they are marked as single peaks. Solid spheres in Figure 3 represent peaks which are not included in either pattern. Some or all of this unassigned absorption may be due to methylene vibrations, or it may be carboxylate vibrations as discussed at the end of the 1070–710 cm^{-1} section on saturated salts.

Unsaturated, Cis. Unsaturated salts have fewer peaks in this region than saturated salts of the same total chain length. See Figures 1 and 6, and Tables II and V. The similarity of oleate and palmitoleate spectra suggests that the number and the position of the peaks is related to the length of the carboxylate segment. The double bond must prevent coupling of the methylene vibrations in the two segments. The wagging vibrations which are parallel to the chain naturally will be more sensitive to bending of the backbone than the rocking vibrations which are perpendicular. The twisting vibrations will also be affected.

Figure 7B outlines the distribution pattern for the methylene wagging vibrations in sodium salts of the *cis*-unsaturated acids. The silver salts have peaks at all the same positions, plus others whose positions and strengths are satisfactory for assignment to twisting-rocking vibrations. This is additional support for considering all of these peaks as caused by vibrations of the carboxylate methylenes. Some of the unassigned peaks may represent twisting-rocking vibrations of the carboxylate segment, or wagging vibrations of the methyl segment, but they are only weak peaks.

Assignments for the *cis*-6-C_{18} salts are the most uncertain, especially the peaks about 1320 and 1330 cm^{-1}. The former is the stronger in the sodium salt and the latter the stronger in the silver salt; thus the 1320 cm^{-1} peak is assigned to a wagging vibration although it does not fit the phase relationship curve so well as the other point. No assignment is made for the 1330 cm^{-1} peak.

Figure 11 shows the phase relationship curves for the *cis*-unsaturated silver salts. Values of m are calculated from the number of carbons in the carboxylate segment minus two, as in the low wavenumber region. Values of k are assigned according to the distribution pattern for saturated salts, since peak separations are the same for corresponding values of m. Table V lists the resultant values of ϕ/π. The phase relationship curves for the unsaturated salts agree quite well with those of the saturated salts, except for a shift of about 10 cm^{-1} on the wavenumber scale. Thus the saturated phase relationship curves may be used for the determination of unsaturated carboxylate segment chain lengths if the shift is taken into account.

Unsaturated, Trans. The *trans* salt spectrum in Figure 6 has the same number of peaks as the *cis* salt spectrum, so this region gives vibrations of the carboxylate segment methylenes. See also Tables V and VIb. In all respects in this region, elaidate spectra agree with those of oleate salts. Silver elaidate has peaks assigned to twisting-rocking and wagging vibrations, while the sodium salt has only the wagging peaks, etc. Figure 11 shows the close correspondence of the phase relationship curves for the silver salts.

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Glyceride Distribution in Adipose and Liver Glycerides of Animals[1]

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Abstract

The glyceride distribution in depot fats from a series of animals was determined by pancreatic lipase hydrolysis, isolation of hydrolytic products by thin-layer chromatography (TLC), and fatty acid analysis by gas-liquid chromatography (GLC).

Distribution of the principal types of glycerides (S_3, S_2U, SU_2 U_3) in the internal and external adipose tissue fats from the same pig was nonrandom. The percentages of palmitic acid at the 2-position in these adipose fats were comparable. However, liver glycerides from this same animal differed strikingly from adipose glycerides, having, for example, only ca. 15% of its palmitic acid in the 2-position compared with $> 80\%$ for adipose fats. The liver glycerides of lamb, rabbit, and dog also differed considerably from adipose glycerides in glyceride distribution and in percentages of individual fatty

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acids in 2-position.

The composition of adipose glycerides from lamb, beef, deer, rabbit, chicken, and dog in terms of the four principal glyceride types approached closely the values calculated for random. When positional isomers were considered, however, only the adipose glycerides of the dog conformed to random distribution.

Introduction

DEVELOPMENT OF NEW TECHNIQUES in fat analyses, including hydrolysis of glycerides with pancreatic lipase as a means for determining the composition of acids esterified on the 2-position of the glycerides, has made possible more comprehensive studies on the glyceride distribution in animal fats (1–3). In a recent publication from this laboratory (4) it was demonstrated that the glyceride distribution could be obtained by these techniques with only 5–50 mg of sample. In the present investigation this semimicro technique was employed to determine the composition of the fatty acids in 2-position of glycerides from adipose and liver tissues of several animals. The glyceride distributions in these animal fats are also discussed.

No attempt was made here to discusss the history, development, and applications of the lipase method, since these have been reviewed recently (5–7).

Experimental

Fresh adipose and liver tissues were obtained from a number of animals and kept frozen until extraction of fat could be undertaken. Adipose tissue from animals other than pig was mainly internal tissue.

Extraction of Fat. Ten grams finely-minced tissue were extracted by triturating in a mortar with successive 200 ml portions of acetone, Delsal solvent (3:1 Methylal-Methanol) and ethyl ether. After removing the solvent from the combined decanted extracts the crude residue was thoroughly extracted with Skellysolve F, and the proteinaceous fines were removed by centrifugation. The solvent was removed from the extracts and the residue brought to constant weight.

Treatment of Adipose Fat. The extracted adipose fats were subjected to column chromatographic treatment to remove traces of free fatty acids, unsaponifiables, and phospholipids. The apparatus and method of packing column have been described in another publication (8). Approximately 400 mg fat were fractionated on a 30 g Silicic Acid – Supercel (80/20) column. The fractions containing only triglycerides, as judged by showing a single spot when examined by TLC, were combined. Usually, 550 ml Skellysolve F containing 4% ethyl ether was required to elute the triglycerides. The yields of triglycerides were ca. 95% of the weight of adipose fat placed on column.

Treatment of Liver Lipids. It was found convenient to employ preparative TLC to isolate triglycerides from liver lipids. The method was similar to that employed for the isolation of lipase hydrolysis products and has been described (4). Approx 25 mg lipid were chromatographed on each of four Silica Gel G plates. The developing solvent was a mixture of Skellysolve F – ethyl ether (85:15) containing 1% acetic acid. The pig and rabbit liver lipids contained 25% triglycerides, the dog about 15%.

Lamb liver lipids contained only ca. 6% triglycerides. Hence, it was necessary to fractionate the lipids first on a silicic acid column (8), then to purify the glyceride fraction by preparative TLC.

TABLE I

Composition of Monoglycerides from Lipase Hydrolysis of Pig Adipose and Liver Glycerides

		Fatty acid composition, Mol %							
		<16:0[a]	16:0	18:0[b]	16:1	18:1	18:2[c]	S[d]	U[e]
External	Triglyc.	1.5	26.2	9.4	3.5	45.8	13.6	37.1	62.9
	Monoglyc.	3.8	68.9	2.2	6.6	14.8	3.7	74.9	25.1
	% in 2 Pos.	84.4	87.7	8.1	62.9	10.7	9.7	67.3	13.3
Internal	Triglyc.	2.1	30.4	15.7	2.2	39.3	10.3	48.2	51.8
	Monoglyc.	4.4	76.5	3.2	3.1	10.2	2.6	84.1	15.9
	% in 2 Pos.	69.8	83.9	6.8	46.9	8.7	8.4	58.2	10.2
Liver	Triglyc.	2.2	31.7	7.3	4.1	35.5	19.2	41.2	58.8
	Monoglyc.	1.5	14.2	0.9	3.9	51.4	28.1	16.6	83.4
	% in 2 Pos.	22.7	14.9	4.1	31.7	48.3	48.7	13.4	47.3

[a] Predominantly 14:0
[b] Includes trace amts. 17:0
[c] Includes < 1% 18:3
[d] Total saturated
[e] Total unsaturated

Enzymatic Hydrolysis. The procedures employed for hydrolysis, isolation of hydrolytic products, conversion of monoglycerides and fatty acids to methyl esters, along with conditions for GLC analysis of the latter have been described in a recent publication (4). Fifty-milligram samples of adipose glycerides and 5 mg samples of liver glycerides were employed in the lipase hydrolysis.

Owing to the multiplicity of fatty acids in lamb adipose and liver glycerides, it was necessary to employ both polar and nonpolar columns in GLC analysis. The polar column was an 8 ft × 3/16 in. O.D. (I.D. = 0.118 in.) stainless steel coiled tube packed with 42–60 mesh acid and base washed Chromosorb W coated with 25% ethylene glycol succinate polyester. The nonpolar column was a 2 ft × 3/16 in. (I.D. = 0.124 in.) stainless steel tube packed with 42–60 mesh acid and base washed Chromosorb W coated with 15% silicone polymer SE-30 (General Electric).

Calculation of Glyceride Composition. The mol % of each component fatty acid of the fat which is esterified in the 2-position was determined as described by Mattson (3) (mol % in 2-position = mol % in monoglyceride/3X mol % in triglyceride).

The values determined experimentally for the acids released by the lipase were in good agreement with the calculated values based on analyses of the monoglycerides and original triglycerides. The average of these values for 1,3 fatty acids were used in the calculations of glyceride distribution (1), the individual acids being grouped as saturated (S) and unsaturated (U). Random glyceride distribution for each fat was calculated from percent saturated and percent unsaturated in the triglyceride as described by Vander Wal (9).

Results and Discussion

The results (Table I) show that the external and internal adipose glycerides differ appreciably in

TABLE II

Glyceride Distribution of Pig Liver and Depot Fats

		Glyceride type				Isomeric forms			
		S_3 %	S_2U %	SU_2 %	U_3 %	SUS %	SSU %	USU %	SUU %
External	F	2.4	23.0	57.7	16.9	0.8	22.2	50.3	7.4
	R	5.1	26.0	44.0	24.9	8.7	17.3	14.7	29.3
Internal	F	7.7	36.8	47.8	7.7	1.4	35.4	41.0	6.8
	R	11.7	36.1	38.3	13.9	12.0	24.1	12.8	25.5
Liver	F	4.9	32.5	44.9	17.7	24.3	8.2	3.5	41.4
	R	7.0	29.9	42.8	20.3	9.9	20.0	14.2	28.6

F = From values found for 2-position acids (1).
R = Random calc. (9).

TABLE III

Composition of 2-Position Acids of Some Animal Fats

Animal	Glyc. Source		Fatty acids,[a] Mol %							
			<16:0	16:0	18:0	16:1	18:1	18:2	S	U
Lamb	Adipose[b]	TG	4.4	21.0	31.7	2.9	35.5	4.5	57.1	42.9
		MG	6.7	13.1	14.9	3.4	54.9	7.0	34.7	65.3
		%2	50.7	21.1	15.7	39.0	51.5	51.9	20.0	51.3
	Liver[c]	TG	11.7	21.6	29.1	4.2	25.0	5.7	65.1	34.9
		MG	11.9	26.3	15.0	7.1	32.1	7.6	53.2	46.8
		%2	33.9	40.5	17.2	56.3	42.8	44.4	27.2	44.6
Dog	Adipose	TG	2.7	22.5	9.0	3.9	51.8	10.1	34.2	65.8
		MG	6.0	25.1	3.2	6.3	45.9	13.6	34.3	65.7
		%2	74.1	37.2	11.9	53.8	29.5	44.8	33.3	33.3
	Liver	TG	2.3	28.4	13.0	3.2	42.4	10.7	43.7	56.3
		MG	2.3	18.5	2.8	5.2	47.0	24.2	23.6	76.4
		%2	33.4	21.7	7.2	54.1	36.9	75.3	18.0	58.3
Rabbit	Adipose[d]	TG	6.8	32.2	5.3	4.8	28.0	19.2	44.3	55.7
		MG	7.0	26.0	0.7	6.6	34.6	21.4	33.7	66.3
		%2	34.3	26.9	4.4	45.8	41.1	41.2	25.4	39.7
	Liver[e]	TG	3.7	40.1	5.7	3.3	28.1	16.9	49.5	50.5
		MG	1.7	13.6	0.8	4.2	43.3	33.7	16.1	83.9
		%2	15.3	11.3	4.7	42.4	51.3	66.5	10.8	55.4
Beef	Adipose	TG	6.3	26.5	24.4	3.3	37.4	2.1	57.2	42.8
		MG	10.6	14.0	12.5	5.7	53.9	3.3	37.1	62.9
		%2	56.1	17.6	17.1	57.6	47.9	54.8	21.6	50.0
Chicken	Adipose	TG	1.0	26.7	4.9	6.9	46.1	14.4	32.6	67.4
		MG	0.8	12.7	4.6	4.8	58.3	18.8	18.1	81.9
		%2	25.9	15.9	31.6	22.9	42.0	43.5	18.5	40.5
Deer	Adipose	TG	2.9	23.6	32.8	3.7	35.9	1.1	59.3	40.7
		MG	4.3	16.0	13.8	4.1	59.5	2.3	34.1	65.9
		%2	50.1	22.6	14.0	37.4	55.6	68.4	19.2	54.0

[a] Trace components treated as in Table I.
[b] <16:0 also includes 14:Br and 15:Br; 18:0 also includes 17:Br and trace of an unknown acid (possibly multibranched).
[c] Acids combined as in b except for 2.7% of an unknown acid (possibly multibranched) in TG, which was not included with 18:0. This acid was not found in MG.
[d] TG contained 3.8% 18:3, MG 3.7% 18:3 not included with 18:2.
[e] TG contained 2.2% 18:3, MG 2.7% 18:3 not included with 18:2.

fatty acid composition, the latter containing more saturated acids but with a lesser proportion of these in 2-position. In general, the data show the same trend reported previously (4) for commercial lard which is a mixture of internal and external fat. More than 80% of the palmitic but only about 8% to 11% each of stearic, oleic, and linoleic acids occurred in 2-position. Strikingly different results, however, were obtained on liver glycerides from the same pig, where only about 15% of the palmitic but nearly 50% each of oleic and linoleic acids occurred in 2-position. The liver glycerides also contained considerably more linoleic and less oleic acid than the adipose fats. Similar results were obtained on glycerides of liver from a different pig.

This difference between pig adipose and liver glycerides could also be seen in terms of glyceride distribution calculated from lipase hydrolysis data (Table II). The external and internal adipose glycerides exhibited a nonrandom pattern, whereas a random distribution of liver glycerides was approached when only the four principal glyceride types were considered. When the amts of isomers were examined, the liver glycerides had greater proportions of symmetrical disaturated and unsymmetrical monosaturated glycerides than calculated for random distribution, while the adipose glycerides had less than random proportions of these isomers.

The dissimilarity in composition of 2-position acids from adipose and liver fats was not unique in the pig, but was also shown in the lamb, rabbit, and strikingly in the dog fats. In the latter, 33-1/3% of total saturated or unsaturated fatty acids of the adipose glycerides were in the 2-position, i.e., they were distributed randomly. The saturated and unsaturated acids of dog liver glycerides, however, were not distributed randomly. The 2-position acid compositions of these glycerides together with those of beef, chicken, and deer adipose glycerides show in Table III. Table IV gives the glycerides distribution in these fats.

TABLE IV

Glyceride Distribution of Some Animal Fats

Animal	Glyceride source		Glyceride types				Isomeric forms			
			S_3 %	S_2U %	SU_2 %	U_3 %	SUS %	SSU %	USU %	SUU %
Lamb	Adipose	F	16.6	46.2	31.1	6.1	31.4	14.8	3.3	27.8
		R	18.6	42.0	31.5	7.9	14.0	28.0	10.5	20.0
	Liver	F	25.3	45.0	25.2	4.5	22.2	22.8	5.1	20.1
		R	27.6	44.3	23.8	4.3	14.8	29.6	7.9	15.9
Dog	Adipose	F	3.9	22.7	44.4	29.0	7.4	15.3	15.1	29.3
		R	4.0	23.1	44.5	28.4	7.7	15.4	14.8	29.7
	Liver	F	6.9	34.2	42.9	16.0	22.4	11.8	4.9	38.0
		R	8.4	32.2	41.6	17.8	10.8	21.5	13.9	27.7
Rabbit	Adipose	F	8.2	32.8	41.9	17.1	16.0	16.8	8.7	33.2
		R	8.7	32.8	41.2	17.3	10.9	21.9	13.7	27.5
	Liver	F	6.4	40.7	41.4	11.5	33.2	7.5	2.2	39.2
		R	12.1	37.1	37.8	12.9	12.4	24.7	12.6	25.2
Beef	Adipose	F	16.6	44.4	32.1	6.9	28.0	16.4	4.1	28.0
		R	18.7	42.0	31.4	7.8	14.0	28.0	10.5	20.9
Chicken	Adipose	F	2.9	21.5	45.8	29.8	12.9	8.6	6.6	39.2
		R	3.4	21.5	44.4	30.6	7.2	14.3	14.8	29.5
Deer	Adipose	F	17.6	47.8	29.4	5.2	34.0	13.8	2.7	26.7
		R	21.1	43.0	29.3	6.6	14.3	28.7	9.8	19.5

F = From values found for 2-position acids (1).
R = Random calc. (9).

Lamb liver triglycerides contained an acid which, from its behavior on both polar and nonpolar GLC columns, appeared to be multibranched (10). Neither the monoglycerides nor the fatty acids from the lipase hydrolysis of lamb glycerides contained this acid. However, the diglycerides contained appreciable amts of it. Therefore, the multibranched acid was assumed to have been esterified on one of the terminal positions and was resistant to hydrolysis by lipase. It has been reported that branching of the aliphatic chain in the vicinity of the carboxyl group hinders lipase action (5).

Perkins, in a recent report (11), stated that rat carcass fat might be designated as a randomly distributed fat if only the four glyceride classes were compared with random values. However, when the amts of isomers were considered, deviation from random was noted. Similar observations were made during the present study. Of all fats examined, only pig adipose fat showed a clearly nonrandom distribution in terms of the principal glyceride classes. However, only dog perinephric fat would be classified as being randomly distributed after the proportions of isomers were compared to random.

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