TABLE I

Vitamin A Reference Standard

Property	Physicochemical Requirements	Biological Requirements	Committee's Present Opinion
Form of Vitamin A	The substance $C_{20}H_{29}OH$ or ester	Same	Pure vitamin A acetate
Concentration	As high as possible to give little interference with physicochemical tests	As low as possible to simplify dilution with carrier oil	3,000 µg. of $C_{20}H_{29}OH$ per gram (about 10,000 U.S.P. units/gram)
Diluent	A non-volatile oil which will not interfere with physicochemical tests	A non-volatile oil which will not interfere with biological tests	Refined and deodorized cottonseed oil
Antioxidant	Must be oil soluble and not interfere with physicochemical tests	Tocopherol	1 to 3 mg. of tocopherol/g. (perhaps the natural content of cottonseed oil will be sufficient)
Container	Ampoule or capsule opaque to actinic light	Same	One-piece gelatin capsules

time it appears that it cannot be assayed by a method similar to that in Table II. The sub-committee on vitamin A of the National Association of Margarine Manufacturers is developing a special method for this product, and there is close cooperation between that committee and our own.

A few members of this committee are experienced in biological work, and we hope to make amendments to the present existing biological assay procedures. The most important problem here also seems to be the adoption of a suitable vitamin A reference standard.

TABLE II

Tentative Method for the Physicochemical Assay of Vitamin A

	Procedure	Detail
Basic Method	Determination of extinction coefficient at 326 to 328 mµ; calculate vitamin A content by direct proportion	Solvent to be isopropanol. Calibrate instrument with new vitamin A standard. Report in gravimetric units.
Necessary confirming data	(I) Determination of extinction coefficients at 300 and 350 mµ.	The ratios, E(300/E(328) and E(350)/E(328), to have values in prescribed ranges.
	(II) Determination of depth of color, at 620 mµ, of $SbCl_3$ reaction product; potency to be calculated by comparison with color produced by vitamin A standard	Potency to be determined by comparing reaction product of test solution with that of test solution fortified with standard. The color of the reaction product must fade at a prescribed rate. The potency by the color method must agree (within prescribed tolerance) with potency by U.V. method.
Purification Methods	(I) Saponification	Probably will be required
	(II) Distribution between solvents	Might be optional
	(III) Chromatography of unsaponifiable fraction	Might be optional
	(IV) Molecular distillation	Might be forbidden, except under conditions that would prevent pyrolysis of kitol into vitamin A

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Fractionation of Lard and Tallow By Systematic Crystallization[†]

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FRACTIONAL crystallization from solvents has been employed for more than a century in efforts to determine glyceride structure and composition of natural fats. The early work in this field, in addition to recent work up to 1939, has been discussed in considerable detail in a monograph by Hilditch (1) and more recently summarized by Brown (2). Practically all the early efforts were concerned primarily with the isolation of pure individual glycerides by fractional crystallization from solvents, usually at temperatures from room temperatures to 0°C. Much painstaking work showed that it was frequently possible by numerous crystallizations to isolate some of these glycerides in relatively pure form but gave little quantitative information concerning the glyceride composition of any fat.

About 1936, however, Hilditch and his associates employed crystallization technique from a somewhat different viewpoint. Instead of attempting to use it as a means of complete separation of fats into pure component glycerides, they applied it as a preliminary step in their chemical method of determining glyceride composition. The fat was separated by fractional crystallization from acetone at 0°C. into several fractions of less complexity than the original. Each fraction was then examined for its fatty acid composition, fully saturated glyceride content, and content of tri-C_{18} glycerides by determining the tristearin after partial or complete hydrogenation. With this analytical information available, they were able, with limitations, to deduce the approximate glyceride composition of the original fat on the basis of the assumption that the individual fatty acids are distributed as evenly as possible among the glycerol molecules. One of the limitations of this procedure is the inability to differentiate between the various types of mixed unsaturated glycerides, such as stearodioleins and stearooleolinoleins, and stearodilinoleins, which after complete hydrogenation are determined in the form of tristearin.

More recently (3, 4, 5), crystallization at much lower temperatures, —40°C. or lower, has been shown to effect considerable fractionation of the liquid glycerides. Indeed, Hilditch and Maddison (4) considered that sufficient separation had been achieved to justify

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[†] Presented at the 19th fall meeting of the American Oil Chemists' Society, Chicago, Ill., Nov. 7-9, 1945.

TABLE 1

Analytical Characteristics of Fractions Obtained by Crystallization of Lard

Fraction No.	Fraction Wt.[1]	Sap. Eq.	I. V. (Wijs)	T. V. (0.1 N, 24 hrs.)	Unsap.	Melting Points	
						Wiley	Capillary
	g.				%	°C.	°C.
P_2	19.3	285.2	4.45	3.6	....	64.0-65.0	63.5-64.2
P_4	191.7	287.6	35.6	29.4	....	42.8-43.0	42.5-44.0
P_6	343.5	287.2	61.3	54.2	....	20.7-21.2	16.0-19.5
P_8	299.0	290.2	81.1	67.2	0.11	1.8	1.0-3.7
P_{10}	65.8	294.0	95.6	75.8	0.76	....	—17 to —10
P_{12}	45.1	291.6	114.4[2]	83.1[2]	1.12	....	—20 to —16
F_{11}-F_{12}	35.6	302.8	130.6[2]	85.9[2]	5.17	....	—32 to —28
Initial Lard	1000.0	288.1	68.0	56.0	0.35	36.2-36.5	36-38

[1] Corrected to 100% recovery and for samples removed for analysis. Actual total recovery was 98.6% of starting material.

[2] Fatty acids recovered after removing unsaponifiable material had the following analysis: P_{12} I.V. 119.7; T.V. 86.1; F_{11}-F_{12} I.V. 134.1; T.V. 90.1.

calculation of the glyceride composition of cottonseed oil on the assumption that substantially only two of the four general classes of glycerides may be present in any one fraction, that is, fully saturated (tri-saturated) with di-saturated-mono-unsaturated, di-saturated-mono-unsaturated with mono-saturated-di-unsaturated, and mono-saturated-di-unsaturated with tri-unsaturated. These classes of glycerides will hereinafter be referred to simply as tri-saturated, di-saturated, mono-saturated, and tri-unsaturated glycerides.

The present paper is a report of a preliminary investigation of the use of low-temperature crystallization as an important step in determining glyceride composition of fats and oils. Further work is under way to improve the fractionation by modifying the conditions of crystallization. A sample of lard and one of edible tallow were subjected to fractional crystallization from acetone at successively lower temperatures from 20° to —45°C. In each case six recrystallized precipitates and a final filtrate residue were obtained. Fatty acid composition of each fraction was determined from spectrophotometric data and iodine value. Comparative data by the thiocyanometric method are included. Calculations of the tri-, di-, and mono-saturated and tri-unsaturated glyceride contents were made on the assumption that not more than two such classes of glycerides were present in any one fraction. Certain physical properties of the crystallized fractions are also given.

Experimental

The lard used in this study was a commercial steam-rendered product of good quality, consisting of 25% killing fats and 75% cutting fats. The tallow was a good grade of edible kettle-rendered beef ruffle fat.

Crystallization. The general outlines of the crystallization steps are given in Charts 1 and 2. An external cooling bath was used for each crystallization. Mechanical stirring was employed in the crystallizations at temperatures below 3°. The rate of cooling was such that about an hour was required to reach the temperatures indicated, after which the temperature was maintained for about an hour before filtering. The solutions crystallized at 20° and 3° were held overnight, with only occasional stirring during the initial cooling. Suction filtration with an ordinary Büchner funnel sufficed for the removal of Fractions P_2, P_4, and P_6, the funnel being sufficiently cooled by carefully pouring most of the supernatant liquid through first. In the filtration of fractions at lower temperatures a tin jacket fitted to a Büchner funnel and containing alcohol cooled with solid carbon dioxide to slightly below the temperature of crystallization served adequately to keep the funnel and contents at about the proper temperature. In all cases the precipitates were pressed and then washed with several small portions of acetone previously cooled to the temperature of crystallization. Anhydrous acetone was employed, but undoubtedly some moisture from the air was absorbed during the crystallizations at low temperatures. Care was taken during the removal

TABLE 2

Spectrophotometric Analysis of Fractions Obtained by Crystallization of Lard

Fraction No	Non-Conjugated Acids			Conjugated Acids		
	Linoleic	Linolenic	Arachidonic	Diene	Triene	Tetraene
	%	%	%	%	%	%
P_2	0.16	0.00	0.00	0.03	0.000	0.000
P_4	6.27	0.31	0.16	0.11	0.000	0.000
P_6	7.53	0.48	0.15	0.17	0.000	0.000
P_8	14.32	0.99	0.30	0.24	0.000	0.000
P_{10}	21.01	1.57	0.39	0.33	0.001	0.000
P_{12}	33.65	2.40	1.25	0.54	0.002	0.000
F_{11}-F_{12}	35.75	2.40	4.69	0.76	0.005	0.001
Initial Lard	12.54	0.82	0.42	0.21	0.000	0.000

TABLE 3

Fatty Acid Composition of Fractions Obtained by Crystallization of Lard, Calculated From I. V. to T. V., and From Spectrophotometric Data and I. V.

Fraction No.	Composition Calc. From I.V. and T.V.			Composition Calc. From Spectrophotometric Data and I.V.					
	Linoleic[1]	Oleic	Saturated	Linoleic	Linolenic	Arachidonic	Oleic	Saturated	Unsap.
	%	%	%	%	%	%	%	%	%
P_2	0.9	3.2	95.9	0.19	0.00	0.00	4.8	95.0	....
P_4	7.3	26.7	66.0	6.38	0.31	0.16	27.0	66.2	....
P_6	8.3	54.7	37.0	7.70	0.48	0.15	53.7	38.0	....
P_8	16.3	61.3	22.3	14.56	0.99	0.30	60.8	23.3	0.1
P_{10}	23.4	63.6	12.2	21.34	1.57	0.39	61.9	14.0	0.76
P_{12}	37.9[2]	55.5[2]	5.5[2]	34.20	2.41	1.25	52.1	8.9	1.12
F_{11}-F_{12}	45.5[2]	47.7[2]	1.6[2]	36.51	2.41	4.69	50.8[3]	0.4	5.2
Initial Lard	14.2	50.6	35.2	12.75	0.82	0.42	49.6	36.1	0.35

[1] All polyunsaturated calculated as linoleic.

[2] Determined on fatty acids after removal of unsaponifiable material.

[3] The iodine value of fatty acids after removal of unsaponifiable material was used in the calculation of oleic acid.

TABLE 4

Fatty Acid Composition Determined From Analysis of Fractions and That Determined From Analysis Directly on the Initial Lard

Fraction No.	Wt.[1] of Fraction	Linoleic	Linolenic	Arachidonic	Oleic	Saturated	Unsap.
	g.	*g.*	*g.*	*g.*	*g.*	*g.*	*g.*
P_2	19.3	0.04	0.00	0.00	0.93	18.33	0.00
P_4	191.7	12.23	0.59	0.31	51.72	126.85	0.00
P_6	343.5	26.40	1.65	0.52	184.46	130.47	0.00
P_8	299.0	43.70	2.96	0.90	181.51	69.63	0.30
P_{10}	65.8	14.04	1.03	0.26	40.74	9.23	0.50
P_{12}	45.1	15.42	1.09	0.56	23.51	4.01	0.51
F_{11}-F_{12}	35.6	13.00	0.86	1.67	18.08	0.14	1.85
Totals	1000.0	124.83	8.18	4.22	500.95	358.66	3.16
Percent	100.0	12.48	0.82	0.42	50.1	35.9	0.32
Anal. on Original Lard		12.75	0.82	0.42	49.6	36.1	0.35

[1] Corrected to 100% recovery and for samples removed for analysis. Actual weights of fractions were 98.6% of starting material.

of solvent and in handling to minimize oxidation. The solvent was removed by distillation from a type of flask used in deodorization, most of it at ordinary pressures and the remaining under reduced pressure according to a procedure for removal of alcohol from fats described in a previous publication (6). The fractions were then stored under nitrogen in a refrigerator.

The temperatures and solvent ratios were selected somewhat arbitrarily, but only after first determining that sizable fractions could be obtained under these conditions and that appreciably lower temperatures in each instance were required to cause more precipitation.

Analytical Characteristics. Analytical and physical constants were determined by standard methods. The thiocyanogen values (T.V.) were determined with 0.1 N solutions of thiocyanogen, an absorption period of 24 hours at 20-21°C. (7) being used.

Fatty Acid Composition. The content of polyunsaturated acids of each fraction was determined spectrophotometrically by a modification (8, 9) of the method described by Mitchell, Kraybill, and Zscheile (10). The oleic acid content was calculated from the difference between total unsaturation as determined by iodine value and that due to polyunsaturated components. This calculation is subject to a slight error because of the presence of small amounts of lower molecular weight monoethenoic acids. The saturated acids were then obtained by difference. Summations were made of the weights of each acid in each fraction as determined from spectrophotometric data and iodine values and expressed in percent for comparison with the composition found by direct analysis on the original lard and tallow.

For comparison, calculations of composition were made from the iodine values and thiocyanogen values on the assumption that oleic and linoleic are the only unsaturated acids present.

Glyceride Composition. The amounts and percentages of tri-saturated, di-saturated, mono-saturated, and tri-unsaturated glycerides were estimated from the fatty acid composition of each fraction on the assumption that substantially only two of these glyceride classes are present in any one fraction. For this purpose, particularly when it was desired to make a comparison with glyceride composition calculated on the basis of random distribution of the fatty acids on the glycerol molecules, the fatty acid composition was calculated as molar percent.

The method of estimating the amounts of the four classes of glycerides from the experimental data is best explained by citing an example:

Fraction P_2—Table 5

19.3 gms. = wt. of fraction

285.2 = sap. eq. of fraction

$$\frac{19.3}{285.2} \times 100 = 6.8 = \text{moles of fraction } (\times 100)$$

95.2 = % (mol) of saturated acids (as glycerides).

100.0 = % (mol) of saturated acids (as glycerides) in tri-saturated glycerides.

2/3 or 66.67 = % (mol) of saturated acids (as glycerides) in di-saturated glycerides.

TABLE 5

Glyceride Composition of Fractions Obtained by Fractional Crystallization of Lard

Fraction No.	Mols of Fraction	Saturated Acids (as glycerides)			Class of Glycerides			
		Sap. Eq.[1]	Mols	% (Mol)	Tri-saturated	Di-saturated	Mono-saturated	Tri-unsaturated
	× *100*		× *100*		*mols*×*100*	*mols*×*100*	*mols*×*100*	*mols*×*100*
P_2	6.8	284.6	6.4	95.2	5.8	1.0	....	....
P_4	66.7	284.0	44.7	67.0	0.7	66.0	....	....
P_6	119.6	275.5	47.4	39.6	....	22.6	97.0	....
P_8	103.0	275.3	25.3	24.6	....	....	76.0	27.1
P_{10}	22.5	266.3	3.5	15.5	....	....	10.4	12.0
P_{12}	15.5	236.2	1.7	11.0	....	....	5.1	10.3
F_{11}-F_{12}	11.5	236.2[2]	0.06	0.5	....	....	0.2	11.3
Total	345.6	....	129.1	37.4	6.5	89.6	188.7	60.7
Calculated from Analysis of Fractions					*% (mol)* 1.9	*% (mol)* 25.9	*% (mol)* 54.6	*% (mol)* 17.6
					% (wt.) 1.9	*% (wt.)* 25.7	*% (wt.)* 54.4	*% (wt.)* 18.0
Calculated on Basis of Random Distribution					*% (mol)* 5.2	*% (mol)* 26.3	*% (mol)* 44.0	*% (mol)* 24.5

[1] Calculated from the saponification equivalents of the fractions and their fatty acid compositions.

[2] The small amount of saturated acids in this fraction was assumed to have the same saponification equivalent as those in the preceding fraction.

TABLE 6

Analytical Characteristics of Fractions Obtained by Crystallization of Tallow

Fraction No.	Fraction Wt.[1]	Sap. Eq.	I. V. (Wijs)	T.V.(0.1 N, 24 hrs.)	Unsap.	Melting Points	
						Wiley	Capillary
	g.				%	°C.	°C.
P_2	190.2	284.8	7.0	4.9	....	63.3-63.5	59.0-60.0
P_4	381.2	286.9	33.7	32.1	....	37.5-37.6	33.2-35.2
P_6	209.1	286.6	52.5	49.5	....	21.9-22.0	10.0-14.2
P_8	179.4	287.3	61.6	57.9	1.02	8.0-9.2	5-10
P_{10}	17.5	288.7	75.9	72.2	1.05	....	—11 to — 9
F_{12}	9.5	287.6	81.7[2]	74.8[2]	1.54	....	—15 to —12
F_{11}-F_{12}	13.1	303.5	97.1[2]	75.8[2]	7.66	....	—33 to —28
Initial Tallow	1000.0	286.4	40.2	38.0	0.29	47.2	46.0-48.0

[1] Corrected to 100% recovery and for samples removed for analysis. Actual total recovery was 97.0% of starting material.

[2] The fatty acids recovered after removing unsaponifiable material had the following analysis: P_{12} I.V. 85.5, T.V. 77.9; F_{11}-F_{12} I.V. 93.4, T.V. 79.0.

By inspection it is apparent that the fraction is largely tri-saturated glyceride, and the assumption is made that the minor component is di-saturated.

$100.0 - 66.67 = 33.33\%$ (mol) difference in saturated content between tri- and di-saturated glycerides.

$100.0 - 95.2 = 4.8\%$ (mol) difference in saturated content between theory for tri-saturated and that found for P_2.

$\frac{4.8}{33.33} \times 6.8 = 1.0$ moles ($\times$ 100) of di-saturated glyceride.

$6.8 - 1.0 = 5.8$ moles ($\times$ 100) of tri-saturated glyceride.

Random Distribution. For comparison with the glyceride composition estimated from the analysis of the fractions as described above, the amounts of the four classes of glycerides can be calculated on the basis of random distribution of the fatty acids on the glycerol molecules if it is assumed that each hydroxyl group of the glycerol molecule has equal ease of esterification and also that each fatty acid has the same degree of reactivity in glyceride formation.

These classes of glycerides may be represented by the following:

Tri-saturated	Di-saturated	Mono-saturated	Tri-unsaturated
A	B	C	D

By interchanging the positions of the fatty acids on the structural formulas, it can be seen that there is only one way of forming A or D but three ways of forming B or C. The statistical weights of A, B, C, and D then are 1, 3, 3, and 1, respectively.

TABLE 7

Spectrophotometric Analysis of Fractions Obtained by Crystallization of Tallow

Fraction No.	Non-Conjugated Acids			Conjugated Acids		
	Linoleic	Linolenic	Arachidonic	Diene	Triene	Tetraene
	%	%	%	%	%	%
P_2	0.29	0.00	0.00	0.07	0.01	0.000
P_4	0.98	0.34	0.01	0.35	0.02	0.002
P_6	1.81	0.61	0.09	0.57	0.02	0.004
P_8	2.04	0.81	0.10	0.77	0.03	0.005
P_{10}	2.61	1.48	0.13	1.16	0.03	0.004
P_{12}	3.80	1.35	0.35	1.35	0.05	0.005
F_{11}-F_{12}	4.90	1.68	1.51	2.05	0.10	0.018
Initial Tallow	1.35	0.43	0.06	0.46	0.02	0.003

The probability of one hydroxyl of a glycerol molecule reacting with a saturated acid is equal to the mol fraction of saturated acids in the total fatty acid mixture, i.e.,

$$x = \frac{n_1}{n_1 + n_2}$$

where x = mol fraction of saturated acids

n_1 = mols of saturated acids

n_2 = mols of unsaturated acids

Similarly, the probability of one hydroxyl of a glycerol molecule reacting with an unsaturated acid is equal to the mol fraction of unsaturated acids, y, where

$$y = \frac{n_2}{n_1 + n_2}$$

Then the probability that all three hydroxyls of a glycerol molecule will be esterified with saturated acids will be x^3, and the fraction of tri-saturated glycerides (A) formed will be the product of the probability times the statistical weight of A, namely,

$$1 \cdot x^3 = \text{mol fraction of A}$$

TABLE 8

Fatty Acid Composition of Fractions Obtained by Crystallization of Tallow Calculated From I. V. and T. V., and From Spectrophotometric Data and I.V.

Fraction No.	Composition Calc. From I. V. and T. V.			Composition Calc. From Spectrophotometric Data and I.V.					
	Linoleic[1]	Oleic	Saturated	Linoleic	Linolenic	Arachidonic	Oleic	Saturated	Unsap.
	%	%	%	%	%	%	%	%	%
P_2	2.5	3.1	94.4	0.37	0.01	0.00	7.35	92.3	....
P_4	1.7	35.7	62.6	1.33	0.36	0.01	35.3	63.0	....
P_6	3.3	54.3	42.4	2.38	0.63	0.10	55.6	41.3	....
P_8	4.1	63.3	32.6	2.81	0.84	0.11	62.9	32.3	1.0
P_{10}	4.0	80.1	15.9	3.77	1.51	0.13	75.5	18.1	1.0
P_{12}	8.3[2]	77.2[2]	11.5[2]	5.15	1.40	0.36	79.0	12.6	1.5
F_{11}-F_{12}	14.9[2]	65.9[2]	11.5[2]	7.03	1.78	1.53	78.6[3]	3.4	7.7
Initial Tallow	2.4	41.9	55.7	1.81	0.45	0.07	41.4	55.5	0.29

[1] All polyunsaturated calculated as linoleic.

[2] Determined on fatty acids after removal of unsaponifiable material.

[3] The iodine value of fatty acids after removal of unsaponifiable material was used in the calculation of oleic acid.

TABLE 9

Fatty Acid Composition Determined From Analysis of Fractions and That Determined From Analysis Directly on the Initial Tallow

Fraction No.	Wt.[1] of Fraction	Linoleic	Linolenic	Arachidonic	Oleic	Saturated	Unsap.
	g.	g.	g.	g.	g.	g.	g.
P_2	190.2	0.70	0.02	0.00	13.98	175.50	....
P_4	381.2	5.07	1.37	0.04	134.56	240.16	....
P_6	209.1	4.98	1.32	0.21	116.26	86.33	....
P_8	179.4	5.04	1.51	0.20	112.90	57.95	1.79
P_{10}	17.5	0.66	0.26	0.02	13.21	3.17	0.18
P_{12}	9.5	0.49	0.13	0.03	7.50	1.20	0.15
F_{11}-F_{12}	13.1	0.91	0.23	0.20	10.30	0.45	1.01
Totals	1000.0	17.85	4.84	0.70	408.71	564.76	3.13
Percent		1.79	0.48	0.07	40.87	56.48	0.31
Anal. on Original Tallow		1.81	0.45	0.07	41.40	55.5	0.74

[1] Corrected to 100% recovery and for samples removed for analysis. Actual weight of fractions was 97% of starting material.

In the same manner,* the following expressions for the other classes of glycerides can be derived.

$$3 \cdot x^2y = \text{mol fraction of B}$$
$$3 \cdot y^2x = \text{mol fraction of C}$$
$$1 \cdot y^3 = \text{mol fraction of D}$$

Applying these equations for the calculation of the glycerides of lard which has 37.4% (mol) of saturated and 62.6% (mol) of unsaturated acids, we obtain the following:

Tri-saturated $= (.374)^3 \times 100 = 5.2\%$ (mol)
Di-saturated $= 3\,(.374)^2(.626) \times 100 = 26.3\%$ (mol)
Mono-saturated $= 3\,(.626)^2(.374) \times 100 = 44.0\%$ (mol)
Tri-unsaturated $= (.626)^3 \times 100 = 24.5\%$ (mol)

Similar calculations made for the tallow are reported in Table 10.

Results and Discussion

The analysis and data obtained from the crystallization of lard are given in Chart 1 and Tables 1-5; similar data for tallow are given in Chart 2 and Tables 6-10.

Spectrophotometric analyses (Tables 2 and 7) show a much greater ratio of linolenic and arachidonic

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* The distribution of the glyceride classes A, B, C, D can be conveniently expressed as the terms in the binomial expansion of $(x+y)^3 = 1$, i.e.,

$$x^3 + 3x^2y + 3y^2x + y^3 = 1$$

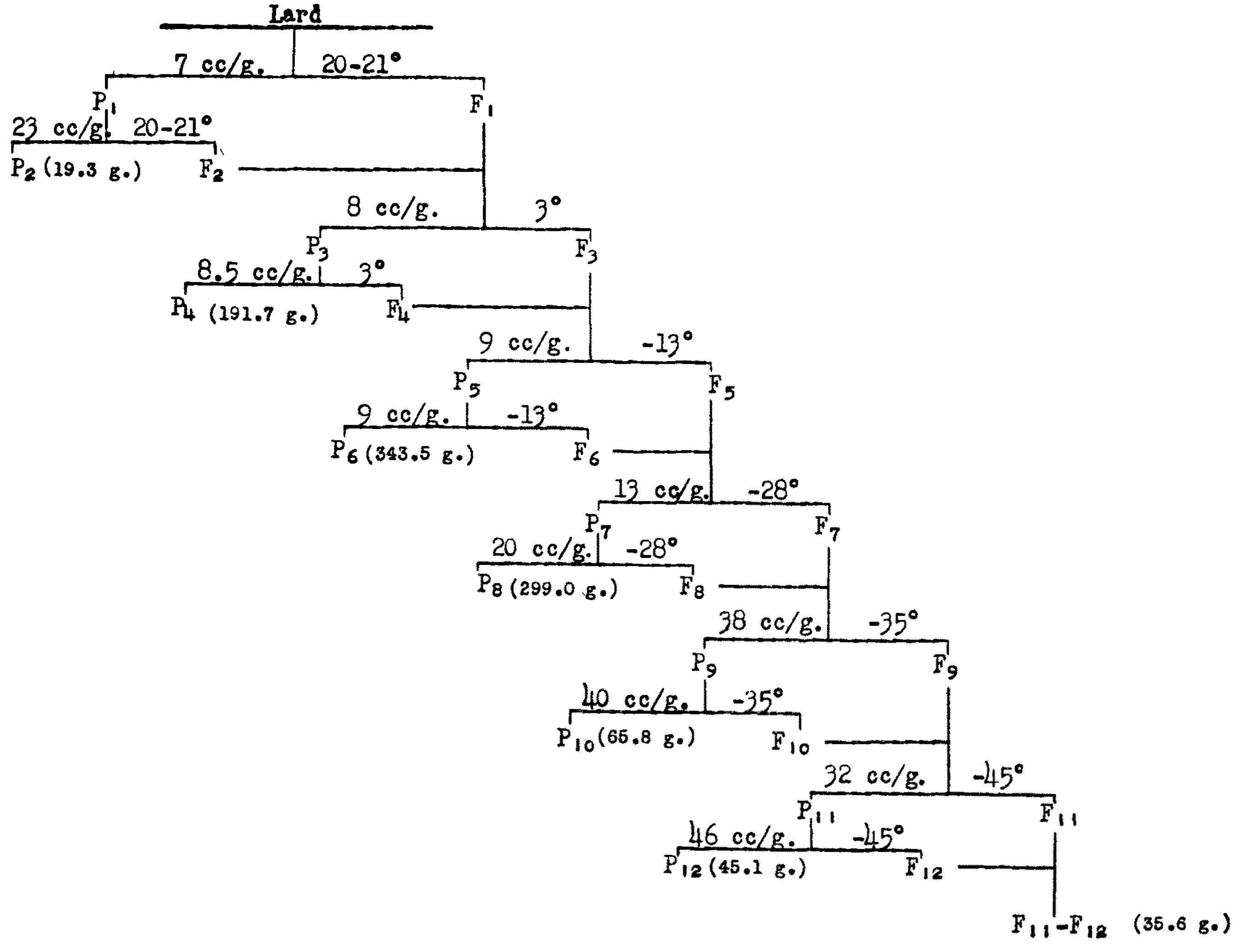

CHART 1. Fractional crystallization of lard from acetone.

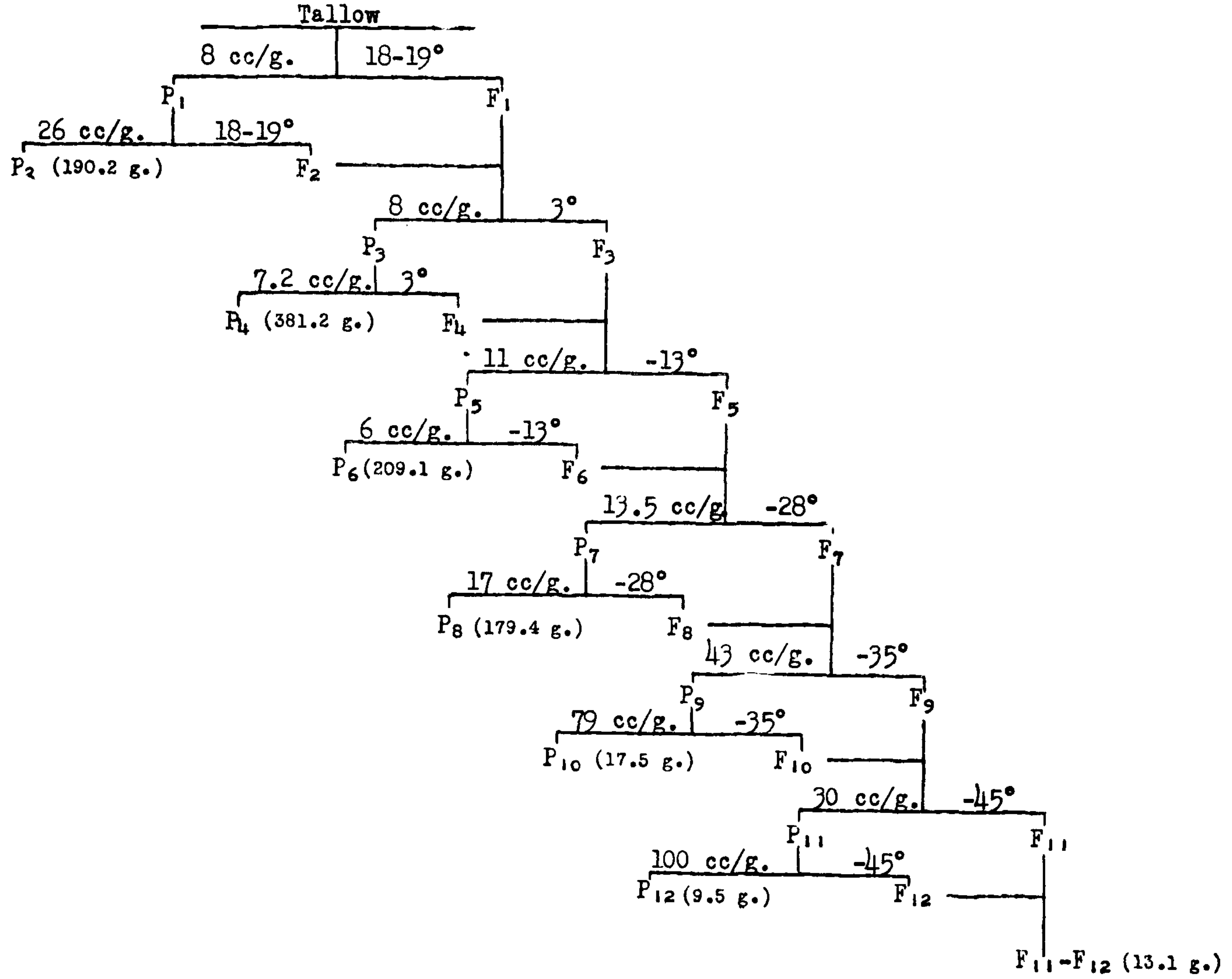

CHART 2. Fractional crystallization of tallow from acetone.

acids to linoleic acid in tallow than in lard although the actual amount of these more highly unsaturated acids in tallow is less. The amount of conjugated acids in the tallow is much greater than in the lard.

Fatty acid composition of the fractions from both lard and tallow (Tables 3 and 8) determined thiocyanometrically and spectrophotometrically are in reasonably good agreement, considering that in the former method all the polyunsaturated acids were calculated as linoleic acid. In the P$_{12}$ and F$_{11}$-F$_{12}$ fractions, as would be expected from their higher linolenic and arachidonic acid content, the differences are greatest. It is of interest to note the consistent trend throughout the crystallization steps for the fractions removed at the lower temperatures to have a higher ratio of polyunsaturated acids to oleic or to saturated acids. The unsaponifiable material also tended to concentrate into the final filtrate fraction.

Remarkably close agreement was obtained in fatty acid composition determined by summation of the amounts of each acid in each fraction with that determined by direct analysis of the original lard and tallow (Tables 4 and 9).

While it would be extremely difficult to prove that each fraction did not contain more than two classes of glycerides, certain indications found in work now in progress lead us to believe that this assumption is substantially correct. For example, independent determination of tri-saturated glycerides by oxidation in acetone solution with potassium permanganate (1) showed good agreement with the amount found by crystallization. Moreover, fatty acid analysis of material in Filtrate F$_2$ corresponded well with that required for di-saturated glycerides. Considerations of solubility and solidifying points of the glyceride classes in connection with the general scheme of crystallization further indicate that the assumption is not unreasonable.

The estimated glyceride compositions shown in Tables 5 and 10 indicate that tallow contains much higher percentages of tri-saturated and di-saturated glycerides than does lard, and correspondingly lower percentages of mono-saturated and tri-unsaturated glycerides. These results indicate further that lard contains considerably larger amounts of tri-unsaturated glycerides than information in the literature would lead one to believe. The percentages of the four classes of glycerides calculated from the experimental data are in close enough agreement with the values calculated on the basis of random distribution

TABLE 10

Glyceride Composition of Fractions Obtained by Fractional Crystallization of Tallow

Fraction No.	Mols of Fraction	Saturated Acids (as glycerides)			Class of Glycerides			
		Sap. Eq.[1]	Mols	% (Mol)	Tri-saturated	Di-saturated	Mono-saturated	Tri-unsaturated
	× *100*		× *100*		*mols×100*	*mols×100*	*mols×100*	*mols×100*
P_2	66.8	284.1	61.8	92.5	51.8	15.0	....	....
P_4	132.8	282.3	85.1	64.1	....	122.6	10.2	....
P_6	73.0	275.5	31.3	42.9	....	20.9	52.1	....
P_8	62.5	264.8	21.5	34.4	....	2.0	60.5	....
P_{10}	6.1	251.3	1.3	21.3	....	....	3.9	2.2
P_T	3.3	222.7	0.5	15.2	....	....	1.5	1.8
F_{11}-F_{12}	4.3	222.7[2]	0.2	4.7	....	....	0.6	3.7
Total	348.8	....	201.7	57.9	51.8	160.5	128.8	7.7
					%(mol)	*%(mol)*	*%(mol)*	*%(mol)*
Calculated From Analysis of Fractions					15.0	46.0	36.9	2.2
					%(wt.)	*%(wt.)*	*%(wt.)*	*%(wt.)*
					14.7	45.9	37.1	2.2
					%(mol)	*%(mol)*	*%(mol)*	*%(mol)*
Calculated on Basis of Random Distribution					19.4	42.3	30.8	7.5

[1] Calculated from the saponification equivalents and fatty acid composition of the fractions.

[2] The small amount of saturated fatty acids in this fraction was assumed to have the same saponification equivalent as those in the preceding fraction.

to indicate that the general pattern is probably random formation in animals such as the pig, perhaps altered somewhat by requirements of the body.

Systematic crystallization affords a direct means of characterizing fats, at least approximately, in respect to their content of the main glyceride fractions or classes. It also affords a means of obtaining appreciable quantities of these fractions for special investigations, such as a study of factors influencing the physical behavior of shortenings during and after plasticizing. Perhaps it may also be useful in following changes in glyceride composition during hydrogenation. The data obtained from crystallization investigations of this type serve somewhat as a background from which processes may be developed for manufacturing "tailor-made" fats for specialized uses.

Summary

Lard and edible tallow were subjected to a series of fractional crystallizations from acetone at temperatures from 20° to —45°C. Six recrystallized precipitate fractions and a filtrate residue were obtained from each fat. In addition to determining the more common physical and chemical characteristics, fatty acid composition of each fraction was calculated from spectrophotometric data, iodine value, and thiocyanogen value. The consistent results obtained throughout by the spectrophotometric method of fatty acid analysis lend further confirmation to the reliability of this method for composition studies of natural fats. The approximate amounts of tri-saturated, di-saturated, mono-saturated, and tri-unsaturated glycerides of the lard and tallow were estimated from the analysis of each fraction on the assumption that not more than two of these classes of glycerides were present in any one fraction. The tallow contains much higher proportions of tri-saturated and di-saturated glycerides and correspondingly lower proportions of the mono-saturated and tri-unsaturated glycerides than does lard. The amount of tri-unsaturated glycerides in lard was found to be significantly greater than meager information in the literature would indicate. The data indicate that the general pattern of glyceride formation in animals such as the pig and cow is probably of random character.

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