Holden, W. D., *ibid.*, 1948, v68, 511.
4. Wiseman, R., Jr., Baltz, B. E., *Endocrinology*, 1961, v68, 354.
5. Gregersen, M. I., Rawson, R. A., *Physiol. Rev.*, 1959, v39, 307.
6. Berlin, N. I., Huff, R. L., Van Dyke, D. C., Hennessy, T. G., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 176.
7. Kaliss, N., Pressman, D., *ibid.*, 1950, v75, 16.
8. Montgomery, P. O.'B., *ibid.*, 1951, v77, 445.
9. Masouredis, S. P., Melcher, L. R., *ibid.*, 1951, v78, 264.
10. Wish, L., Furth, J., Storey, R. H., *ibid.*, 1950, v74, 644.

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Microsomal Lipids of Various Tissues in the Rat.* (28782)

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Considerable data indicates the functional importance of lipid components of protoplasm in normal, physiological activities of cells and tissues (Fleischer and Klounen(1); Gray and MacFarlane(2); Johnson and Albert(3)). Believing that the fundamental lesion involved in the malfunction of organs and tissues must lie within the functional mechanisms of constituent cells, it is pertinent that a base line of lipid constituents in normal cells must be accurately defined by quantitative analysis.

The lipid composition of rat liver cell sap has been described by Getz(4). MacFarlane *et al*(5) have described the fatty acids of phospholipids from mitochondria and microsomes of rat liver. A study similar to that herein described has recently been published by Getz *et al*(6).

Materials and methods. Sprague-Dawley rats 300-400 g fed standard purina laboratory chow were fasted for 24 hours and sacrificed by decapitation. Liver was perfused *via* the dorsal aorta with Locke's solution and homogenized in cold, 0.25 M sucrose(7).

Cell debris, mitochondria and nuclei were separated from soluble phase proteins and microsomes by low temperature centrifugation at 11,000 × *g* for 15 minutes. Fatty layer on the surface of supernatant was aspirated and treated separately. Microsomes were isolated by centrifugation of remaining supernatant in the Spinco "L" at 105,000 × *g* for 1 hour, washed 3 times with 0.25 M sucrose, lyophilized and subsequently extracted.

Microsomes were extracted overnight in chloroform-methanol (2:1 v/v) and washed according to Folch *et al*(8). Total lipid/extract was determined gravimetrically and a sample (300-400 mg) diluted in 10 ml of hexane, placed on a 16 gram silicic acid (Unisil 200-325 mesh, Clarkson Chem. Co., Williamsport, Pa.) column apparatus described by Hirsh and Ahrens(9). Lipid classes were eluted in accordance with a scheme devised by E. C. Horning, modified by D. A. Turner, Nat. Inst. Health, Sinai Hospital, Baltimore, Md., shown in Table I. Only data on lipid classes and fatty acid constituents of liver microsomes is here reported. All solvents analytical reagent grade. Hexane was redistilled.

Eluate was monitored by phosphomolybdic acid, spot test and fractions pooled accordingly. Purity of fractions collected by column chromatography was checked in the following way. Each pooled fraction was analyzed quantitatively for cholesterol, ester linkages, glycerol and phosphorus by methods of Hanel and Dam(10), Snyder and Stephens(11), Lambert and Neish(12), and Bartlett(13) respectively. Di-, mono- and triglycerides were determined by calculation of glycerol ester ratios. Following purity check of all fractions, total mass of each was determined gravimetrically. Aliquots of each

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TABLE I. Elution Scheme for Silicic Acid Separation of Lipid Classes.

Methanol	Benzene	Hexane	Chloroform	Total vol	Lipid eluted
	6%	94%		100	Cholesterol esters
	18	82		150–180	" "
	60	40		300	Triglycerides
	100			100	Free chol. unest. FA's
			100	300	Diglycerides
2			98	200	Monoglycerides
5			95	100	"
10			90	200	"
25			75	200–300	Cephalins
38			62	350	p-inositol rich fraction
70			30	200	Lecithins
100				150	Sphingomyelin lysolecithin rich fraction

fraction were hydrolyzed in H_2SO_4 in the presence of dimethoxypropane at 65°C for 2 hours, (neutral lipids) and 4 hours, (phospholipids) to free subsequently methylated fatty acids. This is a method used by D. A. Turner at Sinai Hospital, Baltimore, Md.

Qualitative and quantitative analysis of fatty acid esters was achieved by gas liquid chromatography on a model "10," Barber Colman Company instrument. Standards were obtained from Applied Science Laboratories, State College, Pa.

Results. Results of total lipid analysis on 6 separate masses of liver microsomes and 2 masses each of brain, kidney and testes microsomes are shown in Tables II and III. The proportional amounts of lipid in microsomes appear to be quite uniform regardless of the tissue involved. Average percent lipid for liver masses was 17.5% ranging from 14.6% to 20.0%. Average for brain was 20.4% and for testes 16.9%. Only total lipid of microsomes from kidney was appreciably different, the level of 11.2% being the lowest percent lipid obtained.

Silicic acid separation of total lipid into various classes showed liver microsomal lipid to be made up of similar quantities of tri- and diglycerides with average percentages of 5.0% and 5.6% respectively. Unesterified fatty acids constituted 4.1% of the total lipid present.

The *in vitro* techniques of extraction, silicic acid chromatography and methylation minimize the possibility that portions of the unesterified fatty acids which appear in this fraction may represent fatty acids actually esterified *in vivo*. The major portion of cho-

TABLE II. Total Lipid Determination. Microsomal mass and total lipid in mg.

Tissue	Liver		Brain		Kidney		Testes	
Microsomal mass	2190	2372	30	60	120	340	170	115
Total lipid	340	475.2	6.25	12.0	12.5	40.8	30	18.7
% "	15.5	20	20.8	20	10.4	12.0	17.5	16.3

TABLE III. Total Lipid Determination and Percentages of Total Lipid in Various Lipid Classes in Liver Microsomes. Microsomal mass and total lipid in mg.

Microsomal mass	3561	4138	4309	4632	1160
Total lipid	578	762	864	790	166
% "	17	19	20	17	15
Cholesterol esters	13%	11%	13%	8%	11%
Triglyceride	3	5	9	2	6
Unesterified fatty acids	3	3	5	2	6
" cholesterol	—	4	3	2	2
Diglyceride	3	3	5	8	5
Monoglyceride	7	7	6	32	11
Cephalin	42	37	29	26	45
Inositol rich fraction	18	15	13	16	9
Lecithin	8	12	9	3	5
Sphingomyelin lyso.	3	4	9	2	2

TABLE IV. Fatty Acid Constituents Yielded by Hydrolysis of Lipid Moieties of Rat Liver Microsomes. Values represent percentages of total area under chromatogram peaks. Column characteristics: Glass, hairpin, 6', packed with ethylene glycol succinate on chromasorb "W," 60/80 mesh. Sample: 0.5 to 1 μl in hexane. Voltage 500, flow 15 psi input, column temp. 180°C, cell temp. 205°C, flash heater 225°C.

Fatty acids*	Chol. esters	Triglyc.	Unest. FA's	Diglyc.	Mono-glyc.	Cephalin	Inositol rich	Lecithin	Sphingo. & lyso.
14 Myristate	1.5	1.2	1.3	1.4					
14 16†	.9	1.5	1.5			.9	.6		
16 0 Palmitate	31.7	29.6	21.7	28.8	23.0	27.9	38.7	38.0	44.8
16 1 Palmitoleate	8.6	4.8	4.1	3.3	.5	4.4	2.1	2.1	3.0
16 18‡	1.1	1.5	3.2	5.7	.3	5.2	1.4	1.3	3.1
18 0 Stearate	10.7	13.1	14.0	26.8	35.7	27.5	38.2	30.1	39.9
18 1 Oleate	29.3	22.9	15.0	20.2	19.5	18.0	9.6	9.2	9.1
18 2 Linoleate	13.3	24.4	39.1	13.8	21.0	16.1	5.9	5.2	
18 3 Linolenate		1.0							
20 0 Arachidate	3.1							7.9	
20 4 Arachidonate									
20 4§							3.4	6.3	

* Fatty acids described by number of carbon atoms and number of double bonds.
† Peak material with carbon chain length greater than 14, less than 16. Degree of saturation unknown.
‡ Same as above except chain length greater than 16 and less than 18.
§ Same as above except chain length in excess of 20 C and 4 double bonds. Arachidonate standards were run.

lesterol was found to be esterified, with only 17% present in unesterified form.

While triglycerides of the glyceride group are present to the largest extent in most tissues these analyses show that in liver microsomes they are present in lowest quantities. No explanation can be given for the variation found in monoglycerides present. Range in the 5 analyses was from 7.2 to 31.6%. Whether these variations in monoglyceride levels reflect the metabolic state of the microsomes, or possible enzymatic degradation in the isolation procedure is unknown.

The most abundant lipid moiety was found in the phospholipids. The amino phosphatide group appeared in largest quantity constituting an average of 36% of the total lipid extracted from microsomes. The entire phospholipid portion represented 59% of the total lipid mass. The next highest percentage lipid was found in the inositol rich fraction averaging 14% of the total. Lecithin and sphingomyelin-lysolecithin fractions constituted 6% and 3% respectively.

Table IV indicates the fatty acid moieties, constituent parts of various lipids in microsomes. Standards of fatty acids with chain lengths 12 and below were chromatogrammed but not recorded since no fatty acids of these chain lengths resulted from hydrolysis and methylation of lipid fractions described.

It is interesting to note the maximum chain lengths found and identified to be 20 carbon, arachidate, and the shortest myristate with 14 carbons and 0 double bonds. In both cases they represent 1.0% and 1.8% of the total fatty acids present.

Palmitate appears as the most abundant fatty acid in any fraction, highest (44.8%) in the highly polar sphingomyelin and lysolecithin. Stearate, relatively low as esterified with cholesterol, is abundant in linkage with diglyceride and monoglyceride forms. Stearate constituents of cephalin molecules represent 27.5% of the total. Oleate appeared to be a major constituent of glycerides of all types and in ester linkage with cholesterol. Linoleate was found most abundant as free, unesterified fatty acid and linoleate was found (1.0%) as a small fraction of the total fatty acid makeup of triglycerides. Fatty acids with retention times in excess of those for arachidonate appeared only in the inositol and lecithin fractions.

Discussion. The results of this experiment on fasted animals are similar in some respects and dissimilar in others to those of Getz *et al* (6). Those workers, using fed animals, reported only trace amounts of fatty acids of chain lengths less than "16 0 br" the most abundant being 1.3% in the diglyceride fraction. They also reported considerably more

arachidonate than this investigation shows, particularly in the phospholipid groups where arachidonate made up 20 to 26% of the total fatty acids present. Another variable in addition to the fed condition of the animals, which may partially account for these observed differences includes the separation procedure used by Getz *et al,* which required separation of microsomes from a "mitochondrial pellet." The work here described involved a milder initial centrifugation separating mitochondria cell debris and nuclei from microsomes which remained in the supernatant. Subsequent high speed centrifugation (105,000 × *g*) pulled them out of suspension. The latter procedure does not involve the inclusion of mitochondria and a "fluffy" layer with the microsome separation.

Bucher *et al*(14) showed that both microsomes and soluble cell constituents are required for the biosynthesis of cholesterol *in vitro*. They also showed that the lipid rich membranous or vesicular structures, rather than the smallest granules of ribonucleoprotein particles appear to be the most probable locus of cholesterol forming enzymes. Neither the ability of microsomes, mitochondrial or other cell components to synthesize specific lipids, nor the ability of specific enzymes to incorporate specific or a variety of, fatty acids into phospholipid and/or glycerides has been thoroughly investigated. L. E. Hokin *et al*(15) demonstrated the presence of diglyceride kinase activity resulting in synthesis of phosphatidic acid in the microsomal fraction of rat liver cells, and presented a scheme for participation of phosphatidic acid in the transport of hydrophilic secretory materials across lipoid membranes. Seikovitz and Palade(16) proposed an hypothesis pointing to an important role for lipid components of cells in the process of secretion. In showing that the endoplasmic reticulum is concerned with the transport of newly formed enzymes from attached RNA particles across the lipoprotein membranes into the cisternal spaces bounded by the endoplasmic reticulum, they suggested an important role of phospholipids in the transport of enzymes across the membranes involved. The work of Redman and Hokin(17) points up the importance of phospholipid turnover in microsomal membranes in relation to the mechanism of pancreatic secretion.

1. Fleischer, S., Klounen, H., *Biochem. and Biophys. Res. Comm.,* 1962, v5, 378.
2. Gray, G. M., MacFarlane, M. J., *Biochem. J.,* 1961, v81, 480.
3. Johnson, R. M., Albert, S., *J. Biol. Chem.,* 1960, v235, 1299.
4. Getz, G. S., *Biochem. J.,* 1961, v81, 214.
5. MacFarlane, M. D., Gray, G. M., Wheeldon, L. W., *ibid.,* 1960, v74, 43.
6. Getz, G. S., Bartley, W., Stirpe, F., Notton, B. M., Renshaw, A., *ibid.,* 1962, v83, 181.
7. Anderson, N. G., *Exp. Cell. Res.,* 1955, v8, 91.
8. Folch, J., Lees, M., Sloane-Stanley, G. H., *J. Biol. Chem.,* 1957, v226, 497.
9. Hirsch, J., Ahrens, E. H., Jr., *ibid.,* 1958, v233, 311.
10. Hanel, H. K., Dam, H., *Acta Chem. Scand.,* 1955, v9, 677.
11. Snyder, F., Stephens, N., *Biochem. Biophys. Acta,* 1959, v34, 244.
12. Lambert, M. L., Neish, A. C., *Can. J. Res.,* 1956, v28, 83.
13. Bartlett, R. G., *J. Biol. Chem.,* 1959, v234, 466.
14. Bucher, N. L. R., McGarrahan, K., *ibid.,* 1956, v222, 1.
15. Hokin, L. E., Hokin, M. R., *ibid.,* 1959, v234, 1387.
16. Seikovitz, P., Palade, G. E., *J. Biophys. and Biochem. Cytol.,* 1959, v4, 557.
17. Redman, C. M., Hokin, L. E., *ibid.,* 1959, v6, 207.

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