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The Unsaturated Fatty Acids of the Alga Chlorella[1]

R. F. PASCHKE and D. H. WHEELER, General Mills Inc., Minneapolis, Minnesota

THE purpose of this paper is to elucidate further the composition of the unsaturated fatty acids of the fresh water alga *Chlorella pyrenoidosa* grown in pure culture in pilot plant quantities. In 1948 Milner (1) had shown the effect of certain environmental conditions on the yield of *Chlorella* and its lipid content. An excellent discussion of these results and the economic aspects of the problem appeared in 1951 (2) in this journal. Significant to us was the observation that the cell yield increased with increased nitrogen content of nutrient media but that the cells thus obtained had a low content of fatty acid. These however were highly unsaturated as indicated by iodine numbers of over 160. Restriction of nitrogen in the media gave poor yield of *Chlorella,* but the fatty acid content of the cells increased tenfold to over 60%. Iodine number of the fatty acids dropped to 125. The one sample which we examined was the low-lipid, high-iodine number type. The sample was obtained from Arthur D. Little Inc. Pilot plant studies at the laboratory were apparently aimed at high cell and high protein yields.

Milner found that the fatty acids were almost all of C_{16} and C_{18} chain length. He noted that the iodine number of the C_{16} fraction indicated at least 17% of triene fatty acids. Polyunsaturated C_{16} fatty acids are relatively rare. Heyes and Shorland (3) have recently reported a 7,10,13-hexadecatrienoic acid from rape leaf glycerides. Toyama and Tsuchiya in 1935 isolated by way of the hexabromide a 6,10,14-hexadecatrienoic (higaronic) acid as a minor component of sardine oil (4). Tutiya in 1940 reported the presence of a hexadecatetraenoic acid in another sardine oil (5). Permanganate oxidation showed the double bonds to be in either the 4,8,11,14 or the 4,8,12,15 positions (6). It will be noted that the Heyes-Shorland acid is of the normal unconjugated or singly methylene interrupted type while both higaronic acid and the Tutiya acid from fish oils contain the doubly methylene interrupted unsaturated systems. Farmer has shown that 1,5 diene systems react very slowly under conditions of alkali isomerizations used for linoleic and linolenic acids (7). Therefore the acids found by the Japanese workers would not be expected to show triene or tetraene absorption peaks in the ultraviolet spectrum after alkali isomerization. Heyes and Shorland, on the other hand, did obtain triene peaks after treatment of their hexadecatrienoic acid. (3).

Of the C_{18} fatty acids, no unsaturated octadecatetraenoic acids of plant origin are known except the conjugated acid, parinaric acid, which has unsaturation at the 9,11,13, and 15 positions. From Japanese sardine oil Toyama and Tsuchiya obtained 4,8,12,15-octadecatetraenoic (moroctic) acid. This, like higaronic acid, has two 1,5 diene systems.

In view of the possible culture and use of *Chlorella* for food or livestock feed, it is interesting to consider the relation of the composition of dietary fatty acids to that of fatty acids deposited in the body. Shorland (10) has recently pointed out that the ability of animals to transform dietary fatty acids before storing them in fat depots is greatest for those high in the evolutionary scale. Lower animals, such as fish, store dietary fats largely unchanged. (Species, temperature, amount of food intake, and other environmental factors may affect this relationship to some extent.) Higher forms have body fats more independent of diet fat.

Lovern (12) has shown that green marine algae are richer in C_{16} and C_{18} unsaturated acids while the brown and red algae are richer in the C_{20} and C_{22} unsaturated acids. The green fresh water algae *Chlorella* resemble the green marine forms in being rich in C_{16} and C_{18} unsaturates. Although the green algae are found in salt water habitats, they are the predominant fresh water type. On the other hand, the brown and red algae are the predominant salt water types (24). This effect appears to be due to the wavelength of light received rather than salinity. It has been experimentally proved by Montford (25) that the color of an alga is complimentary to the color of the light which it absorbs. Montford also concluded that the quality and intensity of the light form the limiting factors in determining the depth at which an alga can live. As would be expected therefore, the red algae are more adapted to life at the greater depths to which only the strong, deep-going, blue-green light waves will penetrate. Since the average depth of the seas is much greater than that of fresh water, it is normal that red algae are predominantly marine types. It is interesting to note that the deeper waters are usually the colder and that Japanese workers have found that oil of red algae was more unsaturated than that of the green algae (26).

The fat of fresh water fishes and of marine fishes follows that of the predominant algae of their environment since fresh water fish fats are rich in C_{16} and C_{18} unsaturated fatty acids contrasted to marine fish fats, which are richer in the C_{20} and C_{22} unsaturates (9, 11).

Higher forms of animals including mammals, except ruminants, incorporate dietary fatty acids in the body fat to a lesser degree. Still however the highly unsaturated fatty acids of *Chlorella* would be expected to be present in the fat of such animals fed on *Chlorella* to an extent which might have definite effects on the oxidative storage stability of the fat.

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Cattle and other ruminants apparently deposit fat of a composition quite independent of that of the feed. *Chlorella* as a cattle feed should therefore not affect the composition or presumably the oxidative stability of the body fat.

Since high nitrogen in the nutrient promotes the most rapid growth of *Chlorella,* this type of culture appears most promising for artificial growth of *Chlorella.* Such *Chlorella* would have a low fat content, but the fat might be undesirable in the diets of livestock (except perhaps cattle) because of highly unsaturated acids, as discussed above. If the fat could be extracted in a practical manner, this possible disadvantage would be overcome. The unsaturated acids of *Chlorella* fat should be valuable for use in air-drying protective coatings.

Milner made no attempt to study the effect of environmental temperature on the degree of unsaturation. It is possible that, as in the case of fish (13) and other animals and as in the case of plants (14, 15), lower growing temperatures would, at least to a slight extent, cause increase in unsaturation in *Chlorella* fatty acids.

Since it is inevitable that in the not too distant future mankind must turn more and more from the limited and exhaustible land areas to the bountiful sea to satisfy its need for food and raw materials, a thorough study of the composition of aquatic plants is as desirable as it would be interesting.

Experimental

The *Chlorella* as received from A. D. Little Inc., was a frozen slurry containing 20.9% solids. The slurry contained 1.68% N or 8.04% on the dry basis. This is equivalent to approximately 50% protein, dry basis. By acid hydrolysis the slurry contained 2.6% total lipid or 12.4% of the solids.

A similar *Chlorella* extracted by a process of azeotropic extraction-dehydration by the Viobin process (28) yielded a lipid of approximately 10% of the *Chlorella* on the dry weight. This lipid analyzed as 9.5% unsaponifiables and 48.5% free fatty acids. This leaves 42% of the lipid as water-alcohol soluble saponification products and indicates the complex nature of the molecules. Most of the fatty acids in the lipids are apparently bound to a large residue which is water-soluble after saponification.

The fatty acids from the Viobin-extracted lipid had an iodine value of 188.0. Extinction coefficients[2] were 7.4 at 234 mμ, 2.9 at 268 mμ, and 0.8 at 315 mμ. After alkali isomerization (16) these values were 44.0, 24.4, and 4.3, respectively. It is quite possible that conjugated fatty acids were not present in the original fatty acids but developed during extraction, handling, shipping, and saponification and are due principally to oxidation.

As mentioned by Milner (1), the lipids of *Chlorella* are exceedingly difficult to extract. Emulsification with ethyl ether combined with alternate freezing and thawing, successful with some biological material, failed completely. Very little success was achieved with hydrochloric acid hydrolysis. Since our interest was primarily with the fatty acids, the procedure eventually employed was saponification with alcoholic potassium hydroxide.

About 4,500 g. of *Chlorella* was heated overnight on the steam bath in a 12-l. flask with 3 l. of 95% ethanol, 1,500 ml. of water, and 1,008 g. of KOH (about 2 N). Since the material filtered slowly and emulsified badly when extraction of unsaponifiables was attempted, an additional 500 g. KOH in 1,000 ml. 50% ethanol were added and the mixture was heated again overnight at reflux. The material was filtered and washed, and the combined filtrates were extracted 10 times with 1,600 ml. of Skellysolve A to remove unsaponifiables. The solution was then acidified with dilute HCl and extracted as before with Skellysolve F. The combined extracts were concentrated to 2,000 ml. and washed thoroughly with dilute HCl. The HCl washings were again extracted with Skellysolve, and this was combined with the first Skellysolve solution and the whole was washed free of mineral acid with water. The solution was dried by filtering it through a column of anhydrous Na_2SO_4 and was stripped of solvent under high vacuum. The 62 g. of fatty acid obtained were crystallized three times from 10 volumes (620 ml.) of acetone at −40°C. (27). The final precipitate of solid acids (11.5 g.) had an iodine value of 6.5 and an acid number of 202.2 and corresponds to 17.2% saturated acids. The saturated acids were not examined further.

The final filtrate contained 50 g. of liquid acids. This was heated for 80 minutes in 10 volumes (500 ml.) of refluxing methanol containing 2% p-toluene sulfonic acid. The methyl esters recovered weighed 51.5 g. They were flash vacuum distilled to give 49.5 g. of a light yellow distillate (acid No. = 1.5, $n_d^{30} = 1.4636$). The 2 g. of residue were dark in color and contained some solid, presumably chlorophyll.

The distilled methyl esters had an iodine value of 218.1, and k values of 9.2 at 234 mμ and 1.2 at 268 mμ. After alkali isomerization (16) these values were 54.7 at 234 mμ, 30.9 at 268 mμ, and 2.0 at 315 mμ. The conjugation before isomerization may have been due to the prolonged alkali treatment used in isolating the acids, or to oxidation in handling. The values after isomerization qualitatively indicate the presence of diene, triene, and tetraene unsaturation. In order to obtain fractions suitable for quantitative spectral analysis, the esters were fractionally distilled.

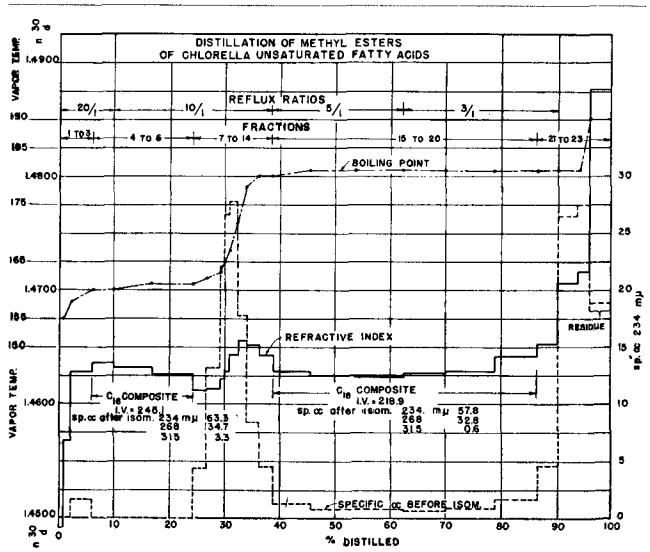

FIG. 1

[2] Extinction coefficient, k, or specific alpha, is in terms of 1 gm. per 1,000 cc. at 1 cm. cell thickness, measured in 95% ethanol.

TABLE I

Ultraviolet Absorption Coefficients, k, (Specific Alpha)
After Alkali Isomerization in 6.5% KOH in Glycol at 180°, 25 minutes (air)
k at 1 gm. per 1,000 cc., 1 cm. *(Calculated from data of Brice *et al.* (17) for all-cis acids)

No. of Carbons in Acid or Esters	Monoene		Diene			Triene				Tetraene				
	Mol. Wt.	I.V.	Mol. Wt.	I.V.	233 Mμ	Mol. Wt.	I.V.	233 Mμ	268 Mμ	Mol. Wt.	I.V.	233 Mμ	268 Mμ	315 Mμ
C_{16}	254.4	99.8	252.4	201.2	102.1	250.4	304.2	69.2	57.1	248.4	408.8	70.2	64.4	27.0
C_{17}	268.4	94.6	266.4	190.6	96.7	264.4	288.0	65.5	54.1	262.4	387.0	66.5	60.9	25.5
C_{18}	282.5	89.9	280.4	181.0	91.9*	278.4	273.5	62.2*	51.4*	276.4	367.4	63.1	57.8	24.2
C_{19}	296.5	85.6	294.5	172.4	87.5	292.4	260.4	59.2	48.9	290.4	349.6	60.1	55.0	23.1
C_{20}	310.5	81.8	308.5	164.6	83.5	306.5	248.5	56.5	46.7	304.5	333.5	57.3*	52.5*	22.0*
C_{21}	324.5	78.2	322.5	157.4	79.9	320.5	237.6	54.0	44.6	318.5	318.8	54.8	50.2	21.0
C_{22}	338.6	74.98	336.5	150.9	76.6	334.5	226.6	51.8	42.8	332.5	305.4	52.5	48.1	20.1

The distilled methyl esters were fractionated in a Podbielniak miniature Hypercal column at 5 mm. pressure (for log see Figure 1). The charge was 48.62 g. and the pot residue 4.64 g. The residue was quantitatively transferred to a micro-Vigreaux-Claisen flask and two more fractions were taken (Nos. 22 and 23). The final residue (No. 24) weighed 1.27 g. As shown in the graph, the C_{16} and C_{18} fractions were the only important fractions obtained and the conjugated material was readily removed. As indicated, two composites were made, one containing the non-conjugated C_{16} and the other the non-conjugated C_{18} methyl esters.

From examination of the boiling point curve it appears that there could be no more than 1 to 2% of unsaturated acids shorter than C_{16} in chain length, about 30% of C_{16}, 64% C_{18}, and possibly 4 to 5% greater than the C_{18}. This last is only an apparent value since it would include any thermal polymer formed during the distillation. Polymers, if present, would be formed mostly at the expense of C_{18} fraction, especially the tetraene portion of that fraction.

In order to calculate the composition of the C_{16} and C_{18} fractions by spectral analysis, extinction coefficients (after isomerization) were calculated for acids or esters of various chain lengths. The data of Brice *et al.* (17) on the normal all-cis acids, linoleic, linolenic, and arachidonic, was used as determined by them for the isomerization conditions of Kraybill *et al.* (16), namely, 6.5% KOH in glycol at 180° for 25 minutes in air. Table I shows these calculated values for C_{16} to C_{22} acids or esters made by merely allowing for molecular weight. Separate listings are not necessary for acids and esters since, *e.g.*, ethyl palmitoleate has the same molecular weight as oleic acid, and methyl linoleate the same as a C_{19} acid with two double bonds. Molecular weights and iodine values are also included in the table. Thus the values for C_{16} methyl esters would be found in the C_{17} line, and so forth.

The isomerization method used was essentially that of Kraybill *et al.* (16) as mentioned above. The samples, in microbeakers, were added to the preheated glycol reagent, and the mixture was stirred with a thin stirring rod. The reaction mixture was stirred two more times at about one-minute intervals. The stirring rod was left in the tube, being washed off carefully with solvent at the end of the reaction. Timing was started after the first stirring.

Compositions were calculated from the observed k values at 234, 268, and 315 mμ after isomerization as described. Tetraene was calculated by dividing the k found at 315 mμ by the appropriate value from Table I. Triene was similarly calculated from the k found at 268 mμ after subtracting the contribution of tetraene at 268 mμ. Diene was similarly calculated from the k found at 234 mμ, after subtracting the contribution of tetraene and triene at 234 mμ.

The C_{16} composite fraction after isomerization showed: $k = 63.3$ at 234 mμ, $k = 34.7$ at 268 mμ, and $k = 3.3$ at 315 mμ.

The C_{18} composite fraction after isomerization showed: $k = 57.8$ at 234 mμ, $k = 32.8$ at 268 mμ, and $k = 0.6$ at 315 mμ.

Table II gives the amounts of mono-, di-, tri-, and tetra-unsaturated methyl esters in both the C_{16} and C_{18} fractions thus calculated from the constants in Table I. Included are the iodine values as determined by the rapid Wijs procedure (26) and iodine values calculated from the fatty ester composition of each of the two fractions.

TABLE II

	Me C_{16}	Calculated Contribution to I.V.	Me C_{18}	Calculated Contribution to I.V.
Tetraene	12.9	50.1	2.6	9.1
Triene	49.7	143.2	64.3	167.4
Diene	22.9	43.6	20.8	35.9
Monoene (by diff.)	14.5	13.7	12.3	10.5
Total	100.0%	250.6	100.0%	222.9
Rapid Wijs I.V. determined		245.1		218.9

The excellent agreement between the determined and calculated iodine values is probably fortuitous. But even so the differences are in the direction that one might expect if small amounts of saturated esters were present. This is, no doubt, the case since the saturates were originally crystallized as the free acids. Under such conditions some saturates would tend to remain in the filtrate as eutectics with at least the mono- and di-unsaturated acids.

It is doubtful that any *trans* double bonds are present. Infrared curves indicate their absence as shown by no band at 10.32μ. Also ultraviolet spectral values would then have been low (18), which, in turn, would give much lower calculated iodine values.

It is also doubtful that the esters contain any doubly-methylene interrupted unsaturation (—CH=CH—CH_2—CH_2—CH=CH—), as is found in Japanese sardine oil (4, 5, 6). The alkali isomerization rate of such systems would be only a fraction of that of the normal singly interrupted systems (—CH—CH—CH_2—CH=CH—) as found in linoleic, linolenic, and arachidonic acids. In the alkali conjuga-

tion of poly-unsaturates, doubly methylene interrupted systems are actually formed, and, once formed, they appear to be very resistant to isomerization to higher degrees of conjugation. For example, Brice and Swain (19) have shown in time studies on the alkali isomerization of linolenic acid that, once maximas are reached, there is no conversion of diene conjugation to triene conjugation.

In addition to showing the absence of *trans* double bonds, infrared indicates that no terminal unsaturation is present. Infrared shows no unsaturation conjugated with the carbonyl.

Bromination Studies

C_{18} Fraction. One g. of the C_{18} composite was saponified to give 0.93 g. of the free acid. This was brominated according to the procedure of White and Brown (21) to give 0.31 g. of dry hexabromides (hexabromide number = 33) melting at 177.5-178.2° C. To determine approximate solubility losses, this procedure was repeated on 1 g. of known hexabromide from linolenic acid, using solvent but no bromine. The recovered known hexabromide weighed 0.98 g. and melted at 180.5 to 180.8°C., using melting point procedures previously described (22). A mixture of equal parts of the two bromides melted at 178.3-178.6°C., indicating that the *Chlorella* C_{18} triene apparently is largely 9,12,15-cis,cis,cis-octadecatrienoic acid (linolenic).

The combined filtrates from the hexabromide determination (volume about 30 ml.) was allowed to crystallize slowly at —26°C. (Rapid cooling caused the bromides to "oil out.") Crystals weighing 0.26 g. were obtained. They softened below 80°C. and melted and cleared at about 95°C. Crystallization from 5 ml. Skellysolve B and washing with 7 ml. more of solvent at room temperature gave 0.17 g., which softened on immersion at 92°C. and melted and cleared at 103-105°C. Another crystallization from 5 ml. of Skellysolve B containing 0.4 ml. ethyl ether gave 0.09 g., melting at 103.7°C., and clearing at 106.5°C. A mixed melting point of 3 parts of this material with 1 part of pure tetrabromide of linoleic acid (M.P. 115°C.) showed a melting range of 105-110°C. No depression indicates the presence of considerable amounts of 9,12-cis,cis-octadecadienoic acid (linoleic) in the C_{18} unsaturates of *Chlorella*. The tetrabromide of linoleic acid and the hexabromide of linolenic acid form an eutectic containing about 9% of the hexabromide (22) and melting at 112°C. In addition to hexabromide, the above 0.09 g. of impure tetrabromide no doubt contains some solid octabromides and possibly some liquid bromides which are sparingly soluble in Skellysolve. It is probable that any of these would tend to lower the melting point from that (112°C.) of the known tetra-hexa eutectic. The presence of positional isomers of linoleic acid is not excluded, but it appears fairly certain that considerable amounts of the 9,12-cis,cis acid are present. Otherwise the melting point would have been depressed rather than raised 4° by the small amount of authentic linoleic tetrabromide added.

An additional crystallization from 1 ml. of ethyl ether at —4°C. raised the melting point 1.5°. The yield was 0.05 g. The material appeared to soften only very slightly when placed in the bath at 100°C. It melted and cleared at 108°C.

All temperatures are corrected.

C_{16} Fraction. Since the C_{16} composite contained 12.9% tetraene as contrasted to only 2.6% in the C_{18} composite, no attempt was made to separate any bromide fraction as the free acid. It is unlikely that any relatively pure hexabromide or octabromide could have been obtained. The first material crystallizing in any quantity would probably be a hexabromide-octabromide eutectic. The methyl esters were therefore brominated without previous saponification according to the polybromide estimation procedure of White, Orians, and Brown (23). Gain in weight of the total esters indicated that only about 80% of the theoretical amount of bromine had been absorbed.

From 1.045 gm. of methyl ester brominated in 35 cc. ether at 0°C., 0.138 gm. of insoluble bromides (M.P. 140-160°) were obtained. Recrystallization from 20 cc. of ether at 0° afforded 0.085 gm. of precipitate and 0.042 gm. of filtrate material. The filtrate material melted at 132-135° and analyzed 62.9% bromine, presumably a mixture of methyl tetrabromopalmitate (theory = 54.6% Br) and methylhexabromopalmitate (theory = 64.5% Br).

The precipitate from above was recrystallized from 20 cc. of ether at 25° to afford 0.025 gm. of precipitate and 0.055 gm. of filtrate material. The precipitate melted at 181-190° and showed 65.5% bromine, presumably methyl hexabromopalmitate (theory = 64.5% Br) contaminated with some methyl octabromopalmitate (theory = 71.0% Br). The filtrate material melted at 151-155° and showed 64.7% bromine, very close to theory for methylhexabromopalmitate (64.5%).

Discussion

The C_{16} tetraene is the most interesting of the *Chlorella* fatty acids. In view of the observations made, the acid must have cis double bonds either in the 3,6,9,12, in the 4,7,10,13, or in 5,8,11,14 positions. The first isomer is perhaps the least probable since fatty acids with the double bonds in the 3,4-position are rare in nature. Mycomycin has such a double bond, but it is stabilized by conjugation with another double bond in the 5,6-position (20). The 4,7,10,13 is perhaps the most probable. This would relate it to the 7,10,13-hexadecatrienoic acid of Heyes and Shorland (3). Also it would possess a terminal saturated chain of three carbons. Most common nonconjugated acids have terminal saturated carbon chains of multiples of three carbons, *e.g.*, oleic has nine, linoleic and arachidonic have six, and linolenic has three.

The C_{18} tetraene is probably an analog of the C_{16} acid. Perhaps the most probable structure is the 6,9,12,15 isomer. The situation would thus be analogous to the relationship between the 7,10,13-hexadecatrionic acid of rape leaf glycerides and linolenic acid (3).

It is quite likely that the mono-, di-, and tri-unsaturated C_{18} acids of *Chlorella* are identical with well known unsaturates (oleic, linoleic, etc.). Since the amounts of these acids available in the C_{16} and C_{18} fractions were small (less than a gram in some cases), further elucidation of the structure awaits perfection of micro methods for oxidative cleavage of double bonds and identification of subsequent products. At the present time paper chromatography seems to offer the greatest hope for success in this direction.

Summary

The fatty acids of alga *Chlorella* have been examined. The cells used were grown in high concentrations of fixed nitrogen and therefore contained only 12.4% lipid on a dry weight basis. The lipid contained only some 50% fatty acids, about 80% of these containing unsaturation. All double bond systems appear to be of the all-cis, singly methylene interrupted type. The C_{18} diene and triene appear to be largely normal linoleic and linolenic acids. Perhaps the most probable structure for the unusual hexadecatetraene is 4,7,10,13-cis,cis,cis,cis-hexadecatetraenoic acid. The estimated composition of the fatty acids of *Chlorella* is:

Below C_{16}	less than 2%
Saturated	17%
C_{16} monoene	4%
C_{16} diene	6%
C_{16} triene	12%
C_{16} tetraene	3%
C_{18} monoene	7%
C_{18} diene	11%
C_{18} triene	34%
C_{18} tetraene	1%
Above C_{18}	less than 4%

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The Glyceride Structure of Natural Fats. III. Factors Governing the Content of Fully Saturated Glycerides

A. R. S. KARTHA,[1,2] Maharaja's College, Ernakulam, India

IN part I of this series (1) a procedure was described for the quantitative determination in fats of the four glyceride types GS_3, GS_2U, GSU_2, and GU_3.[3]

In part II (2) a rule was stated for calculating the glyceride type distribution in natural fats. It was based on the assumption that there is a maximum proportion of GS_3 which may be present in each species of fat. This limit varies, according to circumstances, up to the proportion of GS_3 which may be produced by random or chance distribution of the component fatty acids among the glyceryl radicals. When the proportion of GS_3 which can exist is less than that which could be synthesized by chance distribution of the saturated fatty acids, the excess S is distributed according to chance among the remaining glyceryl radicals without formation of any more GS_3. In the present contribution some factors governing the quantity of GS_3 which may be present in a fat will be discussed.

Any hypothesis accounting for glyceride type distribution must explain the operation, with remarkable accuracy, of the rule for calculation of the glyceride type distribution in natural fats. It must also show how restriction of chance distribution, when this occurs, is effected. It must agree with what appears to be the fact that fatty acid synthesis and esterification take place in all vegetable and animal fat depots (3).

It must also agree with certain evidence that in adipose tissues as well as in mammary glands of animals fat can be deposited from ingested foods without affecting the normal glyceride type distribution. Kartha and Menon (4) have shown that in buffalo and cow milk fats, and in ox depot fat, the ratio of GS_3 actual to GS_3 chance retains a value of very nearly unity in spite of variations in saturated acid content from about 50% to about 70% due to variations in food fat, etc. Kartha (4) has shown that samples of the same depot fats from different sources, varying in saturated acid content possibly because of differences in diet, all give analytical results in agreement with those obtained by application of the rule for calculation of glyceride type distribution.

Furthermore in any hypothesis the mechanism of esterification postulated must agree with the known specificities of the lipases effecting the esterification under normal *in vivo* conditions (5). It must also agree with the fact that lipolytic esterification is reversible and proceeds according to the law of mass action (6).

It is well known that the melting ranges of refined fats vary. This variation may be due to several causes, such as the relative proportions of saturated

[1] Present address, Indian Agricultural Research Institute, New Delhi, India.

[2] Edited and revised by R. J. Vander Wal, Armour and Company, Research Division, Chicago, Ill.

[3] The following abbreviations will be used in the text:
G = Glyceryl radical.
S = Saturated fatty acid(s) or saturated fatty acid group(s) according to context.
U = Unsaturated fatty acid(s) or unsaturated fatty acid group(s) according to context.