The Glyceride Structure of Natural Fats. II. The Rule of Glyceride Type Distribution of Natural Fats[1]

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THE distribution of the constituent fatty acids of fats has been the subject of much investigation. The most extensive work was done by Hilditch and coworkers, who discovered that some fats conform approximately to what they call the "rule of even distribution." This rule has been expressed most recently as follows (1):

1. When a given fatty acid A forms about 35% (mol) or more of the total fatty acids (A + X) in a fat, it will occur at least once $G(AX_2)$ in practically all the triglyceride molecules of the fat in question.[3]
2. If it forms from about 35 to about 65% (mol) of the total fatty acids (A + X), it will occur twice $G(A_2X)$ in any given triglyceride molecule in some instances, and of course more frequently the higher the proportion of this acid in the total fatty acids.
3. If it forms 70% or more of the total fatty acids, the remaining fatty acids (X) can at most only form mixed glycerides $G(A_2X)$, and the excess of A then, and broadly speaking then only, appears as a simple triglyceride $G(A_3)$.
4. A minor component acid which forms much less than about a third of the total fatty acids (*e.g.*, 15% or less), will not occur more than once in any triglyceride molecule (and, of course, not at all in many of the triglyceride molecules).

Hilditch (1) is of the opinion that the rule holds in the initial synthesis of animal as well as vegetable fats and that in the former some of the triglycerides are modified by a secondary reaction. It has been suggested that some marine fats such as whale oil and herring oil conform to the rule (2). According to Lovern (3) porpoise body fat does not conform to the rule, and Green and Hilditch (4) state that green turtle depot fat also does not obey it.

Longenecker (5) and Norris and Mattil (6) favor the hypothesis that in animal fats the fatty acids are distributed in an approximately random manner. Doerschuck and Daubert (7) state that the acids in corn oil are distributed according to a "partial random" pattern.

In Part I of this series (8) a procedure for the determination of the four glyceride types, GS_3, GS_2U, GSU_2, and GU_3, was described.

The glyceride compositions of 27 natural fats determined by this procedure are given in Table I.

In Table II are presented the glyceride-type compositions of 46 natural fats calculated from crystallization data according to the procedure of Hilditch and Meara (9). The data are from various sources (10 to 42 inclusive). In many cases the mol percentage of saturated acids reportedly found by analysis does not agree with the glyceride-type composition found by fractional crystallization. In such cases the mol per cent of S has been calculated from the results of glyceride-type analysis by the latter method.

In Table II are also given the results obtained by calculation according to a new rule for glyceride-type distribution, which will be presented later in this report. These results were calculated from the reported GS_3 contents and the reported or calculated S contents.

The results given in Tables I and II show a number of characteristic features:

1. Throughout almost the entire series values for GS_2U and GU_3 are higher by the present procedure than those calculated from crystallization data, or according to the rule of even distribution, whereas those for GSU_2 are lower. In cases where the amount of GU_3 present is small, the

TABLE I

Glyceride Type Composition of Some Natural Fats as Obtained by the Present Method

No.	Fat	S (Mol %)	Found: Present Procedure (Mol %)				Calculated: Present Procedure (Mol %)			Calculated: Even Distribution (Mol %)			
			GS_3	GS_2U	GSU_2	GU_3	GS_2U	GSU_2	GU_3	GS_3	GS_2U	GSU_2	GU_3
1.	Apricot Kernel Oil	8	0	1	22	77	1	22	77	0	0	24	76
2.	Linseed Oil	13	0	5	29	66	5	29	66	0	0	39	61
3.	Sesame Oil	16	0	6	36	58	7	34	59	0	0	48	52
4.	Samadera Indica Oil	16	0	6	37	57	7	34	59	0	0	48	52
5.	Peanut Oil	20	0	9	42	49	10	40	50	0	0	60	40
6.	Foenugreek Seed Oil	20	0	11	38	51	10	40	50	0	0	60	40
7.	Almond Kernel Oil	22	0	11	47	42	13	42	45	0	0	66	34
8.	Corn Oil	22	0	12	43	45	13	42	45	0	0	66	34
9.	Cottonseed Oil	23	0	13	44	43	14	41	45	0	0	69	31
10.	Calophyllum Oil	23	0	15	39	46	14	41	45	0	0	69	31
11.	Shark Liver Oil	24	0	14	33	43	15	42	43	0	0	72	28
12.	Pengamia Glabra Oil	26	0	15	48	37	18	44	39	0	0	78	22
13.	Duck Fat	31	2	19	48	31	21	44	33	0	0	93	7
14.	Thevetia Nerufolium Oil	33	0	26	47	27	27	45	28	0	0	99	1
15.	Terminalia Cattappa Oil	38	Trace	34	46	20	35	44	21	0	14	86	0
16.	Ter: Bellerica Oil	40	Trace	39	41	20	39	43	19	0	19	81	0
17.	Neem Oil	40	Trace	39	41	20	39	43	19	0	19	81	0
18.	Mowhra Oil	43	Trace	47	36	17	45	39	16	0	29	71	0
19.	Sarcostigma Kleinii fat	50	Trace	59	30	11	58	33	9	0	50	50	0
20.	Palm Oil	54	9	54	27	9	53	30	8	0	62	38	0
21.	Vateria Indica Fat	57	1	76	18	5	73	23	3	0	71	29	0
22.	Garcinia Cambogia Fat	58	1	78	18	3	78	18	2	0	74	26	0
23.	Garcinia Indica Fat	61	1	84	13	2	82	15	1	0	83	17	0
24.	Ox Depot, Australian	61	16	55	26	3	56	24	4	0	83	17	0
25.	Ox Depot, Indian	63	17	58	22	3	59	21	3	0	89	11	0
26.	Goat, Ditto	69	22	63	14	1	64	13	1	10	90	0	0
27.	*Actinodaphne* Hookeri Fat	99	97	3	0	0	3	0	0	97	3	0	0

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[3] The following abbreviations will be used in the text:
G=Glyceryl radical.
S=Saturated acid radical or saturated acids according to context.
U=Unsaturated acid radical or unsaturated acids according to context.
A=Azelaic acid radical, except when otherwise identified.

TABLE II

Glyceride-Type Composition of Natural Fats by Fractional Crystallization Methods

No.	Fat	S (Mol %)	Found by Fractional Crystallization (Mol %)				Calculated, Present Hypothesis (Mol %)		
			GS_3	GS_2U	GSU_2	GU_3	GS_2U	GSU_2	GU_3
1.	Almond Oil (10)	6	—	—	17	83	1	15	84
2.	Safflower Seed Oil (11)	6	—	—	18	82	1	15	84
3.	Niger Seed Oil (12)	9	—	—	26	74	2	22	76
4.	Olive Oil I (10)	15	—	—	45	55	6	32	62
5.	Tobacco Seed Oil (11)	15	—	—	45	55	6	32	62
6.	Gray Seal Oil (13)	17	—	—	51	49	8	35	57
7.	Peanut Oil (10)	19	—	1	56	43	10	38	52
8.	Olive Oil II (10)	21	—	—	61	39	12	39	49
9.	Neats Foot Oil (14)	22	—	7	51	42	13	40	47
10.	Herring Oil (15)	23	—	4	61	35	14	41	45
11.	Cottonseed Oil I (16)	28	—	13	59	28	21	43	36
12.	Sacred Baboon Fat (17)	29	—	6	74	20	22	34	44
13.	Cottonseed Oil II (18)	31	—	19	56	25	25	44	31
14.	Whale Oil (19)	31	—	9	75	16	25	44	31
15.	Neem Oil (20)	32	—	14	67	19	26	44	30
16.	Sapota Oil (21)	34	—	8	87	5	28	45	27
17.	Ceylon Bear Fat (17)	34	2	7	82	9	25	44	29
18.	Lard (22)	37	2	26	54	18	31	44	23
19.	Minusops Hekelii Fat (23)	42	1	34	55	10	42	41	16
20.	Mowhra Oil (24)	43	1	28	71	0	43	40	16
21.	Palm Oil, Grand Bassa (25)	45	4	41	43	12	42	39	15
22.	Pig External (26)	46	5	32	60	3	42	39	14
23.	Shea Butter (27)	46	4	36	55	5	43	39	14
24.	Lophira Alata Fat (28)	46	1	37	62	0	49	37	13
25.	Hodgsonia Capniocarpa Fat (29)	50	2	60	25	13	55	34	9
26.	Garcinia Morella Fat (30)	50	2	46	51	1	55	34	9
27.	Kangaroo Fat (17)	51	1	51	48	0	59	32	8
28.	Ewe External (31)	51	5	42	53	0	52	34	9
29.	Belgian Congo Palm Oil (32)	51	5	44	51	0	52	34	9
30.	Mangifera Indica (33)	51	14	24	61	1	38	36	12
31.	Pig Perinephric (26)	52	9	43	45	3	48	43	10
32.	Palm Oil Cameroons (25)	55	8	54	32	6	55	31	9
33.	Shorea Robusta Fat (34)	55	2	63	34	1	66	27	5
34.	Allanblackia Stuhlmannii (35)	56	1	66	33	0	70	25	4
35.	Allanblackia Parviflora (36)	57	2	66	32	0	70	25	3
36.	Vateria Indica Fat (37)	58	2	69	29	0	72	24	2
37.	Ewe Perinephric (31)	58	14	48	38	0	52	28	6
38.	Beef Tallow (22)	58	15	46	37	2	51	28	6
39.	Garcinia Indica Fat (30)	59	1	76	21	2	77	20	2
40.	Heifer, English (38)	61	17	49	34	0	54	25	5
41.	Allanblackia Floribunda (36)	62	2	81	17	0	83	15	1
42.	Cacao Butter (39)	61	2	77	21	0	80	17	1
43.	Phulwara Butter (40)	62	8	69	23	0	72	18	2
44.	Borneo Tallow (41)	63	5	79	16	0	79	16	1
45.	Ox Depot Fat, Calicut (42)	69	28	52	20	0	53	17	2
46.	Ox Depot Bombay (42)	75	36	52	12	0	54	9	1

present results agree well with those derived from crystallization data.

2. Whereas crystallization data does not show the presence of any GS_2U in oils with a saturated acid content of less than 22 mol per cent, the present results show that GS_2U is present; and with an S content between 12-22 mol per cent, the proportions of GS_2U are considerable.
3. The present results show that in oils with S content less *than about 30 mol per cent, where GS_3 according to chance* would be less than 2-3%, the proportions of all the glyceride types are approximately those required by chance, regardless of biologic source or fatty-acid composition.
4. In oils and fats with very small GS_3 contents, the present results show a progressive increase in the GS_2U content with increase in saturated acid content until the latter reaches about 66.6%. No such regular increase in GS_2U content is shown by the results derived from crystallization data, which frequently give widely differing GS_2U values for fats of the same GS_3 and S content.
5. Until the unsaturated acid content falls below about 45 mol per cent, the GU_3 values found by the present procedure agree closely with chance values. Where the unsaturated acid content falls below this level, the GU_3 values tend to be lower than chance values by an amount dependent on the difference between GS_3 chance and GS_3 actual. No such results are found in the calculations from crystallization data. These frequently show widely differing GU_3 contents for oils and fats with the same unsaturated acid content.
6. According to the rule of even distribution the GSU_2 content of a fat can be 100%. Crystallization data have indicated values as high as 87% although generally they indicate values above about 55 mol per cent for fats containing 19-48 mol per cent of saturated acids (Table II). The present results, Table I, show no values for GSU_2 content higher than 45-47%. Further, as with GU_3, the GSU_2 values agree with chance requirements until the unsaturated acid content falls below about 45 mol per cent. Below this they tend to be lower than chance values by an amount dependent on the difference between GS_3 chance and GS_3 actual.
7. The present results show that when GS_3 actual is about the same as GS_3 chance, the values for all the other glyceride types also agree well with those required by chance. No case has been found where GS_3 actual is higher than GS_3 chance. When GS_3 actual is lower than GS_3 chance, the values for all other types also differ from chance values. GS_2U, GSU_2, and GU_3 values always differ in the same way from chance values when GS_3 actual is less than GS_3 chance. There is always an increase in the GS_2U content to above chance values and decrease in the GSU_2 and GU_3 values to below chance values. No such relationships are evident among the results derived from crystallization data.

The experimental results obtained by the present method can be predicted from the S and GS_3 values if it is assumed that there is a limit to the amount of GS_3 which may be present in each species of fat and that when this limit is reached, any further saturated acids produced replace unsaturated acids in GSU_2 and GU_3 strictly according to chance requirements. The U in GS_2U cannot, of course, be replaced to produce GS_3 if there is a limit to the amount of GS_3 which can be present.

Mathematical study of the results shown in Table I supports this assumption. Further support will appear in Part III of this series. The relationships hold for vegetable, animal, bird, and marine fats, making it likely that they hold for all natural fats regardless of biologic source or fatty-acid composition. The following rule of glyceride-type distribution in natural fats is therefore proposed:

> The glyceride-type composition of any natural fat is that obtained by interchange according to chance of one-third of the saturated acids represented by the difference between the GS_3 content required by chance and that actu-

> ally present, with the unsaturated acids in the fat, without allowing the formation of any further GS_3.

According to this rule, where the GS_3 actual is equal to GS_3 chance, the other glyceride types will be in chance proportions. The variation of chance values with S is shown in Figure 1. GS_3 has never been found by the present procedure in greater than chance proportions.

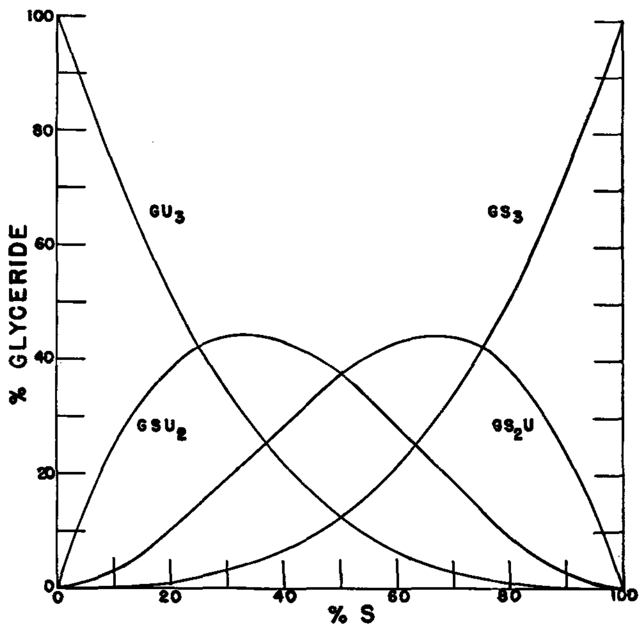

FIG. 1. The variation of glyceride-type distribution, with component saturated acid content, according to chance.

The method of calculation follows: Let the amount, in units per cent, of S substituting for U in GSU_2 be a, and that substituting for U in GU_3 be b. Then the amount of GSU_2 converted to GS_2U is equal to 3a. Similarly 3b mols of GU_3 may be assumed to be converted to GSU_2. The substitution of U by S in GU_3 will give rise to a small amount of GS_2U also, but this may be disregarded for practical purposes. The factor (GS_3 chance — GS_3 actual) is entirely converted to GS_2U by this interchange. The final composition of the fat will hence be as follows:

GS_2U actual = GS_2U chance + (GS_3 chance — GS_3 actual) + 3a
GSU_2 actual = GSU_2 chance — 3a + 3b
GU_3 actual = GU_3 chance — 3b
GS_3 actual has been found by experiment (8).

The following example illustrates the application of the method of calculation.

The acid components of a sample of low-melting vegetable fat containing zero mol per cent of GS_3 have been shown to contain 50 mol per cent of saturated acids. What is the mol per cent of each of the four glyceride types, GS_3, GS_2U, GSU_2, and GU_3, in the mixture, according to the rule stated previously?

If the S were distributed at random, the glyceride-type content would be 12.5% GS_3, 37.5% GS_2U, 37.5% GSU_2, and 12.5% GU_3. Actually no GS_3 has been found so the difference between GS_3 chance and GS_3 actual is 12.5%.

According to the rule, one-third of the saturated acids in this quantity of GS_3 will be interchanged according to chance with unsaturated acids in the fat without formation of more GS_3. In other words, $12.5/3 = 4.2$ mol per cent of S from GS_3 is interchanged, according to chance, with an equivalent quantity of U present in the chance values of GSU_2 (37.5%) and GU_3 (12.5%). The U in GS_2U will not be replaced since this would result in formation of more GS_3 which is contrary to the rule.

Two-thirds of the component fatty acids in GSU_2 are unsaturated so the unsaturated acids represented by the chance value (37.5%) of GSU_2 comprise $(37.5)(2)/3 = 25\%$ of the total fatty acids in the sample. Similarly the unsaturated acids represented by the chance value of GU_3 (12.5%) comprise 12.5% of the total fatty acids in the fat. The ratio of the unsaturated fatty acids in GSU_2 chance to those in GU_3 chance is therefore 25:12.5 or 2:1, and the 4.2% of S will be distributed in that ratio. Therefore 2.8 of the 4.2% of S will replace one U in GSU_2 and 1.4% will replace one U in GU_3. These are values a and b in the formulae given earlier. Theoretically $(1.4)(100)/12.5$, or about 12% of the U present in GU_3 chance, is interchanged with S. Therefore $(12)(12)/(100)(100)$, or about 1.5% of the total GU_3 molecules are converted to GS_2U instead of to GSU_2. This quantity represents only $(1.5)(12.5)/(100)(100)$, or 0.17% of the total quantity of fat, and can be ignored.

The glyceride composition is finally calculated by substitution in the formulae as follows:

GS_3 = zero (by analysis)
$GS_2U = 37.5 + 12.5 + (3)(2.8) = 58.4$ mol %
$GSU_2 = 37.5 + (3)(2.8) + (3)(1.4) = 33.3$ mol %
$GU_3 = 12.5 - (3)(1.4) = 8.3$ mol %.

A comparison between the experimental results found on 27 natural fats, ranging from 8-99 mol per cent of saturated acid content, and the corresponding values, calculated from the S and GS_3 contents, is shown in Table I. The experimental results were obtained by the procedure described in Part I of this series (8).

The high degree of correlation between the experimental and calculated results is self-evident and supports the proposed rule of glyceride-type composition.

The last three columns of Table II show the glyceride-type compositions, calculated in accord with the proposed rule, of the 46 fats, the compositions of which have also been calculated by the method of Hilditch and Meara (9). It can be seen readily that there is poor correlation between the two sets of results.

Summary

It has been shown that the glyceride-type composition of a natural fat can be calculated from the total mol per cent of saturated acids and the total mol per cent of trisaturated glycerides, in accord with a new rule of glyceride-type distribution, which is stated as follows:

> The glyceride-type composition of any natural fat is that obtained by interchange, according to chance, of one-

third of the saturated acids represented by the difference between the GS_3 content required by chance and that actually present with the unsaturated acids in the fat, without allowing the formation of any further GS_3.

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Sesame Oil. X.[1] The Stability of Vitamin A in Sesame Oil and in Margarine Stock Containing Sesame Oil

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CONSIDERABLE divergence of opinion exists in the literature regarding the stability of vitamin A in the presence of sesame oil. Conflicting views have been summarized by Budowski and Markley in an earlier paper in this series (2). Correspondence received in this laboratory not only indicates that confusion still exists but expresses a need for its clarification. Therefore it seemed desirable to include, in the investigations of the composition and properties of sesame oil, a study of the stability of vitamin A in the presence of this oil.

In many European countries the addition of small quantities of sesame oil to margarine is required by law to afford ready identification by means of the well known Villavecchia test. For this reason the stability of vitamin A used to fortify margarine, which contains a small quantity of sesame oil, is also of some practical interest. The stability of vitamin A added to margarine stock containing a small quantity of added sesame oil has been determined and compared to the stability of the vitamin in margarine stock containing no sesame oil. Results of stability studies on the sesame oil and margarine stock at two different temperatures are reported in this paper.

Description and Preparation of Samples

The lipide sample materials used in the investigations were: sesame oil, margarine stock, and a mixture of margarine stock and sesame oil.

The sesame oil was obtained from Colombian-grown seed by solvent extraction with commercial pentane. Following removal of the solvent at a low temperature, the crude oil was refined, bleached, and deodorized. The finished oil had the following characteristics:

Specific gravity	0.9219 25°/25°
Refractive index, $n_D^{40°}$	1.4673
Free fatty acid content	0.13%
Unsaponifiable matter	2.63%
Iodine value	116.2
Thiocyanogen value	78.0
Hydroxyl value	8.7
Peroxide value	0.0
Sesamoline content	0.35%
Sesamin content	1.06%
Color, Lovibond units	35 yellow and 0.47 red

Fresh margarine stock was obtained from a manufacturer of margarine oils. It was a mixture of 77.5% cottonseed oil and 22.5% soybean oil hydrogenated to margarine consistency.

The third sample was a mixture of the two samples just described in the proportion of 5% sesame oil to 95% margarine stock.

The sesame oil was divided into two portions. One was used as a control and the other was fortified with vitamin A concentrate.[4] Approximately 20 grams of the control portion was put into each of 14 1-oz. wide-mouth bottles fitted with plastic covers. They were stored in the dark, seven of them at a temperature of 32°F. and the other seven at 78°-80°F. Vitamin A was added to the second portion to give a fortified sample having a potency of 49.8 U.S.P. units per gram. This fortified sample was divided similarly into 20-gram lots and stored at the same two temperatures.

Portions of the margarine stock and the mixture of the margarine stock and sesame oil were fortified

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[1] For the preceding paper in this series see Analytical Chemistry, vol. 24, page 668, April 1952.

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[4] "Myvapack," natural ester form, 50,000 U.S.P. units per gram. (See footnote 5.)