differs somewhat from that reported by Harman *et al.* (4), particularly in the ratio of the absorbance at the maximum (244 or 245 mμ) to the absorbance at the inflection at 238 or 240 mμ.

Discussion

The toxic substance which we have isolated from triolein resembles that recovered by Harman *et al.* (4) from animal feed tallows. However the divergences in their properties suggest either that we are dealing with two different but closely related compounds, or that one or both of the preparations is still a mixture of related compounds despite the fact that only a single spot could be obtained on paper chromatography in a number of solvent systems. In view of the manifest difficulties involved in isolating a pure compound in minute quantities from a myriad of substances with similar properties it would be hazardous, as Harman warns, to infer chemical structures from spectral data. Nevertheless it should be pointed out that the spectra obtained by us and by Harman *et al.* are strongly reminiscent of those exhibited by highly substituted naphthalenes (7). Furthermore the toxic factor occurs in association with a bewildering array of aromatic naphthalene and phenanthrene derivatives, as we have previously noted (2). The detection of chlorine in large proportions in a toxic preparation, and the ultraviolet spectrum observed, suggest a possible relationship with chlorinated naphthalenes. Pentachloronaphthalene possesses an absorption maximum at 243 mμ and a secondary maximum at 312 mμ (8), and it has been shown to cause hyperkeratosis in cattle (9) and several other species of animals including chickens (10). Other chlorinated naphthalenes also are toxic (11).

The possibility that the chick edema factor is a chlorinated naphthalene derivative cannot be ignored. Samples of tetrachloronaphthalene and hexachloronaphthalene, kindly provided by Engel and Bell of the Virginia Polytechnic Institute, who had demonstrated that these compounds could produce hyperkeratosis in cattle, were without effect in the chick edema test. Furthermore these compounds, despite the similarity of their ultraviolet spectra and their chromatographic behavior to the toxic substance, showed considerable difference in the microcoulometric gas chromatograph. For instance, the chlorinated pesticides, aldrin and heptachlor, showed retention times of 10 min., tetrachloronaphthelene 9 min., and hexachloronaphthalene 14 min., whereas the toxic substance, as well as its inactive analogue with the absorption maximum at 248 mμ, had retention times of 37–38 min. It is tempting to speculate that the greater retention-time of the toxic material is related to a greater molecular weight or to a substituent conferring different solubility and polarity properties.

We are continuing our studies toward the isolation of the toxic factor. It is necessary that the chemical nature of this substance be elucidated to make possible a rapid chemical test for its detection, to clarify its origin, to verify the suggestion of its severe toxicity to primates, and to study its action in other species.

Acknowledgment

Numerous individuals in addition to those mentioned in the text have contributed directly or indirectly to this project. We are particularly grateful to O.L. Kline for his sustained interest, encouragement, and helpfulness in the course of these investigations; to Benjamin Webb, who conducted the extensive molecular distillations; to Donald F. Flick and Linda Gallo for the chick bioassays; and to Raymond J. Gajan for the microcoulometric gas chromatography. The cooperation of the various commercial laboratories that were also working on this problem, in discussing their work with us, is also greatly appreciated.

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[Received October 17, 1960]

Determination of the Glyceride Structure of Fats[1,2]

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A method has been described for the quantitative determination of the following six glyceride types in fats: SSS, SSU, SUS, SUU, USU, and UUU. The method involved a quantitative oxidation of the unsaturated acids in the whole fat to the corresponding dicarboxylic acids. The oxidized fat was separated on a liquid-liquid partition column into two fractions, the first containing glycerides having no dicarboxylic acid or one dicarboxylic acid and the second containing glycerides with two or three dicarboxylic acids. Analysis of these fractions by gas chromatography coupled with lipase hydrolysis allowed the calculation of the proportions of the above six glyceride types.

The oxidation, fractionation, lipase hydrolysis, gas chromatographic analysis, and the over-all method were checked on natural fats and mixtures of synthetic glycerides. The final glyceride composition appeared to be reliable to within plus or minus 2 unit per cent.

Analyses are given for five natural fats. The compositions found agree very well with those calculated by a distribution theory recently proposed by Vander Wal.

[1] Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan.
[2] Issued as N.R.C. No. 6161.

A SMALL NUMBER of fatty acids gives rise to a comparatively large number of combinations as glycerides, and there is little difference in physical properties between adjacent glycerides of the series. As a result, the quantitative separation of the individual glycerides is extremely difficult, if not impossible. The problem can be simplified, at the expense of obtaining less information, by an oxidative cleavage of the unsaturated acids to form a free acid group at the position of the double bond. If the only distinction then made is between mono- and dicarboxylic acids, corresponding to saturated and unsaturated acids, the number of combinations as glycerides is limited to the following six: SSS, SSU, SUS, SUU, USU, and UUU. No distinction is made between the optical isomers. The oxidative cleavage also has the advantage of amplifying the difference between the various glyceride types, giving molecules with zero to three free acid groups corresponding to the four glyceride types S_3, S_2U, SU_2, and U_3.

This general method was first used by Hilditch and Lea (1), who determined the amount of fully saturated glycerides, S_3, as the neutral fraction recovered after oxidation. Kartha (2,3) in a later study showed that the oxidation with potassium permanganate in acetone, used by Hilditch and Lea, resulted in partial hydrolysis of the glycerides. He avoided this hydrolysis by the addition of acetic acid to the acetone and extended the method by fractionating the oxidized glycerides as their magnesium salts. Since neither the oxidation nor fractionation were entirely quantitative, the accuracy of the results is difficult to assess.

A number of techniques have been developed recently which make possible further improvement and extension of the above general method. von Rudloff (4) has demonstrated the quantitative oxidation of unsaturated acids in glycerides, using a mixed oxidant of periodate and permanganate in a medium containing organic solvents. Gas chromatography (5, 6,7) has made possible the quantitative analysis of fatty acid mixtures on a micro scale. Mattson and Beck (8) and Savary and Desnuelle (9) have demonstrated the specific hydrolysis of the 1 and 3 positions in glycerides by pancreatic lipase. A combination of these techniques with an efficient fractionation of the oxidized glycerides should allow an accurate determination of the six glyceride types, SSS, SSU, SUS, SUU, USU, and UUU. There are however a number of points which should be checked. The oxidation must be quantitative and must not result in any appreciable hydrolysis or ester interchange of the glycerides. The fractionation of the oxidized glycerides must be sharp and clear-cut. The analysis of the fractions must be reliable, and the lipase hydrolysis must not make any distinction between mono- and dicarboxylic acids in the 1 and 3 positions.

Since gas chromatography was to be the basis of all fatty acid analysis, it was the starting point in the present investigation. A liquid phase of butanediol-succinate polyester was found to give a good separation of both saturated and unsaturated methyl esters and the saturated and dicarboxylic methyl esters. Craig and Murty (7) have shown that the response of a "hot wire" detector is on a weight basis for the normal monocarboxylic fatty acid esters, with an average standard deviation of 0.5 unit per cent. The response for the dicarboxylic esters was found to be slightly lower than for the monocarboxylic esters, and a factor was introduced to offset this.

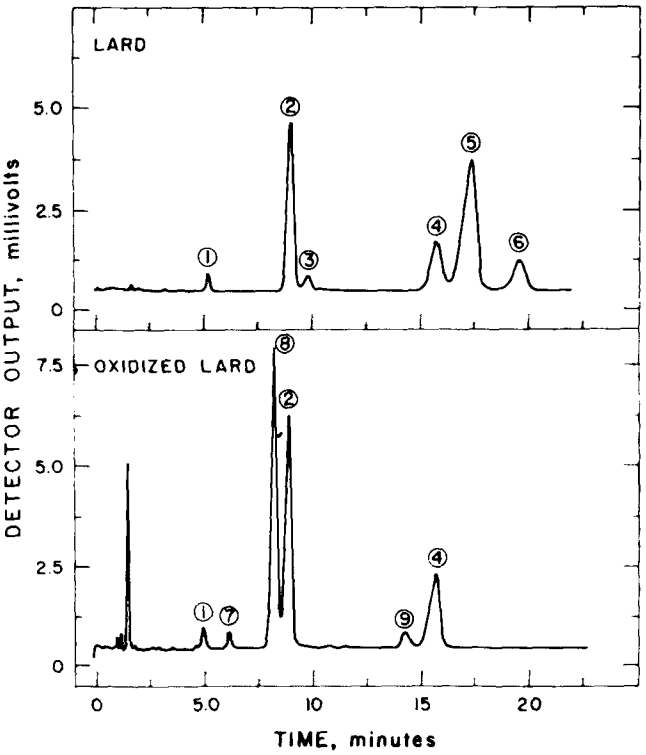

FIG. 1. Gas chromatographic charts for original and oxidized lard:

Peak No.	Acid	Peak No.	Acid
1	Myristic	6	Linoleic
2	Palmitic	7	C_8 dicarboxylic
3	Palmitoleic	8	C_9 dicarboxylic
4	Stearic	9	C_{11} dicarboxylic
5	Oleic		

The oxidation procedure was a slightly-modified form of that described by von Rudloff (4), employing 60% tertiary butanol and 40% water as solvent. Gas chromatography of the methyl esters prepared from a number of fats before and after oxidation of the fats demonstrated that quantitative oxidation was obtained. Typical gas chromatographic charts before and after oxidation are shown in Figure 1, and results for a number of fats are summarized in Table I. Peaks representing the unsaturated acids disappeared completely on oxidation, and dicarboxylic acids were formed in corresponding molar amounts.

TABLE I

Periodate-Permanganate Oxidation of Fats

Fat	Before oxidation		After oxidation	
	Saturated acids	Unsaturated acids	Saturated acids	Dicarboxylic acids
	mol %	*mol %*	*mol %*	*mol %*
Lard	45.9	54.1	45.3	54.7
Chicken	31.3	68.7	31.8	68.2
Rat	19.7	80.3	19.6	80.4
Linseed	7.8	92.2	8.6	91.4
Cocoa butter	40.1	59.9	40.6	59.3

Possible hydrolysis of the glycerides during oxidation was also checked by using gas chromatography. A known proportion of an acid, such as lauric, not present in a fat, was added to a sample of the oxidized fat, and the mixture was treated with diazomethane. The mixture was run on the chromatograph under conditions such that only the methyl esters

formed from free acids came through the column. Comparison of the peaks obtained with the peak for lauric acid showed less than 1% free acids in the oxidized sample, excluding the short-chain monocarboxylic acids formed on oxidation.

The next point considered was the fractionation of the oxidized glycerides. Initially countercurrent distribution was used. A suitable solvent pair was found in 90% ethanol-10% water and Skellysolve "B."[3] A 90-transfer-distribution of oxidized cocoa butter is shown in Figure 2. The short-chain monocarboxylic

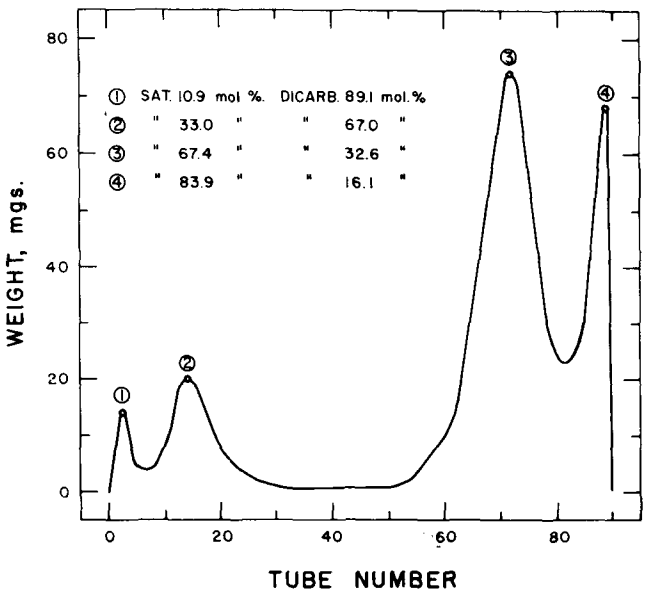

FIG. 2. Countercurrent distribution of oxidized cocoa butter.

acids formed on oxidation had been removed from the sample by placing it in a vacuum oven over-night. Four peaks were obtained corresponding to the U_3, SU_2, S_2U, and S_3 fractions in the original fat. There is a good separation of the U_3 and SU_2 from the S_2U and S_3 with a partial separation within these two groups. Separation into the two groups is however all that is required for the analysis. The first group would contain the three glyceride types, UUU, UUS, and USU. The saturated acids found on total fatty acid analysis of the first group can only be present as SU_2, and the mole fraction of this glyceride type in the fraction would be three times the mole fraction of saturated acids. The remainder of this fraction would be U_3. Saturated acids liberated on lipase hydrolysis of this fraction could only come from the UUS isomer, and the mole fraction of this isomer would be two times the mole fraction of saturated acids found. The amount of USU is then the difference between the total SU_2 and the UUS. Similarly, in the second fraction which would contain SSU, SUS, and SSS, the mole fraction of S_2U is three times the mole fraction of dicarboxylic acids (corresponding to the unsaturated acids) in the total fatty acid composition. The mole fraction of SSU is twice the mole fraction of dicarboxylic obtained on lipase hydrolysis, and the SUS and U_3 are calculated as differences.

This division into only two fractions has the advantage that the relative proportions can be calculated from the composition of the fractions and that of the original oxidized fat. It has the disadvantage that any error in the gas chromatographic analysis is multiplied by three in one instance and by two in another in the calculation of the amount of the glyceride types.

Fractionation by countercurrent distribution is a tedious process, and since only small amounts of the fractions are required for analysis, the solvent system used for the countercurrent distribution was transferred to a liquid-liquid partition column. The 90% ethanol-10% water phase was absorbed on silicic acid as the stationary phase, and the Skellysolve "B" made the mobile phase. Separation of a sample of oxidized lard on such a column is shown in Figure 3. After the first peak, consisting of S_3 and S_2U, had been eluted from the column, the remaining material SU_2 and U_3 was washed from the column with ethyl ether. Calculation from the countercurrent distribution indicated that a very large volume of Skellysolve "B" would have been required to elute this second fraction. The partition column gave a very good separation of the two desired fractions and was used in all subsequent work.

Analysis of the fractions from the partition column was next considered. The gas chromatographic determination of saturated, unsaturated, and dicarboxylic acids had been checked earlier so that the determination of the total composition of the fractions presented no problem. With regard to lipase hydrolysis Mattson and Beck (8) have demonstrated that the 1 and 3 positions are hydrolyzed at a rate independent of whether the acids occupying these positions are saturated, unsaturated, or of varying chain-length over a moderate range. It remained to be seen if this generalization could be extended to include dicarboxylic acids. Synthetic unsymmetrical oleodipalmitin (OPP) and palmitodiolein (OOP) were used as test samples. The compositions of the fatty acids liberated by lipase hydrolysis before and after oxidation of the synthetic glycerides were determined by gas chromatography. The results are given in Table II. If the free acid groups of the dicarboxylic acids formed on oxidation were left as such, hydrolysis of the dicarboxylic acids in the glycerides was less than expected. If however these free acid groups were methylated with diazomethane, lipase hydrolysis did not appear to differentiate between dicarboxylic acids and unsaturated acids. Dicarboxylic acids were released from the oxidized glycerides in an equimolar proportion to the unsaturated acids in the unoxidized fats.

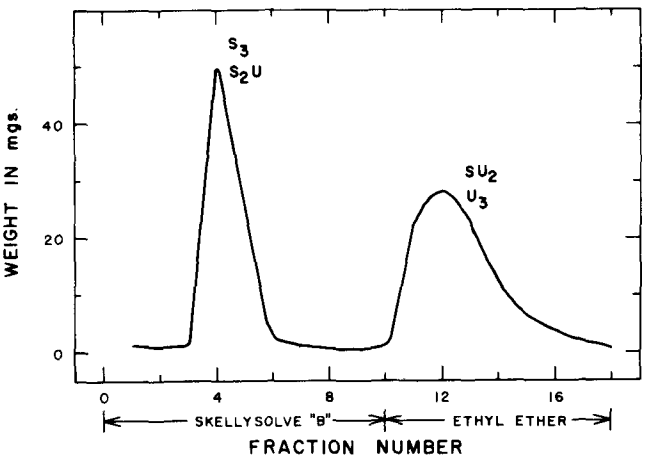

FIG. 3. Fractionation of oxidized lard on liquid-liquid partition column.

[3] Petroleum fraction with boiling range of 58–69°C.

TABLE II

Lipase Hydrolysis of Synthetic Glycerides

	Fatty acids liberated by lipase, mol %		
	Palmitic	Oleic	Azelaic
OPP			
Before oxidation	48.3	51.7	.....
After oxidation	53.6	.....	46.3
After oxidation and methylation	49.1	.....	50.9
OOP			
Before oxidation	47.1	52.9	.....
After oxidation	59.3	.....	40.6
After oxidation and methylation	46.6	.....	53.4

The ratio of palmitic to oleic or azelaic in the fatty acids liberated from the oleodipalmitin are close to the expected 1:1 ratio. In the palmitodiolein the ratio is a little low, possibly because of a small amount of the symmetrical isomer in the sample.

The remaining point to be checked was the possibility of ester interchange during hydrolysis. This and the method as a whole were checked by analyzing known mixtures of synthetic glycerides. Two mixtures were made up from tristearin, oleodipalmitin, palmitodiolein, and triolein. The total fatty acid composition and the fatty acids liberated by lipase hydrolysis were determined for the mixtures before and after oxidation. Good agreement was obtained for the molar proportions of unsaturated acids in the original mixtures and dicarboxylic acids in the oxidized mixtures. The oxidized mixtures were fractionated on the liquid-liquid partition column, and the total fatty acid composition and that resulting from lipase hydrolysis were determined for each fraction. From these data the compositions of the original mixtures were calculated as outlined earlier (Table III). There

TABLE III

Analysis of Mixtures of Synthetic Glycerides

	Mixture 1		Mixture 2	
	Known composition	Calculated composition	Known composition	Calculated composition
SSS	9.5	9.0	3.5	4.0
SSU	40.8	41.5	35.2	37.1
SUU	28.8	29.7	35.2	33.6
USU	1.9	0.8	2.4	4.0
UUU	19.1	18.9	23.7	21.2

is no apparent rearrangement on oxidation, and the method as a whole gives results which are accurate to about ± 2 unit per cent. This is the accuracy expected since there is a possible error of ± 0.5 unit per cent in the gas chromatographic analysis, and this is multiplied by three in the calculation of the glyceride analysis.

The degree of replication that can be obtained for a single fat is illustrated in Table IV by a triplicate

TABLE IV

Triplicate Analysis of Lard

Run No.	1	2	3
SSS	7	7	9
SSU	30	28	28
SUS	...	...	...
SUU	15	17	15
USU	37	37	35
UUU	12	11	13

analysis of lard. The maximum variation is 2 unit per cent, well within the expected limit of accuracy. With regard to the distribution of the fatty acids among the glycerides in lard, the amounts of S_3, S_2U, SU_2, and U_3 are approximately those expected for a "random" distribution. The amounts of the isomers of S_2U and SU_2 are however very different from that predicted by a "random" distribution. Two theories have recently been proposed which give this type of distribution. The first, proposed by the present author (10), is based on a random attachment of the fatty acids to the glycerol in the order 1,2,3, with an intramolecular rearrangement at the 1,2-diglyceride stage. The second theory proposed by Vander Wal (11) is based on a random attachment of one group of fatty acids at the 1 and 3 positions and the random attachment of a different group of fatty acids at the 2 position. The composition of these two groups is determined experimentally by lipase hydrolyses. Both theories predict nearly the same composition for lard. However for four other fats which have been analyzed, chicken fat, rat fat, linseed oil, and cocoa butter, there are differences between the two theories. As shown in Table V, the analytical results agree very well with those predicted by Vander Wal's theory for all five fats analyzed. The differences between the present analytical results and the theory proposed earlier by the author are sufficient to invalidate this theory.

Experimental

Gas Chromatography. All analyses were run on a liquid phase of butanediol-succinate polyester, prepared as described by Craig and Murty (7). The solid support was 60 to 80 mesh, acid-washed, C22 firebrick with a ratio of 6:1 by weight of firebrick to polyester. A commercial gas chromatographic unit, employing a thermal conductivity detector, was used with an 8-ft. column of $\frac{3}{16}$-in. copper tubing. The column temperature was 205°C., and helium flow-rate, measured at the detector outlet, was 40 ml. per minute.

Where the total fatty acid composition of a sample was desired, the methyl esters were prepared from the glycerides by methanolysis. Ten to 20 mg. of sample were refluxed for ½ hr. with 10 ml. of methanol, containing 0.5% anhydrous HCl by weight. The methanol and HCl were then removed under vacuum on a rotary evaporator, and the resulting esters were injected into the chromatographic unit. Preparation of esters from fatty acids released by lipase hydrolysis will be covered under the section on lipase hydrolysis.

Peak areas were measured by drawing tangents to the curves and using the height of the triangle times the width at the base. On test mixtures these areas were found to be proportional to the weight of the components for the saturated and unsaturated monocarboxylic acids. For the dicarboxylic acids, formed by oxidation of the unsaturated acids, the results were consistently lower than for the monocarboxylic acids. Multiplication of the peak areas for dicarboxylic acids by a factor of 1.10 gave a good correlation of the mono- and dicarboxylic acids over a wide range of compositions. All compositions were converted to mole percentages and reported as such.

Oxidation of Fats. The oxidation procedure used was essentially that described by von Rudloff (4). The oxidant solution was 21 g. of sodium periodate plus 25 ml. of 0.1 M potassium permanganate made up to one liter with water; 250 mg. of fat were dissolved in 25 ml. of tertiary butanol and added to a mixture of 67 ml. of the oxidant solution, 83 mg. of potassium carbonate, and 75 ml. of tertiary butanol. The reaction mixture was refluxed for 1 hr. The rel-

TABLE V

Analysis of Natural Fats

	SSS	SSU	SUS	SUU	USU	UUU
Lard						
Found	8	29	0	15	36	12
Vander Wal's theory	6	29	2	12	36	15
Author's earlier theory	10	34	0	14	27	16
Chicken						
Found	3	9	10	38	12	28
Vander Wal's theory	3	10	10	36	9	32
Author's earlier theory	3	7	13	44	0	33
Rat						
Found	2	3	4	33	6	52
Vander Wal's theory	1	5	4	30	9	52
Author's earlier theory	1	3	6	37	0	53
Linseed						
Found	0	0	0	22	4	74
Vander Wal's theory	0	0	1	20	2	76
Author's earlier theory	0	1	1	22	0	77
Cocoa butter						
Found	5	7	66	20	3	1
Vander Wal's theory	5	2	69	22	0	2
Author's earlier theory	12	8	48	32	0	0

ative amounts of fat and oxidant given are sufficient for fats with an iodine value up to 100. For more unsaturated fats the proportion of oxidant must be increased.

After refluxing, the excess oxidant was destroyed by bubbling in ethylene gas until the permanganate was reduced to manganese dioxide. The tertiary butanol was removed on a boiling water bath by a stream of air, and the resulting aqueous phase was acidified by the addition of 3.1 ml. of 10% sulfuric acid. Three extractions of the acidified aqueous phase with ethyl ether served to recover the oxidized fat. The ether was removed in a stream of air, and the short-chain monocarboxylic acids formed by the oxidation were stripped from the sample by placing it in a vacuum oven over-night at 85°C.

When oxidized fat was taken for analysis, the sample was dissolved in ethyl ether and an aliquot was taken as the fully-saturated glycerides tend to crystallize out.

Fractionation of Oxidized Fats. The oxidized fats were divided into two fractions on a liquid-liquid partition column, the first fraction containing the S_3 and S_2U glyceride types and the second fraction the SU_2 and U_3 types. The partition column was prepared by absorbing 40 ml. of 90% ethanol and 10% water (by volume) on 60 g. of Mallinckrodt, 100 mesh, silicic acid. The 90% ethanol was added to the silicic acid in a beaker, and the mixture was worked with a spatula until it regained the appearance of a dry powder. This powder was then slurried with Skellysolve "B" saturated with 90% ethanol and was allowed to settle in a 1-in. diameter column to give a packed column height of about 12 in.

Approximately 200 mg. of an oxidized fat were dissolved in 1 ml. of Skellysolve "B" and 1 ml. of 90% ethanol. This resulted in 2 phases, both of which were pipetted on to the top of the column. The sample was rinsed on to the column with an additional 1 ml. of each phase, and the first fraction was eluted with Skellysolve "B" saturated with 90% ethanol. Elution of material from the column was detected by collecting 10-ml. aliquots, evaporating the solvent, and weighing the residue. The first peak, which contained all of the first fraction, was eluted with 70 to 90 ml. of Skellysolve "B." The Skellysolve "B" was then replaced with ethyl ether, and the second fraction was eluted.

Lipase Hydrolysis. The proportions of the positional isomers in the fractions were determined by a specific hydrolysis of the 1 and 3 positions of the glycerides, using pancreatic lipase. The lipase used was a commercial steapsin, which had been hot-extracted for 6 hrs. with Skellysolve "F"[4] to remove residual fat. Ten to 20 mg. of fat, or of oxidized fat which had been methylated with diazomethane, were placed in the "transition" flask of a VirTis 45 homogenizer.[5] Three ml. of 0.5M K_2HPO_4, buffered to pH 8 with 0.5M Na H_2PO_4, were added to the fat along with 5 mg. of bile salts.[6] If necessary, the flask was warmed until the fat sample was liquid and the homogenizer was then run at high speed to give a stable emulsion. A weight of lipase equal to 40% of the weight of the fat was added, and hydrolysis was allowed to proceed for 30 min. with the homogenizer running at low speed. At the end of the 30-min. period the reaction mixture was added to 3 ml. of 1 N HCl, the resulting solution was saturated with NaCl and the total lipids were recovered by four extractions with ethyl ether. The ether was evaporated in a stream of air, the residue was treated with an excess of diazomethane in ether; after again evaporating the ether and excess diazomethane, an aliquot was injected directly into the gas chromatographic unit. Under the conditions used only the hydrolyzed fatty acids, converted to their methyl esters by the diazomethane, came through the column. The mono-, di-, and triglycerides in the sample remained on the column without any noticeable effect on the operation of the column even after a comparatively large number of samples had been run.

Comparison of peak areas for a given sample size of the hydrolyzed material with the areas for a similar sample size of pure fatty acid methyl esters showed that there was 30 to 40% hydrolysis under the conditions used.

Example of Over-all Analysis. The analysis of a sample of lard will be used as an example of the over-all method. All compositions are expressed as mole percentage.

Gas chromatographic analysis of the methyl esters of the fatty acids of the original sample gave 43.0% saturated acids and 57.0% unsaturated acids. Lipase hydrolysis gave the component fatty acids in the 1

[4] Petroleum fraction with boiling range of 35–58°C.

[5] High-speed, top-entering blender manufactured by the VirTis Company, Gardiner, N.Y.

[6] Bacto-Oxgall, Difco Laboratories, Detroit, Mich.

and 3 positions in the original fats as 29.2% saturated and 70.8% unsaturated. After oxidation the total fatty acid composition was 43.4% saturated and 56.5% dicarboxylic. The composition in the 1 and 3 positions was 28.1% saturated and 71.9% dicarboxylic. This oxidized material was split into two fractions on the liquid-liquid partition column. The analysis of the first fraction was: total composition, 73.1% saturated acids and 26.9% dicarboxylic acids; 1 and 3 positions, 60.0% saturated acids and 40.0% dicarboxylic acids. The analysis of the second fraction was: total composition, 27.6% saturated acids and 72.4% dicarboxylic acids; 1 and 3 positions, 12.8% saturated acids and 87.2% dicarboxylic acids.

The relative amounts of the two fractions were calculated from a material balance of the saturated acids. Let X be the mole fraction of the total glycerides in the first fraction. Then

$$73.1\,X + 27.6\,(1 - X) = 43.4$$
$$X = 0.347$$
$$1 - X = 0.653$$

Fraction 1 contained only S_3 and S_2U glyceride types so that each dicarboxylic acid present (equivalent to the original unsaturated acids) must be associated with two saturated acids as S_2U and the mole percentage of this material in fraction 1 was $3 \times 26.9 = 80.7\%$. The remaining 19.3% was S_3. Of the two possible S_2U isomers, SUS and SSU, only the unsymmetrical isomer would give rise to dicarboxylic acids on lipase hydrolysis. There is one saturated acid for every dicarboxylic acid in the 1 and 3 position of this isomer, and the amount was $2 \times 40.0 = 80.0\%$. Since the total S_2U was 80.7%, there was only 0.7% of the SUS isomer. Multiplying the amounts of each of the three glyceride types by the mole fraction of material in Fraction 1 (0.347) gave the amounts in the total sample as SSS 6.7%, SSU 27.8%, and SUS 0.2%.

Similarly for Fraction 2, which contained SUU, USU, and UUU, the total amout of $SU_2 = 3 \times 27.6 = 82.8\%$. By difference the UUU = 17.2%. The amount of $SUU = 2 \times 12.8 = 25.6\%$ and USU, $82.8 - 25.6 = 57.2\%$. Multiplying by the mole fraction of material in Fraction 2 gives the amounts in the total sample as SUU 16.7%, USU 37.4%, and UUU 11.2%.

Rounding these figures off to the nearest percentage gives the final calculated composition as: SSS 7%, SSU 28%, SUS 0%, SUU 17%, USU 37%, and UUU 11%. The composition expected on lipase hydrolysis of a fat of this composition would be 29% saturated and 71% unsaturated acids. The composition found for the original fat was 29.2% saturated and 70.8% unsaturated acids.

Acknowledgments

Grateful appreciation is expressed to Leo Ruben, Canada Packers Ltd., who donated a number of the synthetic glycerides used in this work, and to David McPhee for his assistance and analytical work throughout the project.

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[Received July 5, 1960]

The Structural Components of Milk Triglycerides[1]

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Pancreatic lipase hydrolysis and gas chromatographic analysis of two samples of butter fat show that the individual acyl groups are not dispersed at random among all the glyceryl carbons. When considered only as saturated or unsaturated, and not as individuals, they appear to be distributed intermolecularly at random, or nearly so, but tend to assume specific positions intramolecularly.

THE LITERATURE contains many accounts of component fatty acid analysis of milk fat triglycerides, and some record of work on the component triglycerides. Much of the work has been referred to by Hilditch (1). Recently Bhalerao, Johnson, and Kummerow (2) have reported results of the triglyceride type of analysis[2] of a sample of butter fat by fractional crystallization. The percentages found were approximately those that would be expected if the S and U were distributed at random throughout the molecules of the fat.

More recently Patton, Evans, and McCarthy (3) have published the results of pancreatic lipase hydrolysis and gas chromatographic analysis of a sample of summer milk fat.[3]

In the present paper the results of similar analyses of milk fat will be reported. The percentages of the triglyceride types and isomeric forms in terms of S and U, calculated by the method of Vander Wal (4), will also be presented and compared with the values calculated in the same way from the data of Patton *et al.*

Experimental

Samples. Sample 1 was commercially produced butter oil made by conventional processes and further clarified by filtration. Sample 2 was obtained from high quality commercial butter by evaporation of the water phase under reduced pressure, extraction of the residue with Skellysolve F, drying with sodium

[1] Presented at the 51st annual meeting, American Oil Chemists' Society, Dallas, Tex., April 4–6, 1960.

[2] The triglyceride types are S_3, S_2U, SU_2, and U_3. S stands for saturated acids or acyl groups, and U stands for unsaturated acids or acyl groups.

[3] In a personal communication Dr. Patton has stated that the initial, rough separation of the fat from the aqueous portion was as butter fat.