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The lipid composition of normal mouse liver*

GARY J. NELSON†

Donner Laboratory of Biophysics and Medical Physics,
University of California,
Berkeley 4, California

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SUMMARY

The lipid composition of normal Swiss laboratory mouse liver has been determined by chromatography and infrared spectrophotometry. The neutral lipids were mainly triglycerides (80%) with cholesterol, cholesterol ester, and an unidentified hydrocarbon also present. No free fatty acids were isolated. The phospholipids were largely lecithin (58%), with phosphatidyl ethanolamine, phosphatidyl serine, monophosphoinositol, sphingomyelin, and cardiolipin also present. A trace of lysolecithin was also detected, although it was impossible to determine whether or not it was an artifact of the isolation procedure.

The fatty acid composition of the isolated lipid fractions was determined by gas-liquid chromatography. The neutral lipids contained mainly palmitic, oleic, and linoleic acid, while the phospholipids contained large quantities of C_{20} and C_{22} polyunsaturated acids and considerably more stearic acid than the neutral lipids. The cardiolipin fraction had a high content of linoleic acid.

Mouse liver lipids have not been previously analyzed in detail with the chromatographic techniques now available. In older studies on the lipid composition of mouse liver, procedures were generally used that determined hydrolysis products from the various lipids and were not particularly sensitive to trace components (1–4). Lee *et al.* (5) and Beher and Anthony (6) have reported more recent values for the total cholesterol in mouse liver. In another recent study, Cornatzer and Reiter (7) have analyzed in detail the phospholipids of mouse liver by chromatography and also the incorporation of P^{32} into the individual phospholipids in mice of different ages. No reports on the fatty acid composition of the lipids in mouse liver could be found in the literature. In this study, gradient-elution silicic acid chromatography, thin-layer chromatography, infrared spectrophotometry, and gas-liquid chromatography were used to separate and analyze the lipids of the normal mouse liver.

EXPERIMENTAL PROCEDURE

Normal male Swiss albino laboratory mice, approximately four months old and in good nutritional balance, were used. They had been fed *ad libitum* on Simonsen's laboratory white diet[1] and water since weaning. The animals were sacrificed by neck fracture and the abdomens opened immediately. Two milliliters of physiological saline were injected into the portal vein after which the intact liver was removed and placed in 160 ml of methanol—chloroform 1:1 (v/v).[2] Livers from two animals were pooled to obtain sufficient material for analysis. The results presented here represent a detailed analysis on one such run. Other runs, performed in less detail, yielded similar results.

Extraction. The tissue was homogenized for 2 minutes in a Waring blendor. The homogenate was transferred to a 250-ml volumetric flask and brought to volume with chloroform, tightly stoppered, and heated for 15 minutes at 60°. After cooling to room temperature, the solution was filtered through fat-free filter paper into a 300-ml separatory funnel fitted with a Teflon stopcock, to which was added 50 ml of distilled water. The funnel was shaken vigorously for 5 minutes, and the phases were allowed to separate by standing overnight in a refrigerator at 0°. The lower phase was transferred to a rotary evaporator, and the solvent was

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† Postdoctoral Fellow of the National Heart Institute, U. S. Public Health Service.

[1] A natural diet supplied by Simonsen Laboratories, Inc., Gilroy, California.

[2] All solvents were reagent grade commercial solvents. The chloroform contained 0.5% to 1.0% ethanol as a preservative. In addition, the purity of all solvents was checked by recording infrared spectra of their nonvolatile residues. The solvents used in gas-liquid chromatography operations were all redistilled, and solvent blanks were carried through the entire sequence.

removed. The total lipid extract, after dessicating for 17 hours in the dark, was weighed and immediately chromatographed or stored at −4°.

Column Chromatography. The column packing was prepared as described earlier (8). The column, containing 20 g of silicic acid—Celite 2:1 (w/w), had a diameter of 20 mm and was 150 mm in height. The total lipid extract was dissolved in chloroform in a 5-ml volumetric flask, and an appropriate portion was pipetted on to the column. The charge was limited to approximately 7 mg of total lipid extract to each gram of column packing.

The charge was washed onto the column with two 2-ml washes of chloroform. The neutral lipid fraction was then eluted from the column with 1000 ml of chloroform and collected as a single batch. The effectiveness of this procedure has been discussed elsewhere (9). The phospholipids were then removed from the column by gradient elution using increasing concentrations of methanol in chloroform. The gradient was produced by a reservoir and mixing-chamber system fed by precision pumps. The mixing chamber fed the column directly by a pump operated at 1 ml per minute. In a typical run, 1000 ml of pure chloroform from the mixing chamber was pumped through the column to remove the neutral lipids. At this time, the pump connecting the methanol resevoir to the mixing chamber was activated, and 1000 ml of a methanol—chloroform gradient was pumped through the column. The methanol was added to the mixing chamber at a continually increasing rate producing a concave gradient shown in Figure 1. The details of this system will be described elsewhere.

The column effluent was collected by an automatic fraction collector coupled to an automatic valve (10), which cut 10-ml fractions during the gradient elution. The collection tubes were 16 x 150 mm screw-cap vials with Teflon-lined caps. The vials were capped as rapidly as possible after filling and stored at low temperature until further analysis.

Analytical Procedures. Chemical phosphorus determinations (11) were performed on the neutral lipid fraction and on 1-ml aliquots from each tube of the gradient elution. The resulting elution pattern is given in Figure 1. Infrared analyses were performed on the neutral lipid fraction and on each of the phospholipid fractions, divided on the basis of phosphorus analysis. A sample of the neutral lipid fraction was rechromatographed on silicic acid to determine the percentage of cholesterol ester, triglyceride, and free cholesterol present (12).

Thin-layer chromatography of the various phospholipid fractions was used to supplement the infrared

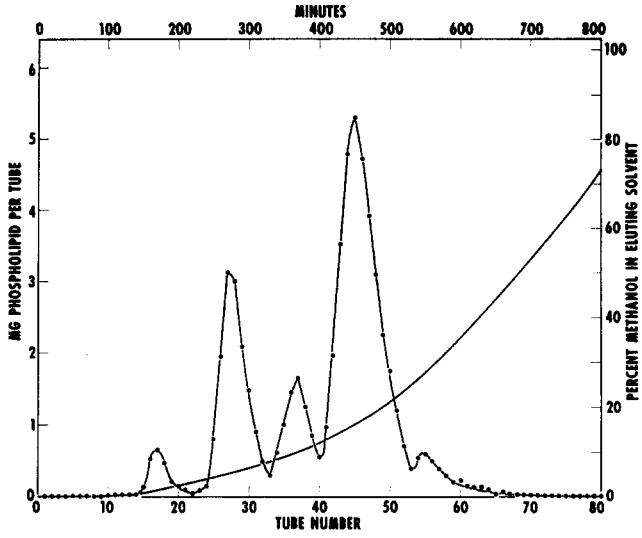

FIG. 1. A typical elution curve obtained by gradient elution of mouse liver phospholipids after the neutral lipids had been eluted from the column with 1000 ml of chloroform. Charge added to the column contained 2.36 mg of phosphorus and 143.4 mg of total lipid. Phosphorus recovery was 105% as determined by chemical means. The calculated concentration gradient of methanol in chloroform is shown. This column was partially deactivated to reduce the elution time of the phospholipids.

analyses. Approximately 25 μg of lipid was applied to the thin-layer plates prepared as described by Stahl (13). The developing solvent was a 1:1 (v/v) solution of methanol—chloroform with 2% water added. The chromatograms were run ascending for 1 hour at room temperature. The plates were then dried in a hood for a few minutes to remove the solvent, sprayed with concentrated H_2SO_4, and heated to locate the spots.

Spots were identified by running standards[3] on the same plate and also by using evidence obtained from the infrared analyses of the same material. The various phospholipids on the thin-layer chromatograms were not cleanly separated. Tailing of the major components may have obscured substances present in small amounts. Several different solvent systems were investigated but failed to give satisfactory resolution. Phosphatidyl ethanolamine tailed quite noticeably in all cases, and some material was usually left at the origin even with synthetic compounds.

Gas-Liquid Chromatography. One-milliliter aliquots from each collection tube in the region of each column peak were pooled for gas-liquid chromatographic analysis. If peaks overlap slightly, as they do in this

[3] Synthetic phosphatidyl ethanolamine and phosphatidyl choline were obtained from the California Corporation for Biochemical Research, Los Angeles, California. Monophosphoinositol and sphingomyelin were purified from liver by rechromatography. No cardiolipin, phosphatidyl serine, or lysolecithin standards were obtained.

TABLE 1. DATA ON COMPLETENESS OF LIPID EXTRACTION FROM MOUSE LIVER WITH METHANOL-CHLOROFORM

Fraction	Weight	Percentage Wet wt. of Liver
	mg	
1. Wet mouse liver	3801.0	
2. Methanol-chloroform extract	241.0	6.35
3. Chloroform wash of aqueous phase	1.32	0.03
4. Lyophilized aqueous phase	55.3	1.45
5. Chloroform-soluble material in aqueous phase	0.56	0.01
6. Methanol-soluble material in aqueous phase	50.3	1.33

TABLE 2. COMPOSITION OF NEUTRAL LIPID AND PHOSPHOLIPID FRACTIONS OF MOUSE LIVER

Neutral Lipid Fraction*	Percentage of Total Neutral Lipid Recovered
Hydrocarbon	3.56
Cholesterol esters	5.74
Glycerides	79.90
Free cholesterol	10.80
Phospholipids†	**Percentage of Total P Recovered**
Cardiolipin (?)	4.14
Phosphatidyl ethanolamine plus serine	22.84
Monophosphoinositol	11.39
Phosphatidyl choline plus lysolecithin	58.14
Sphingomyelin	3.50

* Weight of lipid added to first silicic acid column, 143.4 mg; recovered as neutral lipid, 48.3%.

† Weight of P added to second column, 2.36 mg; recovered as P, 105.0%.

analysis, pooling will result in more contamination than would be obtained from a selected single-peak sample. Pooling, however, is necessary because silicic acid column chromatography of phospholipids with mixed fatty acid composition tends to fractionate phospholipids according to their fatty acid composition (9). Thus, any selected sample from part of the elution peak of a particular phospholipid is unlikely to yield a fatty acid pattern representative of the fatty acid composition of the natural material.

The samples were subjected to transesterification in HCl—methanol as described by Stoffel *et al.* (14) or in H_2SO_4—methanol if sphingomyelin was present (9). The cross contamination from adjacent peaks was less than 5% in all cases except for the "sphingomyelin" sample which contained approximately 30% lecithin and/or lysolecithin.

Eighty micrograms of methyl esters were applied to the column operated as reported earlier (15). The gas-liquid chromatograph was designed and built in this laboratory and uses a beta-ray ionization detector designed by Lovelock (16). The calibration and standardization of this instrument have been reported in detail (17, 18).

RESULTS

The extraction method used in this study is essentially that of Folch *et al.* (19). Getz and Bartley (20) have indicated that, with liver preparations, less total lipid is extracted from the homogenate by methanol—chloroform than by ethanol—ether mixtures. However, they also found that ethanol—ether extraction would yield considerably more nonlipid material than methanol—chloroform. They showed that the lipid extractable with chloroform—methanol and the lipid left behind (extractable only with ethanol—ether) had the same fatty acid composition, suggesting that lipid extracted with chloroform—methanol is a satisfactory representative sample.

In Table 1, an analysis is given of the extracted material into lipid and nonlipid material. Marinetti *et al.* (21) have reported that lysolecithin is lost in aqueous washing of an organic solvent solution of lipid extracts, and Macfarlane (22) has indicated that monophosphoinositol may also be extracted into an aqueous wash. Thus, there was the possibility that lipid material was still retained in the aqueous phase after re-extraction with $CHCl_3$. To check this, the aqueous phase was lyophilized and the nonvolatile components were analyzed further. From the aqueous phase, 55.3 mg of material was recovered of which only 0.56 mg was soluble in chloroform. An additional 50.3 mg was soluble in methanol and had an infrared spectrum similar to glycogen. Getz and Bartley (20) have previously shown that a large part of the additional material extracted from the liver homogenate by ethanol—ether mixture was carbohydrate.

While some lipid was not extracted from the tissue, no exhaustive extraction was attempted. It was believed that oxidative and other deleterious effects to the lipids recovered in this manner would counteract any advantage gained by a more complete extraction.

Table 2 gives the results of silicic acid column chromatographic analysis of the lipid extract. The table presents results of infrared, gravimetric, and chemical phosphorus determinations.

The neutral lipids comprised 48.3% by weight of the lipids extracted and were largely glycerides (79.9%). The glycerides were not analyzed for mono- or di-acyl compounds, but the amount present was apparently quite small. Cholesterol esters and free cholesterol were present to the extent of 5.7% and 10.8%, respectively. An unidentified hydrocarbon component was present to the extent of 3.6%. No free fatty acid was observed, but an amount on the order of 1% would not have been detected with methods used in this work. The chemically determined phosphorus content of this material was less than 0.2%.

The phospholipids showed five distinct elution peaks. The percentage of eluted phosphorus in each peak is given in Table 2. The identification of the major components was made by infrared spectroscopy (8). The infrared spectrum of each peak is given in Figure 2. Trace components were determined by thin-layer chromatography. The material in the first peak could not be definitely identified, although its elution position on a silicic acid column corresponded to that of cardiolipin as reported by several workers. The phosphatidyl ethanolamine peak showed a slight contamination with monophosphoinositol and two unidentified components. The major contaminant is probably phosphatidyl serine, which has been found by other workers in all liver "cephalin" fractions. The remaining component could be either lysophosphatidyl serine or ethanolamine. The monophosphoinositol peak had a slight contamination of phosphatidyl ethanolamine and lecithin but no other observable components.

Tubes 41 to 55 in Figure 1, comprising the lecithin fraction, accounted for 58.1% of recovered phosphorus. The remaining tubes (56–80) contained 3.5%. Thus, 61.6% of total extracted phospholipids were choline-containing compounds. Traces of monophosphoinositol and sphingomyelin were found in this fraction.

The sphingomyelin content of the lipid extract was small. The material in collection tubes 56 to 80 was primarily sphingomyelin, with lecithin and/or lysolecithin in small amounts. Infrared analysis of the pooled material gave an estimate of 70% sphingomyelin. This value coupled with the estimated percentage of sphingomylein in tubes 41 to 55 gives approximately 3.5% sphingomyelin in the phospholipid fraction.

Table 3 presents the fatty acid composition of the total lipid extract of the liver and of the neutral lipid and cholesterol ester fractions separated by silicic acid chromatography. The neutral lipid fraction represents essentially the triglyceride fatty acid composition, as the amount of cholesterol ester present in this fraction was too small to influence the analysis. More polyunsaturated fatty acids are found in the cholesterol esters than in the triglycerides, although oleic and palmitic acids predominate in both compounds. The neutral lipids contain less C_{20} and C_{22} unsaturated fatty acid than do the phospholipids.

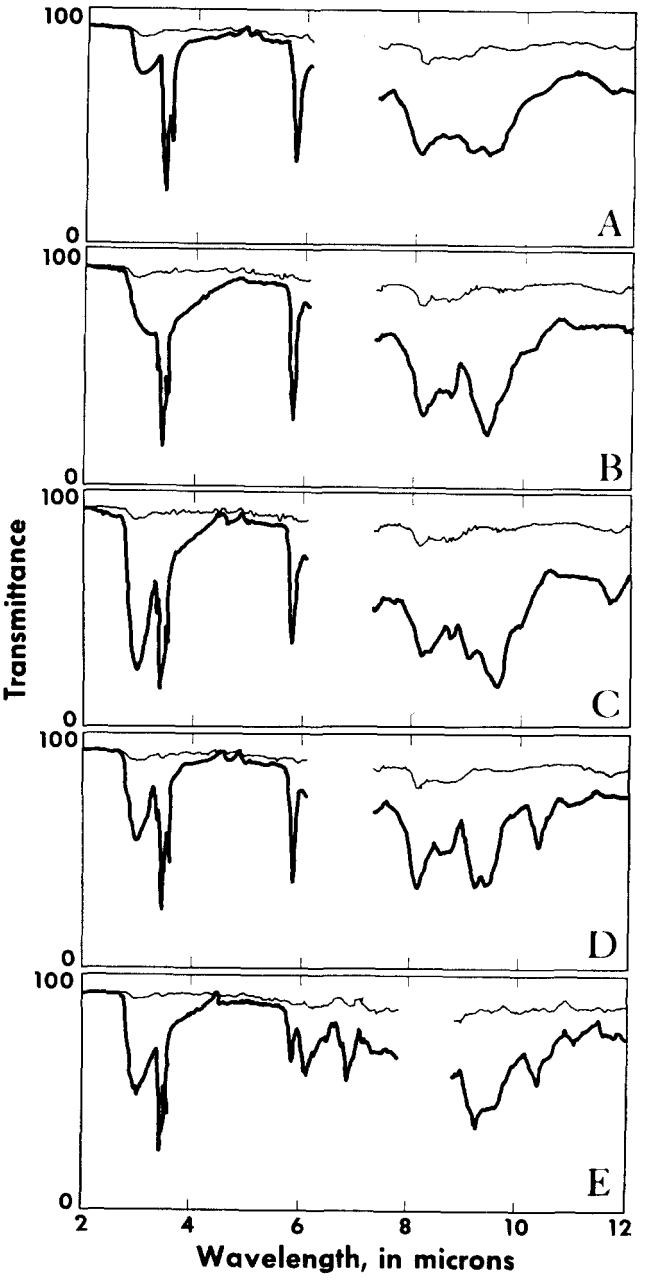

FIG. 2. The infrared spectra of the phospholipids isolated from mouse liver by the chromatographic separation shown in Figure 1. (A) "Cardiolipin" fraction, tubes 14–21; (B) phosphatidyl ethanolamine, tubes 22–33; (C) monophosphoinositol, tubes 34–40; (D) lecithin, tubes 41–55; (E) sphingomyelin, tubes 56–80. The sphingomyelin fraction is obviously contaminated as shown by the absorption at 5.8 μ. The curves represent solution spectra of samples A through D in carbon disulfide, E in chloroform, in the concentration range 5 to 7 mg per ml.

TABLE 3. The Fatty Acid Composition of the Total Lipids, Neutral Lipids, and Cholesterol Esters of Mouse Liver Determined by GLC

Fatty Acid	Retention Time Relative to Stearate	Methyl Esters as Percentage of Total Methyl Esters		
		Total Lipids	Neutral Lipids	Cholesterol Ester
		%	%	%
12:0	0.16	0.07	0.14	0.06
13:0	0.21	0.07		0.10
14:0	0.30	0.44	1.01	1.07
14:1	0.37	0.04		0.50
15:0	0.40	0.22	0.26	0.72
16:0	0.55	38.39	30.75	19.29
16:1	0.63	1.93	4.34	6.33
17:0	0.74	0.39	0.57	0.36
16:2	0.85	0.24	0.44	0.88
18:0	1.00	8.29	3.49	3.19
18:1	1.12	19.52	33.89	37.13
18:2	1.36	14.55	20.11	14.39
—*	1.80	0.30	0.53	0.82
20:0	2.03	0.42	0.58	0.42
20:2	2.45	0.33	0.22	0.39
20:3	2.76	0.91	0.66	0.34
20:4	3.08	8.42	1.61	6.47
22:4	5.55	0.12	0.30	1.20
24:0	6.30	0.31	0.50	1.87
24:1	6.83	0.27		
22:5	7.05	0.17	0.26	1.50
22:6	7.95	4.57	0.34	3.14

* Unidentified.

TABLE 4. The Fatty Acid Composition of the Phospholipids of Mouse Liver

Fatty Acid	Retention Time Relative to Stearate	Methyl Esters as Percentage of Total Methyl Esters			
		Cardiolipin (Tubes 14–21)	Phosphatidyl Ethanolamine* (Tubes 22–33)	Monophosphoinositol (Tubes 34–40)	Lecithin (Tubes 41–55)
		%	%	%	%
12:0	0.16	0.39	0.07	0.04	0.04
13:0	0.21	0.05			
—	0.25		0.08	0.43	0.03
14:0	0.30	0.14	0.21	0.43	0.12
14:1	0.37	0.10			
15:0	0.40	0.11	0.05	0.05	0.11
15:1	0.47	0.12		0.16	
16:0	0.55	3.49	5.82	17.82	30.72
16:1	0.63	1.56	0.61	0.55	0.67
17:0	0.74	2.84	0.36	0.31	0.37
16:2	0.85	0.19		0.12	0.13
18:0	1.00	1.45	40.54	14.78	1.73
18:1	1.12	9.33	4.65	7.79	8.18
18:2	1.36	69.94	12.46	6.02	15.89
19:0	1.57				0.22
—	1.80	0.42		0.17	
20:0	2.03	0.03		0.37	0.38
20:2	2.45	1.36	1.12	0.33	0.57
20:3	2.76	2.64	2.06	0.85	2.37
20:4	3.08	1.83	25.59	21.87	23.34
22:0	3.41	1.24			
22:1	3.80			0.28	0.31
22:4	5.55		0.75	0.71	0.92
24:0	6.30		0.52	0.80	
22:5	7.05			0.40	0.44
22:6	7.95	2.74	5.11	25.73	13.48

* Includes phosphatidyl serine.

Table 4 presents the fatty acid composition of the phospholipid fractions. The material contained in the four pooled samples from tubes 14–21, 22–33, 34–40, and 41–35 were found to contain 95% or more of their principle component by infrared spectral analysis, except that the ratio of phosphatidyl ethanolamine to phosphatidyl serine was not determined in the sample from tubes 22–33. The sphingomyelin samples contained as much as 50% methyl ester derived from lecithin and/or lysolecithin and so results for this fraction are not shown. The "cardiolipin" fraction, tubes 14 to 21, appeared homogeneous by thin-layer chromatography.

DISCUSSION

The older studies on mouse liver lipids by Hodge *et al.* (1, 3, 4) and MacLachlan *et al.* (2) agree quite well with this work for the major lipid classes. The value of 6.34% for total lipid is well within the range (5.8% to 7.7%) found by Hodge *et al.* (1) for normal nonfasting mice. MacLachlan *et al.* (2) obtained values ranging from 41.2% to 76.7% for the percentage of phospholipid in liver lipids in twenty normal mice with an average value of 62.2%. Hodge *et al.* (1) found that the average value for total cholesterol in mouse liver lipids was 6.2%, of which 20% was esterified. More recently, Lee *et al.* (5) found an average value of 2.92 mg per g wet liver which is close to the earlier value, although Beher and Anthony (6) reported somewhat higher values (5.0 to 7.4 mg per g wet liver). Neither of these latter studies estimated esterified and free cholesterol separately.

The glycerides in the earlier studies were estimated by differences. From the data of Hodge *et al.* and MacLachlan *et al.*, it can be estimated that 40% of lipid extracted from mouse liver was glyceride. Recent data on the glyceride content of mouse liver is lacking.

Having utilized the technique of Hanahan *et al.* (23), Cornatzer and Reiter (7) recently reported a fractionation of mouse liver phospholipids. Their results agree

generally with those reported here. They failed to report any polyglycerol phosphatides but claimed to have separated a phosphatidyl serine fraction. In the original procedure of Hanahan *et al.*, the serine and ethanolamine phospholipids were eluted as a single fraction. It is possible that the phosphatidyl serine fraction reported by Cornatzer and Reiter is actually polyglycerol phosphatide, and the phosphatidyl serine was included in the phosphatidyl ethanolamine fraction. They also found a higher percentage (7%) of sphingomyelin in the liver of their animal than was observed here. In addition, these authors investigated the incorporation of radioactive phosphorus into the different phospholipids of the mouse liver and found that the activity was distributed uniformly throughout the various phospholipids.

In other recent work, Getz *et al.* (24) reported values for rat liver phospholipids that are in close agreement to those reported here for the mouse. Getz and Bartley (20) and Macfarlane *et al.* (25) have also analyzed the subcellular components of rat liver and obtained generally similar patterns for the phospholipid composition.

The sphingomyelin content of liver of all species is conceded by all investigators to be low. Lysolecithin has not been reported in liver previously, and, while no distinct lysolecithin peak was observed in the column chromatography, infrared data and thin-layer chromatography indicated that some lysolecithin was present. It was not possible, however, to obtain an accurate value for the amount present or to determine if it was an artifact of the analytic procedure. Recently, Collins and Shotlander (26) were unable to detect any lysolecithin in rat liver by countercurrent distribution.

The fast-eluting material found in collection tubes 14 to 21, which is probably cardiolipin, has been analyzed by Getz and Bartley (20), Getz *et al.* (24) and Macfarlane *et al.* (25) in rat liver phospholipids and by Macfarlane (22) in ox liver preparations. Generally it accounted for 4% of the recovered phosphorus in the case of rat liver but only 1% to 2% in ox liver. Whether or not this material is actually a polyglycerol phospholipid has not been determined.

Recently, Rehnborg *et al.* (27) reported on the fatty acid composition of cholesterol esters, triglycerides, and phospholipids from mouse serum and serum lipoproteins. One major difference between mouse serum and liver is the higher percentage of C_{20} and C_{22} polyunsaturated fatty acids found in the liver phospholipids. The triglyceride fatty acid composition in liver and serum appears to be similar.

Another difference occurs in the cholesterol esters. Whereas Rehnborg *et al.* (27) found cholesterol esters of serum to contain mainly linoleic acid (50%) and arachidonic acid (30%), the liver cholesterol esters contained only 14% and 6% respectively of these fatty acids. Conversely, the oleic acid content of the liver cholesterol esters (37%) was six times that reported in mouse serum. This difference between liver and serum cholesterol ester fatty acid composition has been observed by Swell *et al.* in humans (28) and rats (29). It is particularly striking in the rat where 50% of the fatty acids in the circulating cholesterol esters are arachidonic, while the liver cholesterol esters contain only 2% of this particular fatty acid. These differences indicate that there is a preferential selection process among the cholesterol esters synthesized in the liver and those transferred to the circulation.

Getz *et al.* (24) reported more stearic acid in rat liver than was found here in mouse liver. Dittmer and Hanahan (30) also found a high stearate content in most of the individual phospholipids of rat liver, averaging about 25% for each class with a high of 39% in the monophosphoinositol. Getz *et al.* (24) also found 39% stearic acid in their monophosphoinositol fraction. Macfarlane (22) found even higher values for stearic acid in ox liver monophosphoinositol, 55% and 47% in two separate preparations. In recent studies of monophosphoinositols from various sources, Brockerhoff found 78%, 59%, and 53% stearic acid in beef liver, rat liver, and beef heart preparations, respectively (31). The value of only 15% stearic acid in mouse liver monophosphoinositol found in this study is in contrast to these findings.

REFERENCES

1. Hodge, H. C., P. L. MacLachlan, W. R. Bloor, C. A. Stoneburg, M. C. Oleson, and R. Whitehead. *J. Biol. Chem.* **139**: 897, 1941.
2. MacLachlan, P. L., H. C. Hodge, W. R. Bloor, E. A. Welch, F. L. Truax, and J. D. Taylor. *J. Biol. Chem.* **143**: 473, 1942.
3. Hodge, H. C., P. L. MacLachlan, W. R. Bloor, E. Welch, S. L. Kornberg, and M. Falkenheim. *Proc. Soc. Exptl. Biol. Med.* **67**: 137, 1948.
4. Hodge, H. C., P. L. MacLachlan, W. R. Bloor, E. Welch, S. L. Kornberg, and M. Falkenheim. *Proc. Soc. Exptl. Biol. Med.* **68**: 332, 1948.
5. Lee, C. C., R. G. Herrmann, and R. O. Froman. *Proc. Soc. Exptl. Biol. Med.* **102**: 542, 1959.
6. Beher, W. T., and W. L. Anthony. *Proc. Soc. Exptl. Biol. Med.* **99**: 356, 1958.
7. Cornatzer, W. E., and J. H. Reiter. *Proc. Soc. Exptl. Biol. Med.* **106**: 194, 1961.
8. Nelson, G. J., and N. K. Freeman. *J. Biol. Chem.* **234**: 1375, 1959.
9. Nelson, G. J. *J. Lipid Research* **3**: 71, 1962.
10. Nelson, G. J. *Anal. Chem.* **32**: 1724, 1960.
11. Griswold, B. L., F. L. Humoller, and A. R. McIntyre. *Anal. Chem.* **23**: 192, 1951.

12. Freeman, N. K., F. T. Lindgren, Y. C. Ng, and A. V. Nichols, *J. Biol. Chem.* **227:** 449, 1958.
13. Stahl, E. *Chemiker-Ztg.* **82:** 323, 1958.
14. Stoffel, W., F. Chu, and E. H. Ahrens, Jr. *Anal. Chem.* **31:** 307, 1959.
15. Nelson, G. J., and N. K. Freeman. *J. Biol. Chem.* **235:** 578, 1960.
16. Lovelock, J. E. *J. Chromatog.* **1:** 35, 1958.
17. Upham, F. T., F. T. Lindgren, and A. V. Nichols. *Anal. Chem.* **33:** 845, 1961.
18. Tandy, R. K., F. T. Lindgren, W. H. Martin, and R. D. Wills. *Anal. Chem.* **33:** 665, 1961.
19. Folch, J., M. Lees, and G. H. Sloane-Stanley. *J. Biol. Chem.* **226:** 497, 1957.
20. Getz, G. S., and W. Bartley. *Biochem. J.* **78:** 307, 1961.
21. Marinetti, G. V., M. Albrecht, T. Ford, and E. Stotz. *Biochim. et Biophys. Acta* **36:** 4, 1959.
22. Macfarlane, M. G. *Biochem. J.* **78:** 44, 1961.
23. Hanahan, D. J., J. C. Dittmer, and E. Warashina. *J. Biol. Chem.* **228:** 685, 1957.
24. Getz, G. S., W. Bartley, F. Stirpe, B. M. Notton, and A. Renshaw. *Biochem. J.* **80:** 176, 1961.
25. Macfarlane, M. G., G. M. Gray, and L. W. Wheeldon. *Biochem. J.* **77:** 626, 1960.
26. Collins, F. D., and V. L. Shotlander. *Biochem. J.* **79:** 321, 1961.
27. Rehnborg, C. S., A. V. Nichols, and J. K. Ashikawa. *Proc. Soc. Exptl. Biol. Med.* **106:** 547, 1961.
28. Swell, L., H. Field, Jr., P. E. Schools, Jr., and C. R. Treadwell. *Proc. Soc. Exptl. Biol. Med.* **103:** 651, 1960.
29. Swell, L., H. Field, Jr., and C. R. Treadwell. *Proc. Soc. Exptl. Biol. Med.* **104:** 325, 1960.
30. Dittmer, J. C., and D. J. Hanahan. *J. Biol. Chem.* **234:** 1976, 1959.
31. Brockerhoff, H. *Arch. Biochem. Biophys.* **93:** 641, 1961.