TABLE II
Expansivity and Melting Dilation of the Products

Compound or mixture	Temperature interval, °C.	Polymorphic form	Coefficient of expansion, ml./g./°C.	Melting dilation, ml./g.	Melting point,[a] °C.
2-Palmito-oleostearin	−38 to 0	I	0.000403	0.1161	40.3(40.5)
	−37 to 0	II	0.000485	0.1035	(37.0)
	−30 to −10	III	0.000581	0.0695	(24.0)
	50 to 60	Liquid	0.000881	.....	.....
1-Oleodistearin	−36 to 0	I	0.000441	0.1117	42.2(42.1)
	−32 to −10	II	0.000614	.....	.....
	5 to 20	II	0.001116	0.0554	30.6(30.3)
	45 to 60	Liquid	0.000859	.....	.....
2-Oleopalmitostearin and 2-palmito-oleostearin (1:1)	−36 to −10	I	0.000381	0.1050	36.5
	− 5 to 2	II	0.000543	.....	.....
	−30 to −5	III	0.000586	0.0698	23.4
	42 to 60	Liquid	0.000858	.....	.....
2-Oleodistearin and 1-oleodi-distearin (27.8:72.2)	−36 to 0	I	0.000482	0.0966	42.0
	−30 to 0	II	0.000501	0.0822	38.3
	−30 to −15	III	0.000594	0.0722	29.8
	46 to 60	Liquid	0.000866	..	

[a] Melting points in parentheses are capillary melting points, others are dilatometric melting points.

When the mixture was solidified slowly from the melt by cooling in air at 25°C. and then held at this temperature for several days, melting occurred between the range of the intermediate forms, represented by Curve B, and the highest melting forms, represented by Curve A. Dilatometric curves obtained with slowly cooled samples exhibited slight breaks in the melting portions, indicating that individual melting of components tended to occur. Possibly under the conditions of slow cooling nonhomogeneous compositions were obtained.

The mixture in the highest melting forms, Curve A, was obtained by tempering for 4 days at 28°C. a sample containing seed crystals of the highest melting forms. The tempering of some seeded samples could be effected in less time by using a higher temperature. Only 2 hr. was required in one instance.

The coefficients of expansion in the solid and liquid states, the dilatometric or capillary melting points or both, and the volume changes accompanying melting were determined for the two glycerides and the two mixtures and are recorded in Table II.

The mixing of one triglyceride with another is generally recognized to retard the rate of any polymorphic transformations. In an earlier examination of 2-oleopalmitostearin and 2-oleodistearin, the major components of cocoa butter, this was found to be true (10). Furthermore, 2-oleopalmitostearin and 2-oleodistearin exhibited similar polymorphic behavior, and their mixtures tended to behave like a single compound.

In the present investigation 2-oleopalmitostearin and 2-oleodistearin were each mixed with a positional isomer, and the binary mixtures were examined. The admixed isomer in each case exhibited a much faster rate of polymorphic transformation and a different pattern of transformation. On the basis of the data obtained it must be concluded that the mixing of two glycerides which exhibit widely different rates of polymorphic transformation may actually serve to increase the rate of transformation of the slower one. Possibly the more easily transformed component can serve as "seed" for the other. The mixtures of the isomers which were examined behaved otherwise as would be expected of a triglyceride mixture.

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Further Studies on the Pancreatic Hydrolysis of Some Natural Fats

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A series of animal and vegetable fats has been subjected to hydrolysis with pancreatic lipase. From the results obtained, the triglyceride compositions of the original fats have been calculated by the method previously proposed by Coleman and Fulton.

These compositions show substantial agreement with those obtained by other methods. Similarities and differences between fats are shown to be reflections of similarities and differences in glyceride composition. The middle position of the triglycerides has been shown to be largely occupied by unsaturated acids in the case of shea, illipé, and cocoa butters; and by palmitic acid in the case of lard. From a study of the glyceride compositions of a series of lards, of differing fatty acid content, it is suggested that the glycer-

ide composition of a lard may be predicted from a knowledge of its fatty acid content, alone.

It is concluded that the hydrolysis procedure, and the method of interpreting the results proposed, together provide a simple and rapid method for the analysis of natural fats.

STUDIES on the specificity of pancreatic lipase have been made by a number of workers, notably by Mattson *et al.* in America (1,2,3), and Desnuelle *et al.* in France (4,5,6,7). These investigations have shown that pancreatic lipase brings about the preferential hydrolysis of fatty acid residues from the terminal positions of triglycerides; and this observation has led to the suggestion that pancreatic lipase provides a tool for the study of natural fats (3,7).

Some use has already been made of the data obtained from pancreatic hydrolysis for the investigation of fats (8,9,10,11,12,13). Both Mattson *et al.* (9) and Desnuelle *et al.* (8) have concluded that their fatty acid distribution is not random, confirming investigations previously made by other methods. The data so obtained have been discussed by Youngs (14) and by Vander Wal (15).

In a previous publication, Coleman and Fulton (12) have proposed a simple method of calculating the triglyceride composition of a fat from pancreatic hydrolysis data, and have shown that the results so calculated show substantial agreement with those obtained by fractional crystallization and other methods.

The present work is an extension of that already reported: additional specimens of the fats previously examined, and some samples of other fats have been analyzed in the same way, and the results interpreted in the manner previously proposed.

Experimental

The conditions for the hydrolysis were similar to those already described (12).

The triglyceride fraction was separated from about 1 g. of the fat by the method of Quinlin and Weiser (16), and 50 mg. of this material was saponified with alcoholic potash, acidified, and the fatty acid composition determined by gas-chromatography (17).

The remainder of the triglyceride fraction was dispersed in 10 ml. of a 1.2*M* NH_4Cl/NH_4OH buffer, pH 8.5; 2.0 ml. of a 22% $CaCl_26H_2O$ solution and 0.1 ml. of a 25% bile salt solution were added, together with 50 mg. of a pork pancreatic lipase preparation, purified by the method of Desnuelle *et al.* (29) to the end of Stage II. Hydrolysis was carried out at 37.5°C., and the pH was maintained at 8.5 throughout by the addition of 0.880 S.G. NH_4OH from an "Agla" microburette. When some two-thirds of the fatty acids had been liberated, the pH was brought to 1.0 by the addition of 4*N*/HCl, and the mixture extracted with five 30-ml. portions of ether.

The free fatty acids were removed by passing through a 30-g. column of IR400 "Amberlite" resin, and the neutral glycerides recovered by evaporating the ether under reduced pressure. These were then separated into mono-, di-, and triglycerides (16) and the monoglyceride fraction saponified in alcoholic potash. After acidification the fatty acid composition was determined by gas-chromatography (17).

The samples of pigeon, pheasant, chicken, rabbit, beef, and one of the pig fats (Pig 1) were extracted in the laboratory by macerating the tissue with a little water, and extracting with chloroform. The remaining fats were commercial refined samples.

Results

For convenience in calculating the results, the fatty acids have been grouped as "Palmitic" (C_{16} and shorter, saturated), Stearic, and "Oleic" (unsaturated). This grouping has been used by Hilditch (18); and an examination of the distribution of individual fatty acids between the middle and outside positions of triglycerides suggests that, for a large number of fats, this grouping is appropriate. Other groupings would appear more appropriate for some of the vegetable oils, and in these cases alternative groupings have been used.

TABLE I

Fatty Acid Compositions of Original Fat and Resulting Monoglycerides

Fat	Fatty acid composition (M.%)	
	Triglycerides "P"/St/U	Monoglycerides "P"/St/U
Pig (1)	24.0/ 9.9/66.1	65.6/ 4.2/30.2
Pig (2)	26.5/14.3/59.2	66.4/ 4.4/29.2
Pig (3)	31.1/18.0/50.9	74.6/ 4.6/20.8
Pig (4)	27.8/21.3/50.9	73.0/ 4.1/22.9
Pig (5)	31.2/22.4/46.4	76.9/ 4.4/18.7
Ox	33.7/34.3/32.0	28.4/18.5/53.1
Rabbit	36.2/ 7.6/56.2	35.5/ 3.3/61.2
Chicken	31.6/ 5.9/62.5	16.8/ 4.8/78.4
Pheasant	26.1/ 5.4/68.5	8.9/ 2.8/88.3
Pigeon	23.4/ 9.1/67.5	9.1/ 4.7/86.2
Palm oil	44.5/ 5.6/49.9	17.3/ 1.9/80.8
Shea butter	4.1/41.1/54.8	0 / 3.5/96.5
Cocoa butter	26.2/35.8/38.0	2.2/ 1.0/96.8
Illipé butter	17.0/45.6/37.4	2.3/ 2.1/95.6
	Sat./oleic/unsat. S/O/U	Sat./oleic/unsat. S/O/U
Olive oil	14.9/73.9/11.2	0.7/88.9/10.4
	Sat./Linoleic/Unsat. S/L/U	Sat./Linoleic/Unsat. S/L/U
Soybean oil	18.3/47.4/34.3	4.7/64.0/31.3
	Lauric/Sat./Unsat. La/S/U	Lauric/Sat./Unsat. La/S/U
Coconut oil	36.8/55.8/ 7.4	63.0/25.1/11.9

P–C_{16} and shorter sat. St.–Stearic. S–Saturated. U–Unsaturated. O–Oleic. L–Linoleic. La–Lauric.

Table I gives the grouped fatty acid compositions of the original triglycerides, and the resulting monoglycerides, for a number of natural fats. From the difference between these two sets of values, the composition of the fatty acids occupying the terminal (1:3) positions of the triglycerides may be calculated thus:

	"P"	S	"O"	
Composition of triglycerides	20	30	50	a
Composition of triglycerides × 3	60	90	150	a×3
Composition of monoglycerides	8	12	80	b
Difference	52	78	70	(a×3−b)
% Composition of 1:3 acids	26	39	35	$\left(\frac{a\times3-b}{2}\right)$

The original triglyceride composition may then be calculated by distributing the 1:3 acids at random between the unoccupied positions of the monoglycerides, thus:

Monoglyceride	%	Diglyceride	%	Triglyceride	%
				PPP $2.08 \times \frac{26}{100}$	0.54
		PP-8 $\times \frac{26}{100}$	2.08	PPS $2.08 \times \frac{39}{100}$	0.81
				PPO $2.08 \times \frac{35}{100}$	0.73
				SPP $3.12 \times \frac{26}{100}$	0.81
-P-	8.0	SP-8 $\times \frac{39}{100}$	3.12	SPS $3.12 \times \frac{39}{100}$	1.22
				SPO $3.12 \times \frac{35}{100}$	1.09
				OPP $2.8 \times \frac{26}{100}$	0.73
		OP-8 $\times \frac{35}{100}$	2.80	OPS $2.8 \times \frac{39}{100}$	1.09
				OPO $2.8 \times \frac{35}{100}$	0.98

The calculation is extended to the monoglycerides containing stearic and "oleic" acids to complete the analysis. The results obtained in this way are given in Tables II and III.

Discussion

Fat analyses have, in the past, been reported as the proportions of each of four main triglyceride classes present. For the purposes of comparison, the present data have been summarized in this form, and both the present and previously reported results are summarized in Table IV.

It will be seen that while there is overall agreement, there are differences in detail. Some of these are attributable to differences in fatty acid composition between the samples compared, but this is not always the case. There appears to be no general trend in these differences; thus, whereas pancreatic hydrolysis indicates higher proportions of triunsaturated glycerides in pig fat, shea, olive, and soybean oils, it indicates higher proportions of trisaturated glycerides in ox and chicken fats, and in palm oil.

Since the resolution of triglyceride mixtures by fractional crystallization is never complete, there is always some element of uncertainty in interpretation. Again, there is some evidence to suggest that gas chromatography presents a more accurate method of fatty acid analysis than earlier methods. In view of these considerations, the differences between the present and previous results do not necessarily invalidate the present data.

It should be noted that in making these comparisons, the grouping of the present data, as in Table IV, masks many of their most suggestive features. Thus the preponderance of 2-unsaturated triglycerides in cocoa and illipé butters (and to a lesser degree in shea butter) is immediately apparent from the data of Table III. Similarly the preponderance of 2-palmito triglycerides in pig fat is also obvious from Table III. These findings confirm those previously reported (8,9,10,12).

TABLE II

Glyceride Compositions of Some Animal Fats

Glyc.	Pig (1)	Pig (2)	Pig (3)	Pig (4)	Pig (5)	Ox	Rabbit (wild)	Chicken	Pheasant	Pigeon
PPP	....	0.3	0.7	0.2	0.5	3.7	4.7	2.6	1.1	0.8
PPS	0.5	1.7	3.5	2.3	4.1	8.7	2.5	0.9	0.4	0.6
SPS	1.1	2.4	4.5	6.5	7.6	5.0	0.4	0.1	....	0.1
PSP	....	0.2	....	....	....	2.4	0.4	0.7	0.3	0.4
PSS	....	0.1	0.2	0.1	0.2	5.7	0.2	0.3	0.1	0.3
SSS	....	0.2	0.3	0.4	0.5	3.3	....	....	....	0.1
PPU	3.5	6.5	9.2	4.9	7.8	4.4	13.9	7.1	3.6	3.2
SPU	14.1	18.9	24.3	28.3	29.0	5.1	3.7	1.2	0.7	1.2
PSU	0.2	0.4	0.6	0.3	0.5	2.9	1.3	2.0	1.1	1.7
SSU	0.9	1.2	1.5	1.6	1.7	3.4	0.4	0.3	0.2	0.6
PUP	0.3	0.1	0.2	0.1	0.1	7.0	8.2	11.9	10.6	8.1
PUS	0.2	0.7	1.0	0.7	1.0	16.3	4.4	4.0	4.1	6.0
SUS	0.5	1.1	1.3	2.1	1.8	9.5	0.6	0.3	0.4	1.1
UPU	46.4	36.5	32.3	30.7	27.8	1.3	10.2	5.0	3.1	3.1
USU	2.9	2.4	2.0	1.7	1.6	0.9	1.0	1.4	1.0	1.6
PUU	1.6	2.9	2.6	1.6	1.9	8.3	24.0	33.3	36.0	30.6
SUU	6.5	8.3	6.8	8.9	7.1	9.6	6.4	5.6	6.9	11.3
UUU	21.3	16.1	9.0	9.6	6.8	2.5	17.7	23.3	30.4	29.2

TABLE III

Glyceride Composition of Some Vegetable Fats

Glyceride	Palm oil	Shea butter	Cocoa butter	Illipé butter	Glyceride	Olive oil	Glyceride	Soybean oil	Glyceride	Coconut oil
PPP	5.8	....	0.3	0.1	SSS	0.1	SSS	0.3	LaLaLa	3.5
PPS	1.5	....	0.9	0.7	SSO	0.2	SSL	0.9	LaLaS	21.2
SPS	0.1	....	0.6	1.0	SSU	....	SSU	0.9	SLaS	31.8
PSP	0.6	0.1	0.2	0.1	SOS	4.3	SLS	4.0	LaSLa	1.4
PSS	0.2	0.3	0.4	0.7	SUS	0.5	SUS	2.0	LaSS	8.5
SSS	....	1.2	0.3	0.9	SOO	26.0	SLL	12.6	SSS	12.7
PPU	6.9	....	0.1	0.1	SOU	4.5	SLU	11.5	LaLaU	1.5
SPU	0.9	....	0.2	0.3	SUO	3.0	SUL	6.1	SLaU	4.7
PSU	0.8	0.1	0.1	0.1	SUU	0.5	SUU	5.6	LaSU	0.6
SSU	0.1	1.4	0.1	0.2	OSO	0.3	LSL	0.7	SSU	1.9
PUP	27.3	0.4	14.1	5.7	OSU	0.1	LSU	1.3	LaULa	0.7
PUS	7.0	7.2	39.3	31.4	USU	....	USU	0.6	LaUS	4.0
SUS	0.5	34.6	27.4	43.3	OOO	39.2	LLL	9.8	SUS	6.0
UPU	2.0	....	....	....	OOU	13.7	LLU	17.9	ULaU	0.2
USU	0.2	0.4	....	....	OUO	4.6	LUL	4.8	USU	0.1
PUU	32.3	4.0	6.4	3.9	OUU	1.6	LUU	8.8	LaUU	0.3
SUU	4.2	39.2	8.9	10.7	UOU	1.2	ULU	8.2	SUU	0.9
UUU	9.6	11.1	0.7	0.7	UUU	0.2	UUU	4.0	UUU	....

TABLE IV

Comparison of Present with Previously Reported Results

Fat	Total sat. acids (M%)	Glycerides (M%)			
		Trisat.	Disat.	Monosat.	Triunsat.
Pig (1)	33.9	1.6	19.7	57.4	21.3
(Ref. 19)	36.4	2	26	54	18
Pig (2)	40.8	4.9	28.9	50.1	16.1
(Ref. 20)	46.8	5	32	60	3
Pig (3)	49.1	9.2	38.1	43.7	9
(Ref. 20)	52.9	9	43	45	3
Ox	68.0	28.8	48.6	20.1	2.5
(Ref. 21)	61	16	55	26	3
Chicken	37.5	4.6	26.8	45.3	23.3
(Ref. 22)	34	2	28	41	29
Palm oil	50.1	8.2	43.5	38.7	9.6
(Ref. 23)	44.1	6	41	41	12
Shea butter	45.2	1.6	43.7	43.6	11.1
(Ref. 24)	47	4.5	35	55	4.5
Cocoa butter	62.0	2.7	81.3	15.3	0.7
(Ref. 25)	60	2	77	21	....
Olive oil	14.9	0.1	5	39.4	60.5
(Ref. 26)	13.9	....	....	45	55
Soybean oil	18.3	0.3	7.8	38.4	53.5
(Ref. 27)	19.6	....	2	55	43
Coconut oil	92.6	79.1	19.4	1.5	....
(Ref. 28)	93.9	84	12	4	....

It is interesting to observe that the data for the series of pig fats show a systematic change in triglyceride composition, with change in fatty acid composition. Figure 1 illustrates this systematic variation. From these curves it should be possible to predict the glyceride content of a pig fat from a knowledge of its fatty acid composition.

It will be seen that there are marked departures from the compositions predicted for the random distribution of the fatty acids. Using the trisaturated content given in Table II, application of the restricted random calculation does not give very good agreement with the present results.

The present results confirm the observation of Savary, Flanzy, and Desnuelle (8) that palmitic and stearic acids are not interchangeable in pig fat. Reference to Table I shows that while the stearic acid content in the whole fat varies from 10 to 22%, its level in the monoglycerides is remarkably constant at around 4%; on the other hand the ratio of palmitic acid in the whole fat to that in the monoglycerides remains substantially constant throughout the series.

A number of workers using pancreatic hydrolysis for the study of fats have used the composition of the free fatty acids liberated as the composition of the acids occupying the outside positions of the original triglycerides. Coleman and Fulton (12) have shown that appreciable amounts of glycerol may be produced in these hydrolyses, and for this reason it would seem advisable to calculate the acids of the 1:3 positions from the difference between that of the original triglycerides and that of the resulting monoglycerides, in the way proposed.

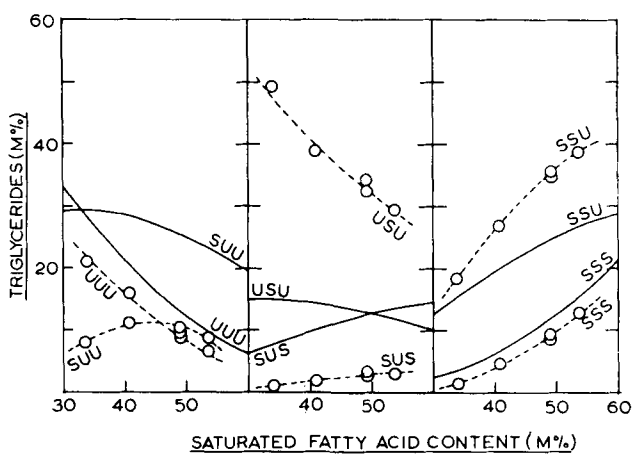

FIG. 1. The triglyceride compositions of a series of lards. Continuous line—Random distribution of fatty acids. Broken line—Calculated from experimental data.

It will be seen from Tables II and III that natural fats may vary. At one extreme is pig fat, where the departure from random fatty acid distribution lies in the direction of high proportions of saturated acids in the middle positions of the triglycerides. At the other extreme are fats like cocoa butter where the converse applies. Between these extremes lie such animal fats as beef and such vegetable fats as palm oil. The similarities between members within such groups as chicken, pheasant, and pigeon fats or shea, cocoa, and illipé butters, and the contrasts between members of different groups, is well exemplified by the present results.

Pancreatic hydrolysis presents a convenient method for the analysis of natural fats, and the method for calculating their glyceride content used here provides a simple method for interpreting the results. Together they possess the merits of speed and simplicity, and they yield information not easily obtained in any other way.

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