ether-insoluble material (P 0·51%, N 3·26%) isolated during the course of preparation of the methyl esters of the "solid" acids. The same material (P 0·56%, N 3·26%) was also found to the extent of 0·3% of "solid" acids in the ether-insoluble fraction of the residue obtained by distillation of the methyl esters of the "solid" acids.

The residues of the methyl esters of the "solid" phosphatide fatty acids were not only dark coloured, but the presence of ether-insoluble material made it preferable to determine equivalents on the fatty acids rather than on the esters. An examination of the residue from the methyl esters of the "liquid" acids of ox liver phosphatide indicated the presence of 0·13% P. The dark

[1] The original material gave a clear solution in chloroform.

Table III. *Pig liver lipoid fractionation data*

(i) *Pig liver: "glyceride"* (30·2% of total lipoid). Sap. equiv. 349·5; I.V. 106·6; 23·4% free fatty acid (as oleic); P, trace only; wt. hydrolysed, 182·5 g.

Lead salt separation (with unsaponifiable extraction)

	g.	%
Solid acids (S)	41·6	23·6
Liquid acids (L)	107·0	60·8
Unsaponifiable matter removed	27·55	15·6
	176·15	

Methyl esters of "solid" acids

No.	g.	B.P./0·2 mm. ° C.	Sap. equiv.	I.V.
S 1	5·77	81–125	269·6	1·7
S 2	6·92	125–126	273·1	1·8
S 3	6·85	126–128	276·3	2·7
S 4	3·97	128–129	277·9	2·8
S 5	6·30	129	287·6	4·1
S 6	4·60	129–140	289·2	5·8
*S 7	3·42	Residue	294·5	6·6

* S 7: 1·31 g. on oxidation yielded 1·15 g. saturated esters, sap. equiv. 292·2.

Methyl esters of "liquid" acids

No.	g.	B.P./0·2 mm. ° C.	Sap. equiv.	I.V.	No.	g.	B.P./0·2 mm. ° C.	Sap. equiv.	I.V.
L 1	7·05	76–127	271·4	78·8	—	—	—	—	—
L 2	21·77	127–132	283·7	91·5	L 21	3·17	72–130	270·9	75·2
					L 22	6·01	130–131	280·6	82·1
					L 23	3·92	131	289·1	91·9
					L 24	5·16	Residue	295·6	113·4
L 3	23·26	132–135	293·6	100·2	L 31	4·67	67–132	286·4	88·8
					L 32	5·14	132	292·0	94·9
					L 33	3·45	132–133	294·6	97·7
					L 34	5·17	Residue	296·4	117·0
L 4	23·59	135–140	294·6	118·0	L 41	5·65	70–132	294·9	101·2
					L 42	5·98	132–133	296·3	103·3
					L 43	4·69	133–134	296·6	115·1
					L 44	3·46	Residue	299·1	149·2
L 5	4·63	140–147	300·2	140·2	—	—	—	—	—
L 6	4·38	147–149	305·5	175·3	—	—	—	—	—
*L 7	7·08	149–163	317·8	216·4	—	—	—	—	—
†L 8	9·81	Residue	490·1	183·5	—	—	—	—	—

* L 7: Esters, freed from unsaponifiable matter, sap. equiv. 309·0.
† L 8: Esters, freed from unsaponifiable matter, sap. equiv. 326·9.

(ii) *Pig liver: mixed phosphatide-glyceride* (10·6% of total lipoid). Sap. equiv. 340·2; I.V. 62·8; P, 1·78%; N, 0·93%; wt. hydrolysed, 68·5 g.

Lead salt separation (with unsaponifiable extraction)

	g.	%
Solid acids (S)	15·91	28·0
Liquid acids (L)	21·27	37·4
Unsaponifiable matter removed	19·64	34·6
	56·82	

Methyl esters of "solid" acids

No.	g.	B.P. 0·2 mm. ° C.	Sap. equiv.	I.V.
S 1	1·86	103–115	272·6	1·6
S 2	2·29	115	275·7	1·9
S 3	1·64	115–120	279·4	3·1
S 4	2·23	120–122	291·1	3·8
*S 5	2·82	Residue	309·8	14·2

* S 5: Esters, freed from unsaponifiable, sap. equiv. 306·6.

Table III (*cont.*)

Methyl esters of "liquid" acids

No.	g.	B.P./0·2 mm. ° C.	Sap. equiv.	I.V.
L 1	2·15	102–120	290·7	81·5
L 2	2·98	120–122	299·8	95·1
L 3	3·34	122–124	307·4	108·7
L 4	2·93	124–132	309·9	128·1
L 5	3·28	132–100	317·0	166·8
*L 6	3·92	Residue	357·5	206·8

* L 6: Esters, freed from unsaponifiable, sap. equiv. 325·6.

(iii) *Pig liver: phosphatide* (59·2% of total lipoid). Sap. equiv. 206·6; I.V. 55·9 (½ hr.), 57·0 (1 hr.); P, 3·62%; N, 2·06%; wt. hydrolysed, 396·5 g.

Lead salt separation (with unsaponifiable extraction)

		Esters	Corresponding acids	
	g.*	g.	g.	%†
Solid acids (S)	71·1	66·7	63·5	24·9
Liquid acids (L)	191·0	187·5	178·8	70·3
Unsaponifiable matter removed	12·2	—	12·2	4·8
			254·5	

* Includes 0·7 g. ether-insoluble material which was removed after esterification (N, 3·26%). Both the "solid" and the "liquid" acids were esterified in the usual way and the acids recovered from the potassium carbonate washings were re-esterified. 1·7 g. from the "solid" acid fraction resisted esterification.

† Calculated from the weights of the corresponding esters.

Methyl esters of "solid" acids

No	g.	B.P./0·2 mm. ° C.	Sap. equiv.	I.V.
S 1	7·08	99–124	277·9	3·1
S 2	8·32	124–127	282·6	3·0
S 3	9·21	127–132	288·0	3·3
S 4	8·71	132–135	292·1	4·1
S 5	12·18	135–138	294·9	4·4
S 6	5·25	138–105	298·6	6·1
*S 7	6·45	Residue	328·8	27·8

* S 7: Esters, freed from unsaponifiable, sap. equiv. 321·9; 3·89 g., on oxidation, yielded 2·50 g. saturated esters, sap. equiv. 323·1.

Methyl esters of "liquid" acids

No.	g.	B.P./0·2mm. ° C.	Sap. equiv.	I.V.	No.	g.	B.P./0·2mm. ° C.	Sap. equiv.	I.V.
L 1	11·27	107–131	274·0	69·5	—	—	—	—	—
L 2	39·77	131–137	288·7	82·5	L 21	8·45	98–128	277·5	54·8
					L 22	10·06	128–130	284·0	69·0
					L 23	10·05	130	293·7	90·8
					L 24	4·91	Residue	302·0	139·1
L 3	42·40	137–139	298·0	112·2	L 31	9·19	103–130	290·5	84·1
					L 32	10·28	130–135	296·0	97·4
					L 33	5·57	135	297·6	106·7
					L 34	6·95	Residue	305·9	168·0
L 4	43·53	139–149	305·9	159·3	L 41	10·07	100–137	298·8	118·5
					L 42	9·05	137–138	300·2	132·6
					L 43	7·05	138–139	307·6	167·3
					L 44	8·38	Residue	312·9	231·0
L 5	11·10	149–151	314·0	234·0	—	—	—	—	—
*L 6	8·60	151–172	323·9	269·2	—	—	—	—	—
†L 7	5·44	172–191	331·6	262·4	—	—	—	—	—
‡L 8	16·03	Residue	364·9	121·4	—	—	—	—	—

* L 6: Esters, freed from unsaponifiable, sap. equiv. 321·6.
† L 7: Esters, freed from unsaponifiable, sap. equiv. 321·9.
‡ L 8: Esters, freed from unsaponifiable, sap. equiv. 325·5.

Table III (*cont.*)

(iv) *Final results of analyses.*

(*a*) Pig liver: "glyceride"

Component acids	"Solid" (23·6%)	"Liquid" (60·8%)	Total	% (wt.)	% (mol.)	Mean unsaturation
Saturated:						
Myristic	0·08	—	0·08	0·1	0·1	—
Palmitic	14·78	3·77	18·55	22·5	24·3	—
Stearic	7·80	—	7·80	9·5	9·2	—
Unsaturated:						
Hexadecenoic	—	7·31	7·31	8·9	9·6	(−2·0H)
C_{18}	0·78	37·84	38·62	47·0	46·0	(−2·4H)
C_{20}	0·16	8·55	8·71	10·6	9·6	(−6·8H)
C_{22}	—	1·15	1·15	1·4	1·2	(−6·8H)
Unsaponifiable:	—	2·18	17·78	—	—	—
(extracted separately)	—	15·60				

(*b*) Pig liver: mixed phosphatide-glyceride

Component acids	"Solid" (28·0%)	"Liquid" (37·4%)	Total	% (wt.)	% (mol.)	Mean unsaturation
Saturated:						
Palmitic	12·90	0·42	13·32	20·6	23·0	—
Stearic	11·83	—	11·83	18·3	18·4	—
Arachidic	1·27	—	1·27	2·0	1·8	—
Unsaturated:						
Hexadecenoic	—	0·34	0·34	0·5	0·6	(−2·0H)
C_{18}	0·62	15·57	16·19	25·1	25·3	(−2·1H)
C_{20}	1·30	19·13	20·43	31·6	29·3	(−4·6H)
C_{22}	—	1·21	1·21	1·9	1·6	(−4·8H)
Unsaponifiable:	0·08	0·73	35·41	—	—	—
(extracted separately)	—	34·60				

(*c*) Pig liver: phosphatide

Component acids	"Solid" (24·9%)	"Liquid" (70·3%)	Total	% (wt.)	% (mol.)	Mean unsaturation
Saturated:						
Palmitic	6·56	4·85	11·41	12·1	13·4	—
Stearic	14·49	—	14·49	15·4	15·3	—
Arachidic	1·69	—	1·69	1·8	1·7	—
Unsaturated:						
Hexadecenoic	—	4·50	4·50	4·8	5·3	(−2·0H)
C_{18}	1·14	36·49	37·63	39·9	40·3	(−2·2H)
C_{20}	0·82	21·89	22·71	24·1	22·4	(−6·5H)
C_{22}	—	1·77	1·77	1·9	1·6	(−6·5H)
Unsaponifiable:	0·20	0·80	5·80	—	—	—
(extracted separately)		4·80				

nature of the residues from the methyl esters of the "liquid" phosphatide fatty acids and probable contamination with phosphorus compounds rendered the saponification equivalent uncertain.

In order to illustrate the method of fractionation it should suffice to give detailed data for pig liver lipoid only (Table III).

The final results for the composition of the component fatty acids of the various fractions are collected together in Table IV.

The mixed phosphatide-glyceride fractions varied considerably in composition, according to the phosphatide content. The mixed fraction from cow liver lipoid contained only 17·9% of the total fatty acids present as phosphatide while that from sheep liver consisted mainly of phosphatide fatty acids. After

Table IV. *Component fatty acids of glycerides and phosphatides in liver and depot fats (wt. %)*

	Saturated				Unsaturated				
	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Ox liver:									
"Glyceride"	1·4	30·4	6·6	—	1·1 (2·0)	9·9 (2·0)	40·3 (3·0)	8·5 (6·9)	1·8 (6·9)*
"Glyceride" [Klenk & Schoenebeck, 1932]	—	25·0	20·0	—	—	9·0 (2·0)	37·0 (2·6)	8·0 (5·0–7·4)	1·0
Phosphatide	1·3	28·2	14·4	0·2	0·7 (2·0)	3·8 (2·0)	31·5 (2·9)	10·8 (7·5)	9·1† (7·5)
Phosphatide [Klenk & Schoenebeck, 1932]	—	12·5	27·0	—	—	5·0	27·0 (2·6–3·4)	18·0 (5·3–8·2)	10·5
Cow liver:									
"Glyceride"	2·8	29·8	5·3	—	0·4 (2·0)	9·7 (2·0)	48·6 (2·4)	3·4 (6·0)	—
Mixed phosphatide-glyceride	0·8	56·6	11·0	—	—	5·4 (2·0)	22·7 (2·4)	2·1 (6·0)	—
Phosphatide	—	21·3	21·8	—	—	4·1 (2·0)	47·8 (2·7)	5·0 (6·0)	—
Pig liver:									
"Glyceride"	0·1	22·5	9·5	—	—	8·9 (2·0)	47·0 (2·4)	10·6 (6·8)	1·4 (6·8)
Mixed phosphatide-glyceride	—	20·6	18·3	2·0	—	0·5 (2·0)	25·1 (2·1)	31·6 (4·6)	1·9 (4·8)
Phosphatide	—	12·1	15·4	1·8	—	4·8 (2·0)	39·9 (2·2)	24·1 (6·5)	1·9 (6·5)
Sheep liver:									
"Glyceride"	0·2	21·9	12·9	—	—	4·9 (2·0)	44·7 (2·8)	11·6 (7·3)	3·8 (7·8)
Mixed phosphatide-glyceride: "$CaCl_2$ ppt."	—	14·9	18·1	0·8	—	5·5 (2·0)	27·8 (2·9)	21·9 (7·1)	11·0 (10·0)
Mixed phosphatide-glyceride: "Insol. glyceride"	—	20·6	17·4	4·1	—	5·5 (2·0)	29·8 (2·7)	14·7 (7·0)	7·9 (10·0)
Mixed phosphatide-glyceride: "Crystalline fraction"	—	28·6	21·2	5·0	—	2·4 (2·0)	25·8 (2·7)	12·6 (5·7)	4·4 (10·0)
Phosphatide	—	12·6	21·8	0·8	—	8·9 (2·0)	27·8 (3·1)	23·6 (6·9)	4·5 (10·5)

* Figures in brackets, e.g. (6·9), denote mean unsaturation per molecule, e.g. (−6·9 H). † Mainly C_{20}.

correction for phosphatide fatty acids it appeared that the glyceride fatty acids present in these mixed fractions were mainly saturated, palmitic acid being the major constituent. The glyceride fatty acids from the mixed fractions of cow and pig liver lipoids respectively contained about 75 and 79 % saturated acids, corresponding to at least 25 and 37 % of fully-saturated glyceride. Although, owing to the semi-quantitative nature of the data for the mixed fractions of sheep liver lipoid, the calculated composition of the glyceride fatty acids in these fractions could not be accurately ascertained, it is almost certain that the proportion of saturated acids was similar to those found in the cases of the cow and pig liver lipoids. The mixed fractions of the cow, pig and sheep liver lipoids represented respectively 10, 6 and 2 % of the total glyceride fatty acids.

The obvious effect of insoluble di- and tri-saturated glycerides in the usual method of phosphatide separation will be to increase the apparent content of saturated acid in the "phosphatide" fraction at the expense of the non-phosphatide ("glyceride") fraction. The relative effect on the fatty acid composition of either of these fractions will be in the inverse ratio of its relative proportion in the liver lipoid. The error introduced is probably not usually serious as is shown by the fact that the corrected data mostly agree quite closely with

data given for the original glyceride fraction. A maximum deviation is shown in the case of the cow liver glyceride, where the observed palmitic acid content is 29·8 % (mol.) as compared with the corrected figure of 34·7 % (mol.).

Although no data are available for a mixed phosphatide-glyceride fraction of ox liver lipoid a crystalline precipitate was observed during acetone separation of the phosphatide fraction. This precipitate is likely to represent insoluble glyceride and would thus account for the low phosphorus content of the "phosphatide". Similar contamination with these glycerides may also be responsible for the low phosphorus contents of phosphatides sometimes recorded in the literature.

It is clear from Table IV that the so-called "acetone-soluble phosphatides" [cf. MacLean, 1914] represented by the "calcium chloride precipitate" of sheep liver lipoid are essentially similar to the acetone-insoluble phosphatides as regards the proportions of component fatty acids. The fatty acids of the former are, however, somewhat more unsaturated. ("Calcium chloride precipitate", 66·2 % unsaturated acids (I.V. 208·4); "acetone-insoluble phosphatide", 64·7 % unsaturated acids (I.V. 199·8).) If, on the basis of the phosphorus content, the fatty acids of the "calcium chloride precipitate" are corrected for the presence of some 8 % contaminating glyceride, then the differences between these fractions will appear rather greater. From considerations of solubility it is likely that any phosphatide present in the "insoluble glyceride" fraction would be more saturated than the so-called "acetone-soluble phosphatide" represented by the "calcium chloride precipitate". These observations together with the data presented in Table IV suggest that in the case of the mixed phosphatide-glyceride fractions it is not unreasonable to calculate the phosphatide component fatty acids on the basis that these acids are present in the same proportions in both the mixed phosphatide-glyceride and the main phosphatide fractions. After ascertaining the proportions of phosphatide in the mixed fractions from the respective phosphorus contents[1] of the phosphatide and the mixed fraction, the percentage of phosphatide fatty acids is calculated as

$$\frac{\%\text{ phosphatide (mixed fraction)} \times \%\text{ fatty acids (phosphatide)} \times 100}{\%\text{ phosphatide (mixed fraction)} \times \%\text{ fatty acids (phosphatide)} + \%\text{ glyceride (mixed fraction)} \times \%\text{ fatty acids ("glyceride")}}$$

If the compositions of the mixed fraction and of the phosphatide respectively are known, the composition of the non-phosphatide ("glyceride") fatty acids may be ascertained by difference. The composition of the component fatty acids present in the main "glyceride" fraction as well as in the glyceride portion of the mixed fraction being thus known it is possible to calculate the proportions of component fatty acids present in the total non-phosphatide (glyceride + free fatty acids + cholesteryl ester) fraction.

(Owing to imperfect separation, it could not be assumed in the case of the ox liver lipoid that the "phosphatide" fraction had the same fatty acid composition as the phosphatide present in the "glyceride" fraction. The phosphatide contents of both "glyceride" and "phosphatide" constituents were therefore ascertained by comparing the respective phosphorus contents with that of a lecithin whose fatty acids would have the same mean mol. wt. as those of the isolated crude phosphatide. After calculating the respective proportions of phosphatide and glyceride fatty acids in the crude glyceride and phosphatide fractions, the percentage of each acid present as glyceride and phosphatide was solved algebraically.)

Corrected data for the fats examined are given in Table V.

[1] Corrected for the presence of unsaponifiable matter.

Table V. *A comparison of the corrected fatty acid compositions of the non-phosphatide ("glyceride") and phosphatide constituents of the ox, cow, pig and sheep lipoids with the available data for the corresponding depot fats. Fatty acids (% mol.)*

	Saturated				Unsaturated				
	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Ox liver:									
"Glyceride"	1·6	32·5	5·0	—	1·5 (2·0)	11·7 (2·0)	40·3 (3·0)	7·4* (6·9)	
Phosphatide	1·5	29·7	17·0	0·2	0·6 (2·0)	1·6 (2·0)	27·7 (2·9)	21·7* (7·5)	
Depot glyceride‡	7·3	29·2	20·5	—	—	—	43·0 (2·1)	—†	—
Cow liver:									
"Glyceride"	3·2	34·7	5·3	—	0·5 (2·0)	9·9 (2·0)	43·6 (2·4)	2·9 (6·0)	—
Phosphatide	—	22·9	21·2	—	—	4·4 (2·0)	46·9 (2·7)	4·6 (6·0)	—
Pig liver:									
"Glyceride"	0·1	25·8	10·3	0·2	—	9·2 (2·0)	44·1 (2·4)	9·2 (6·8)	1·1 (6·8)
Phosphatide	—	13·4	15·3	1·7	—	5·3 (2·0)	40·3 (2·2)	22·4 (6·5)	1·6 (6·5)
Depot glyceride§	1·1	28·1	9·7	—	—	—	61·1 (2·1)	—†	—
Sheep liver:									
"Glyceride"	0·3	24·2	12·5	—	—	5·3 (2·0)	44·1 (2·8)	10·5 (7·3)	3·2 (7·8)
Phosphatide	—	13·9	21·7	0·7	—	9·9 (2·0)	28·0 (3·1)	21·9 (6·9)	3·9 (10·5)
Depot glyceride‖	2·1	24·6	25·6	—	—	—	47·7 (2·2)	—†	—

* Mainly C_{20}. † Traces of C_{20}-acids are also present in these depot glycerides.
‡ Mean values—Banks & Hilditch [1931]; cf. Armstrong & Allan [1924].
§ Mean values—Ellis & Zeller [1930]; cf. Ellis & Isbell [1926], Bhattacharya & Hilditch [1931].
‖ Mean values—Armstrong & Allan [1924]; cf. Collin *et al.* [1929].

It will be seen from Table V that the non-phosphatide liver fatty acids somewhat resemble the corresponding depot fats as regards the relative proportions of component fatty acids [cf. Klenk & Schoenebeck, 1932], but the former are distinguished by the presence of some 5–10% (mol.) of hexadecenoic acid together with 5–15% (mol.) of C_{20} and C_{22} highly unsaturated acids, the proportions in both cases being much greater than in the depot fats.

In view of the fact that hexadecenoic acid had not been reported by previous investigators in the case of the depot fats concerned it seemed desirable both to verify the presence of this acid in the liver "glyceride" and to search for traces in the depot fat. Accordingly the methyl esters of the liquid acids from sheep liver glyceride were fractionated before and after hydrogenation with the following results.

Table VI. *Component fatty acids (% mol.)*

	Before hydrogenation	After hydrogenation
Palmitic	6·7	14·6
Hexadecenoic	8·4	—
C_{18}	64·3	65·3
C_{20}	15·5	14·5
C_{22}	5·1	5·6

The presence of hexadecenoic acid was thus verified by the increase of palmitic acid in the hydrogenated sample as compared with the original esters.

An examination of the methyl esters of the liquid acids from pig depot fat by refractionation and oxidation of the lower-boiling fractions gave the following results.

Table VII

Weight	Sap. equiv.	I.V.	Sap. equiv. of saturated esters
3·12	273·1	54·0	257·9
10·40	279·0	80·4	262·4
9·88	286·7	92·8	271·5
7·71	290·0	102·1	—

These figures suggest that, contrary to previous observations [Dean & Hilditch, 1933], pig depot fat contains a small proportion (3–4 % mol.) of hexadecenoic acid.[1]

The occurrence of such proportions of hexadecenoic acid in depot glycerides together with relatively constant palmitic contents (25–30 %) in both liver and depot glycerides indicate a general similarity between these two classes of fats. It is possible that the final compositions of the depot and liver glycerides may be the result of a selective interchange between the body and liver leading to a higher concentration of hexadecenoic and C_{20}–C_{22} glycerides in the liver than in the body glycerides. Although, as shown by the present data, the mean unsaturation of the C_{18} unsaturated acids is higher in the liver glyceride ($-2·4$ H to $-3·0$ H) than in the corresponding depot fat ($-2·0$ H to $-2·2$ H) these acids are present in smaller proportions in the former, so that the higher I.V. of the liver "glyceride" (non-phosphatide) fatty acids as compared with that of the depot fatty acids is due mainly to the presence of increased proportions of C_{20} and C_{22} acids.

A survey of the data given by Klenk [1935] for the liver glycerides and phosphatides of the frog, turtle and ox respectively, together with the results of this investigation, establishes that liver phosphatides are characterized by the presence of increased proportions of stearic and C_{20} and C_{22} unsaturated acids together with diminished proportions of hexadecenoic acid, as compared with the corresponding liver glycerides. The sheep liver lipoid examined, however, is exceptional in two respects: (1) the phosphatide is richer in hexadecenoic acid than the "glyceride", (2) the greater proportion of stearic as compared with palmitic acid typically found in the depot fat is not reflected in the liver "glyceride". Associated with these exceptions is the high free fatty acid content of 55·9 %.

Table VIII. *Comparison of mean mol. wt. of phosphatide and non-phosphatide ("glyceride") fractions calculated from the results of Table V*

	Mean mol. wt. of fatty acids
Ox liver "glyceride"	254·8
Ox liver phosphatide	262·3
Cow liver "glyceride"	253·9
Cow liver phosphatide	261·6
Pig liver "glyceride"	260·5
Pig liver phosphatide	268·7
Sheep liver "glyceride"	262·4
Sheep liver phosphatide	267·5

[1] Dr H. E. Longenecker and one of us, in a forthcoming communication, will present evidence for the presence of similar small amounts of hexadecenoic acid in ox depot fats.

Table VIII illustrates the marked tendency of the phosphatide as compared with the "glyceride" (non-phosphatide fraction) to contain acids of higher mol. wt. Sinclair [1932] showed in the case of rats a remarkable preferential absorption of more unsaturated acids by the phosphatides from the various tissues. The feeding of small amounts of cod liver oil, sufficient to produce a marked increase in the level of unsaturation of the phosphatides, had no apparent effect on the neutral fat. The present results show that absorption of unsaturated acids into the phosphatide molecules is a selective phenomenon. Whereas the highly unsaturated C_{20} and C_{22} acids and octadecadienoic acid (as shown by the subsequent bromination data) are preferentially selected by the liver phosphatide, myristoleic and hexadecenoic (low mol. wt. monoethenoid) acids tend to concentrate chiefly in the liver glyceride.

As regards the mean unsaturation of C_{18} and C_{20} groups respectively, the values for the "glycerides" and phosphatides do not vary greatly although these groups are frequently more unsaturated in the phosphatide fractions. The mean unsaturation of the C_{22} unsaturated acids has usually been rendered uncertain owing to lack of material in the case of the "glyceride" and to polymerization in the case of the phosphatide. Attention should, however, be drawn to the abnormally high mean unsaturation ($-10{\cdot}5$ H) of the C_{22} acids from sheep liver phosphatide suggesting the possibility of acids even more highly unsaturated than clupanodonic acid or isomerides, which Klenk & Schoenebeck [1932] have shown to exist in ox liver phosphatide.

Examination of the bromo-additive products of the C_{18} unsaturated acids

Recent studies on the composition of C_{18} unsaturated acids by various workers have shown that although oleic acid ($\Delta^{9:10}$-octadecenoic acid) is a common constituent of plant and animal fats, the di- and poly-ethenoid C_{18} acids vary both in nature and in proportion according to the origin of the fat. In seed fats the C_{18} unsaturated acids other than oleic usually consist of linoleic, $\Delta^{9:10,12:13}$-octadecadienoic acid, and frequently linolenic acid, $\Delta^{9:10,12:13,15:16}$-octadecatrienoic acid, is also present. The available data for aquatic fats [Green & Hilditch, 1936; Shorland & McIntosh, 1936] suggest that linoleic and linolenic acids are not present in more than traces, the polyethenoid unsaturation being due in part at least to octadecatetraenoic (stearidonic) acid. The fats from land mammals form another group in which linolenic acid is not usually found and linoleic acid may be present to the exclusion of other C_{18} di- and poly-ethenoid acids, as in some pig depot fats [Hilditch & Stainsby, 1935], or it may appear in traces only. In the latter case there seems to be present, as in butterfat [Green & Hilditch, 1935], an isomeric form of linoleic acid yielding a petroleum-soluble tetrabromide.

As regards the liver lipoids described in this investigation Klenk & Schoenebeck [1932] found linoleic acid in ox liver phosphatide, whilst Hartley [1909] and Turner [1930] observed this acid in the fatty acids of pig and sheep livers respectively. The data regarding the presence of linoleic acid in these fats are not, however, conclusive, for Levene & Simms [1922] in their detailed bromination studies on the fatty acids of beef liver lecithin failed to obtain the solid tetrabromostearic acid M.P. 114° characteristic of linoleic acid.

In the present investigation concentrates of C_{18} unsaturated acids were prepared by repeated fractionation of the methyl esters of the "liquid" acids. The acids (5–10 g.) were separated by successive crystallization of the lithium salts from 95% acetone and 80% alcohol respectively, into three groups which

were brominated as described by Green & Hilditch [1936]. The data finally obtained are summarized in Table IX (*a*, *b*, *c*).

Table IX (*a*). *Separation of acids by lithium salts*

	A. Acetone-soluble		B. Acetone-insoluble, alcohol-soluble		C. Insoluble in both acetone and alcohol	
	%	I.V.	%	I.V.	%	I.V.
Beef liver glyceride (sap. equiv. 295·1, I.V. 126·5)	9·5	210·0	32·0	149·4	58·5	104·7
Beef liver glyceride (61 %) + ground-nut oil C_{18} acids (cf. p. 1513)	15·6	222·6	40·7	150·0	43·7	104·6
Beef liver phosphatide (sap. equiv. 294·6, I.V. 117·2)	10·0	181·8	34·3	146·2	55·7	86·6
Cow liver glyceride (sap. equiv. 294·9, I.V. 97·9)	6·6	172·4	18·5	126·0	74·9	81·6
Pig liver glyceride (sap. equiv. 294·9, I.V. 100·4)	7·9	214·5	18·9	121·3	73·2	89·1
Pig liver phosphatide (sap. equiv. 295·5, I.V. 90·1)	11·2	225·7	25·4	114·8	63·4	62·1
Sheep liver glyceride (sap. equiv. 294·3, I.V. 109·5)	5·6	156·7	50·7	125·4	43·7	99·2
Sheep liver phosphatide (sap. equiv. 292·0, I.V. 113·3)	10·4	163·0	43·3	128·6	46·3	81·1

Table IX (*b*). *Bromo-additive products of each fraction*

		Beef liver				Pig liver		Sheep liver	
		Glyceride	Glyceride + ground-nut acids	Phosphatide	Cow liver glyceride	Glyceride	Phosphatide	Glyceride	Phosphatide
A 1*	M.P.	230°	246°	236°	239°	238°	238°	215–218°	232°‡
	% Br	68·0	69·2	66·7	65·2	67·2	70·0	68·8	67·6
	%†	1·9	2·6	1·3	0·3	1·5	1·8	0·3	0·9
A 2	M.P.	80–90°	Indef.	120–140°	156°	138–140°	135–140°	118–124°	108–135°
	% Br	61·9	52·2	65·8	58·7	61·2	61·9	66·2	52·2
	%	1·7	6·1	1·2	0·6	1·1	2·5	0·7	1·0
A 3	M.P.	—	—	—	—	—	—	—	—
	% Br	48·9	56·4	44·9	48·5	48·3	53·8	44·2	44·7
	%	5·9	6·9	7·5	5·7	5·3	6·9	4·6	8·5
B 1	M.P.	234°	240°	224°	224°	236°	226°	175–176·5°	175–176°
	% Br	65·2	67·3	64·8	65·5	67·8	65·0	64·2	64·0
	%	2·8	1·7	2·1	0·2	0·5	0·3	1·3	1·3
B 2	M.P.	176°‡	106–114°	118–135°	166°‡	149–151°	108–115°	105–135°	106–108°
	% Br	54·6	51·3	42·0§	63·7	63·2	59·1	52·9	53·5
	%	0·8	6·9	2·7	0·6	0·5	2·1	3·3	5·8
B 3	M.P.	—	—	—	—	—	—	—	—
	% Br	45·1	47·7	44·6	41·0	40·4	38·4	41·6	41·7
	%	28·4	32·1	29·5	17·7	17·9	23·0	46·1	36·2
C 1	M.P.	176°‡	200°	208°	200°	—	—	—	160°
	% Br	63·1	—	68·3	—	—	—	—	—
	%	1·0	Trace	0·3	Nil	Nil	Nil	Nil	Trace
C 2	M.P.	172°	106–128°	174°	166°‡	135–150°	—‖	150–170°	—‖
	% Br	61·5	46·3	52·5	58·1	60·7	11·2	56·9	12·0
	%	0·3	1·8	0·6	0·3	0·3	6·0	0·8	1·8
C 3	M.P.	—	—	—	—	—	—	—	—
	% Br	38·1	39·2	34·6	33·3	35·1	28·0	36·7	33·4
	%	57·2	41·9	54·8	74·6	72·9	57·4	42·9	44·5

* 1 = ether-insoluble; 2 = ether-soluble, petroleum-insoluble; 3 = soluble in both petroleum and ether.
† Expressed as a percentage of total C_{18} fraction of liquid acids.
‡ Melted with decomposition. § Decomposed while drying. ‖ Mainly stearic acid.

Table IX (*c*). *Identification of bromo-additive compounds*

	Insoluble in hot petroleum	Soluble in hot, insoluble in cold petroleum	Soluble in cold petroleum
B 2. Ox phosphatide	0·0312 g. M.P. 174°*	0·0050 g. M.P. 127–130°	0·0048 g.
B 2. Ox glyceride + ground-nut oil C_{18} acids (cf. p. 1513)	0·0166 g. M.P. 170–174°	0·1220 g. M.P. 113° (mixed tetrabromo-stearic 113–114°)	0·0620 g.
B 2. Pig glyceride	0·0550 g. M.P. 171°*	0·0180 g. M.P. 109–110° (mixed tetrabromo-stearic 110–112°)	0·0210 g. M.P. 98–102°
B 2. Pig phosphatide	0·0310 g. M.P. 165–170°	0·1308 g. M.P. 112–113° (mixed tetrabromo-stearic 113–114°)	0·0296 g. M.P. 100–104°
B 2. Sheep glyceride	0·0484 g. M.P. 165°*	0·0960 g. M.P. 108–110° (mixed tetrabromo-stearic 110–112°)	0·0226 g. M.P. 60–85°
B 1. Sheep glyceride	Mixed with hexabromostearic acid M.P. 175–176°		
B 2. Sheep phosphatide	0·0824 g. M.P. 115–130° (indefinite)	0·2203 g. M.P. 110–111° (mixed tetrabromo-stearic 111–112·5°)	0·0714 g. M.P. 79–83°
B 1. Sheep phosphatide	Mixed with hexabromostearic acid M.P. 175–176°		

* Melted with decomposition.

With the exception of the A 1 sheep liver glyceride fraction (M.P. 215–218°), the remaining A 1 fractions melted within a range of 230–246° characteristic of highly unsaturated C_{20} acids which were probably, therefore, still present owing to imperfect fractionation. It is noteworthy in this connexion that the relatively low proportion of high-melting bromides in the case of the sheep liver fatty acids is associated with the use of an efficient fractionation column [Longenecker, 1937] in place of the customary Willstätter flask employed in the preparation of the remaining C_{18} fractions. The bromine contents of the A 1 fractions lay mostly between the theoretical values for a mixture of $C_{20}H_{32}O_2Br_8$ (% Br = 67·8) and $C_{20}H_{30}O_2Br_{10}$ (% Br = 71·6). The A 1 fractions of pig liver phosphatide (M.P. 238°, % Br = 70·0) and pig liver "glyceride" (M.P. 238°; % Br = 67·2) may be compared with the ether-insoluble polybromides (M.P. 240°, % Br = 68·3) of pig liver fatty acids described by Irving & Smith [1935].

The B 1 fractions resembled for the most part the A 1 fractions as regards M.P. and bromine content. The B 1 fractions of sheep liver fatty acids were obviously not typical C_{20} polybromides. They melted clearly (without decomposition) at about 176° and when mixed with an authentic specimen of hexabromostearic acid (M.P. 175–176°) there was no depression of M.P. This result suggests the presence of some linolenic acid in sheep liver lipoid.

In order to test for linoleic acid, the B 2 fractions in which the tetrabromide of this acid would tend to concentrate were recrystallized from petroleum (B.P. 40–60°) as indicated by Table IX (*c*). The high M.P. and low concentration of the B 2 fractions in the ox and cow liver "glycerides" suggested the absence of more than traces of linoleic acid. This was confirmed by the fact that, when this method was applied to a mixture of the "liquid" acids from ground-nut oil

(39 %) and ox liver glyceride C_{18} acids (61 %, sap. equiv. 296·7, I.V. 149·8), the added linoleic acid (equivalent to about 12 % of the mixture) was readily indicated by the increased yield of the B 2 fraction as well as by the isolation of a crystalline tetrabromide, M.P. 113°. The yield of tetrabromide was equivalent to 34 % of the linoleic acid originally added as compared with a 40 % yield (M.P. 114°, % Br = 53·5) obtained on bromination of the original ground-nut liquid acids. By comparison with the data given for the ox liver "glyceride"-ground-nut "liquid" acids mixture it is estimated that the "glyceride" and phosphatide C_{18} unsaturated acids from pig liver contain approximately 1 and 4 % of this acid, whilst the linoleic acid content of the sheep liver C_{18} unsaturated acids is somewhat higher, the glyceride and phosphatide fractions containing, respectively, about 6 and 11 %. Although the yield of the B 2 fraction from ox liver phosphatide was relatively large, the low M.P., bromine content and solubility data gave no evidence for the presence of tetrabromostearic acid. The B 2 fractions were shown to contain varying proportions of high-melting bromides insoluble in hot petroleum. The bromide (M.P. 171°) obtained from the B 2 fraction of pig liver "glyceride" contained 63·3 % Br, which accords well with the theoretical bromine content of hexabromostearic acid. The exact nature of this bromo-derivative is unknown, but the failure to obtain a clear M.P. together with the solubility data suggest that it cannot be identical with the hexabromostearic acid from ordinary linolenic acid.

In Table X the apparent linoleic acid content has been calculated from the mean I.V. of the C_{18} esters of the "liquid" acids, presuming that oleic and octadecadienoic acids only are present.

Table X. *The apparent linoleic acid content of the C_{18} unsaturated acids*

		Linoleic acid	
	I.V.	% observed from tetrabromide yield	Calculated
Cow glyceride	103·0	Nil	19·8
Cow phosphatide	120·5	Nil	29·9
Ox glyceride	133·5	Nil	54·9
Ox phosphatide	124·7	Nil	44·7
Pig glyceride	103·0	1	19·8
Pig phosphatide	94·4	4	10·0
Sheep glyceride	124·7	6	44·7
Sheep phosphatide	137·7	11	59·7

The mean I.V. of the C_{18} esters given in the table are probably too high owing to the inclusion of traces of C_{20} acids which cannot be completely removed by the usual fractionation method. Although this would tend to give a high result for the calculated linoleic acid content it is evident that (with the possible exception of pig liver phosphatide) the fractions contained considerable proportions of petroleum-soluble tetrabromides as in the case of cow butterfat.

In order to account for the I.V. in excess of that given by oleic acid, Green & Hilditch [1935] submitted a concentrate of C_{18} unsaturated acids of cow butterfat to an acetone permanganate oxidation [Armstrong & Hilditch, 1925] and concluded from the oxidation products that an octadecadienoic acid structurally similar to linoleic acid was probably present. They also drew attention to the general similarity between the octadecadienoic acids of butterfat and grass [Smith & Chibnall, 1932]. In both instances no evidence of a crystalline tetrabromostearic acid (M.P. 114°) characteristic of linoleic acid could be obtained,

although the grass fatty acids yielded 8·9 g. of oily tetrabromide from 10·4 g. of unsaturated acids. In both cases also the yield of tetrahydroxy-derivatives indicated the presence of less than 4% of ordinary linoleic acid. From these results it was concluded that the peculiarities of butterfat octadecadienoic acid might be the consequence of direct assimilation of the grass glycerides. In view of the sensitivity of the bromination method as indicated in this investigation by the recovery of added linoleic acid even in the presence of considerable amounts of C_{20} highly unsaturated acids, it is not impossible that the tetrahydroxystearic acid recorded both in grass fat and in cow butterfat may be the result of isomerization of part of the octadecadienoic acid to linoleic acid during oxidation.

Cattle and sheep in New Zealand subsist on grass, while the basic diet of pigs consists of dairy products and grass supplemented in winter by meat and root crops, which would probably not contain any appreciable amount of linoleic acid. The definite proportions of linoleic acid in sheep and pig liver lipoids, especially in the phosphatide fractions, suggest that these animals tenaciously store the traces of linoleic acid of the diet or else that they have a mechanism for the conversion of isomeric octadecadienoic acid into linoleic acid which is absent or very imperfectly developed in the ox or cow. This latter hypothesis is in harmony with the possibility that the definite evidence for the existence of linoleic acid (as indicated by the yield of tetrabromostearic acid, M.P. 114°) in the ox liver phosphatide examined by Klenk & Schoenebeck [1932] may be the effect of including bran or linseed meal (containing linoleic acid) in the diet. This evidence also tends to exclude the possibility that the absence of linoleic acid from the ox and cow lipoids reported in this investigation may be due to a destructive action of the liver on this acid.

Summary

The phosphatide and "glyceride" constituents of the liver lipoids of ox, cow, pig and sheep have been studied in detail. It has been shown that the usual method of phosphatide-glyceride separation is incomplete owing to the sparing solubility of di- and tri-saturated glycerides (typically present in animal fats) in acetone. A correction is therefore introduced by use of a third or mixed phosphatide-glyceride fraction. As would be expected the main error involved was to increase the apparent content of saturated acid in the "phosphatide" fraction at the expense of the non-phosphatide ("glyceride") fraction. So far as the individual fatty acids are concerned the error is usually quite small, but the uncorrected palmitic acid content of cow liver "glyceride" is 29·8% (mol.) as compared with the corrected figure of 34·7% (mol.).

The non-phosphatide fatty acids of the liver have been shown to resemble somewhat those of the corresponding depot fats [cf. Klenk & Schoenebeck, 1932]. The former are distinguished, however, by the presence of some 5–10% (mol.) of hexadecenoic acid together with 5–15% (mol.) of C_{20}–C_{22} highly unsaturated acids, the proportions in both cases being much greater than in the depot fats. The evidence for the occurrence of small proportions of hexadecenoic acid (3–4% (mol.)) in the animal depot fats concerned, which had not been reported by previous workers, together with the relatively constant 25–30% (mol.) of palmitic acid of the liver "glyceride", further stress the similarity between these two types of fat.

Survey of the available data [cf. Klenk & Schoenebeck, 1932] together with the results of the present investigation establishes that liver phosphatides are characterized by the presence of increased proportions of stearic, C_{20} and C_{22} unsaturated acids together with diminished proportions of hexadecenoic acid, as

compared with the corresponding liver "glycerides". A marked tendency of liver phosphatides as compared with liver glycerides to contain acids of higher mol wt. is also shown.

Bromination studies established that linoleic acid is not present in detectable amount in cow and ox liver fatty acids respectively, although traces of this acid are found in the pig and sheep liver fatty acids. The presence of linolenic acid in sheep liver fatty acids is also recorded.

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