present data. Thus the birds fed coconut oil and no cholesterol had essentially the same visual score as any of the four groups fed corn oil despite the fact that the latter groups had at least twice the dienoic acid concentration of the aorta as found in the former groups. This does not rule out the possibility that the type of fatty acid does play a role in plaque formation, as suggested by the higher scores but low essential fatty acid intake of the birds fed coconut oil and cholesterol.

The striking similarity of relationships between cholesterol level and polyunsaturated fatty acid content of plasma and each of the aortic segments supports the suggestion of Hirsch and Nailor (15) that aortic lipids reflect passive deposition from plasma. Close inspection of the comparative fatty acid analyses indicate however considerable differences between plasma and aortic fat, the interpretation of which must await further investigation.

The data in Table II show that dietary coconut oil, at 5 weeks, resulted in higher plasma cholesterol values than did dietary corn oil (16). After 5 weeks the cholesterol levels decreased on the coconut oil diets and increased on the corn oil diets. At 20 weeks corn oil-fed groups thus had the higher plasma cholesterol levels. While there is no ready explanation for the steady increase with time in plasma cholesterol for the corn oil groups, the decrease observed on the low protein-coconut oil diets is very likely caused by the increasingly greater ability of the low-protein level to satisfy the protein needs of the older animal. Whereas 10% protein satisfies no more than half the protein needs of a young chick, this amount is fully adequate to meet the requirements of an essentially mature bird at 20 weeks of age. Whatever the explanation for these findings however, they do point up the danger of evaluating short experiments in terms of dietary variables on atherogenesis, a process which presumably continues over the entire life-span of the animal.

Acknowledgments

Thanks are given to Mrs. O. Donis and H. Lutz for excellent technical assistance, also for materials supplied by E. F. Drew and Company Inc., New York, and by Merck, Sharp, and Dohme, Rahway, N.J.

REFERENCES

1. Ahrens, E. H., Insull, W., Blomstrand, R., Hirsch, J., Tsaltas, T. T., and Peterson, M. L., The Lancet, *272*, 943–953 (1957).
2. Beveridge, J. M. R., Connell, W. F., Mayer, G. A., and De Wolfe, M. S., J. Nutrition, *56*, 311–320 (1955).
3. Leveille, G. A., and Fisher, H., Proc. Soc. Exp. Biol. Med., *98*, 630–632 (1958).
4. Sinclair, H. M., The Lancet, *270*, 381–383 (1956).
5. Fisher, H., Feigenbaum, A. S., Leveille, G. A., Weiss, H. S., and Griminger, P., J. Nutrition, *69*, 163–171 (1959).
6. Fisher, H., Weiss, H. S., Leveille, G. A., Feigenbaum, A. S., Hurwitz, S., Donis O., and Lutz, H., Brit. J. Nutrition, *14*, 433 (1960).
7. Weiss, H. S., J. Gerontology, *14*, 19–24 (1959).
8. Fisher, H., and Feigenbaum, A. S., Nature, *186*, 85–86 (1960).
9. Weiss, H. S., and Fisher, H., Am. J. Physiol., *197*, 1219–1223 (1959).
10. Fisher, H., and Johnson, D. Jr., J. Nutrition, *60*, 261–273 (1956).
11. Griminger, P., and Fisher, H., Proc. Soc. Exp. Biol. Med., *99*, 424–426 (1958).
12. Luddy, F. E., Barford, R. A., Riemenschneider, R. W., and Evans, J. D., J. Biol. Chem., *232*, 843–851 (1958).
13. Morris, S. G., Riemenschneider, R. W., and Evans, J. D., Arch. Biochem. Biophys., *78*, 183–185 (1958).
14. Kahn, S. G., and Yacowitz, H., Fed. Proc., *17*, 251 (1958).
15. Hirsch, E. F., and Nailor, R., A.M.A. Arch. Path., *61*, 469–487 (1956).
16. Leveille, G. A., Feigenbaum, A. S., and Fisher, H., Arch. Biochem. Biophys., *86*, 67–70 (1960).
17. Nikkila, E. A., and Ollila, O., Acta Path. Microbiol. Scand., *49*, 177–180 (1957).
18. Stamler, J., Pick, R., and Katz, L. N., Circulation Res., *6*, 447–451 (1958).
19. Nishida, T., Takenaka, F., and Kummerow, F. A., Circulation Res., *6*, 194–202 (1958).

[Received June 8, 1960]

Glyceride Structure of Vegetable Oils by Countercurrent Distribution. V. Comparison of Natural, Interesterified, and Synthetic Cocoa Butter[1]

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The glyceride structure of cocoa butter is of considerable practical importance and of particular theoretical interest. Countercurrent distribution, gas chromatography, and isotopic dilution methods are employed in its study. The observed fractionation of glycerides is accounted for by assuming that palmitic and stearic acids are randomly esterified on the 1 and 3 positions of glycerol and that oleic is on the 2 position, as demonstrated by other workers. Complete randomization of the specific structure of cocoa butter through the application of interesterification catalysts greatly alters its physical properties, including its countercurrent distribution pattern. A glyceride synthesized according to the "1,3 random palmito-stearo-2-olein" concept has properties similar to natural cocoa butter.

A CONFECTIONERY coating fat with properties equal or superior to cocoa butter could provide a new, large market for domestic fats and vegetable oils. Two necessary steps are the learning of the glyceride structure of natural cocoa butter and the synthesizing of a fat of equivalent structure on a laboratory scale.

Fundamental interest in the glyceride structure of cocoa butter stems from a nonrandom distribution of palmitic, stearic, oleic, and linoleic fatty acids among its glyceride molecules (1,2). Recent and current work, which exploits the high-resolving power of countercurrent distribution for fractionating glycerides, has shown that constitutionally different glycerides in linseed (3), soybean (4,5), corn (6), and safflower (7) oils agree with a random pattern. These results are contrary to those obtained by Hilditch

[1] Presented before Division of Agricultural and Food Chemistry, American Chemical Society Meeting, Cleveland, O., April 5–14, 1960.

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and co-workers who used crystallization procedures that led to the concept of "even distribution" as the generalized pattern of glyceride structure (8). It was evident that cocoa butter also needed to be examined by countercurrent distribution.

Part IV of this series (9) presents evidence that the pattern of cocoa butter fat is neither random nor even. By applying recent countercurrent distribution, gas chromatographic, and isotopic dilution techniques, new and more precise information is presented on the glyceride structure of natural cocoa butter. The basic pattern proposed for the glyceride structure of cocoa butter is "1,3 random palmito-stearo-2-olein." Results from fractionating natural cocoa butter are compared with those from a sample of cocoa butter randomized by an interesterification catalyst and also with those determined for a sample of glycerides synthesized according to the proposed concept of glyceride structure.

Experimental

Methods of countercurrent distribution and of determining fatty acid composition by the spectro-iodine value procedure are described in Part IV of the series (9).

Melting range of fractions was measured in a water bath warmed at a rate of 6° per hour. The melting behavior was observed in the same flasks used for evaporating solvent from the fractions. Gas chromatography was used to determine palmitic (P), stearic (S), oleic (O), and linoleic (L) acids after conversion of glycerides to methyl esters. A 5-ft. column of Reoplex 400 on Celite, held at 200°C., was connected to a thermal conductivity detector. The helium flow rate was 45 ml. per minute.

Radioactive triglycerides, C^{14} carboxy-labelled tripalmitin and tristearin, were added to a 15-g. sample of natural cocoa butter. Approximately 10 μc. (3 mg.) each were used. Radioactivity of fractions from the countercurrent distribution apparatus was determined on every fifth tube. The sample used to establish the weight distribution curve was washed into a 20-ml. glass vial with 15 ml. of scintillation solution composed of 3 g. of 2,5-diphenyloxazole and 100 mg. of 1,4-di(2-[5-phenyloxazolyl]) benzene per liter of toluene. Activity was determined with an automatic Tri-Carb scintillation spectrometer.

Interesterified cocoa butter was prepared by heating 20.8 g. of natural cocoa butter in the presence of 0.75 ml. of 6.5% sodium methoxide (10). At the same time tritium-labelled palmitic acid, as the methyl ester (40 mg. and 50 μc.) prepared by the Wilzbach tritium-gas-exposure technique (11) and C^{14} carboxy-labelled stearic acid, as tristearin (1.2 mg., 3.7 μc.), were introduced into the glyceride structure randomly by adding them to the reaction mixture at the start of the interesterification. The mixture was heated to 174°C. over a period of 1½ hrs. and held under vacuum to remove methanol; then the catalyst was destroyed by adding 10 ml. of glacial acetic acid at 100°C. After washing an ether solution of this interesterified and double isotope-labelled cocoa butter three times with water to remove acid and salts, the fat was dried and stored at 0°C. pending countercurrent distribution.

Analysis of palmitic and stearic acids in the interesterified cocoa butter was carried out by isotope dilution methods. Tritiated palmitic and C^{14}-labelled stearic acids were counted simultaneously, using the scintillation spectrometer at voltage tap No. 3 and channel settings of 10–50 and 50 to 100 volts. Calculations were made by the isotopic ratio procedure of Okita *et al.* (12).

Synthetic glycerides having oleic acid in the 2 position with palmitic and stearic acids randomly distributed on the 1 and 3 positions were prepared as follows. The directed interesterification process of Baur and Lange for mono-acid diglycerides (13) was modified in preparing the random mixture of 1,3 diglycerides of palmitic and stearic acids by starting with equal amounts of tristearin and tripalmitin. After crystallization, the diglycerides contained 45.7% palmitic and 54.3% stearic acids. They were esterified on the 2 position with oleyl chloride as described by Youngs (14).

Results and Discussion

Natural Cocoa Butter. Countercurrent distribution and analysis data on natural cocoa butter with added tristearin 1-C^{14} and tripalmitin 1-C^{14} are given in Figures 1 and 2. After 700 transfers had been applied under recycle operation in the pentane-hexane nitroethane-furfural system (6 ml. of upper layer and 40 ml. of lower layer in each tube), 400 upper-layer fractions were collected. The weight curve is similar in shape to that previously published in Part IV (9). Spectro-iodine values for linoleic and oleic contents of fractions (not plotted), while more complete, are also similar to those of the earlier work. The analyses for palmitic, stearic, oleic, and linoleic acids presented in Figure 2 were carried out by gas chromatography. Because of the greater number of these gas chromatographic analyses over the previously published analyses, more accurate calculations of glyceride composition may be made.

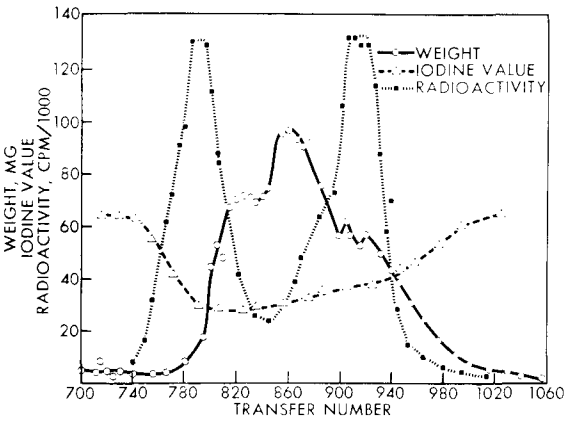

FIG. 1. Countercurrent distribution of natural cocoa butter with added tripalmitin C^{14} and tristearin C^{14}. The peak for radioactivity on the left is tristearin and on the right is tripalmitin.

Peaks for labelled tristearin and tripalmitin (Figure 1) mark the positions in the distribution where these two triglycerides should appear if present. The normality of their shape in the presence of other glycerides gives assurance of nearly ideal operation of the countercurrent distribution system. At the same time the incomplete separation of these two saturated homologues in the present system of immiscible solvents explains the incomplete separations of the more complex cocoa butter triglyceride mixture.

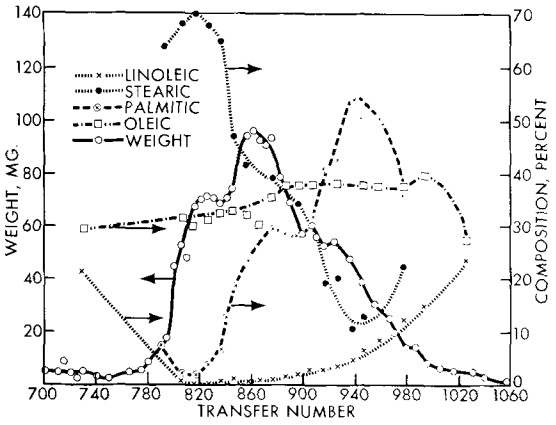

FIG. 2. Fatty acid composition of fractions from the countercurrent distribution of natural cocoa butter presented in Figure 1.

It is believed from the linoleic acid content of transfers 700–830 that during the 700 recycle transfers the saturated leading edge of the band caught up to and passed slightly over the trailing end of the unsaturates. However the weight of fractions in this region is small and does not greatly affect the calculation of glyceride composition.

In agreement with previous work (1,2) and as supported by the position at which labelled tristearin is found to occur (Figure 1), the amount of tristearin in natural cocoa butter is insignificant to the weight distribution curve. The predominant glyceride in this region must be oleo-distearin, which accounts for the peak in the weight curve at stage 825 and which is contained primarily in stages 800–840. The merging of analytical curves for stearic, oleic, and palmitic acids, at a level of approximately one-third in stages 840–910, is indicative of oleo-palmito-stearin, the major triglyceride of cocoa butter, which has its peak at stage 860. From this point on palmitic acid increases more than 33%, indicating the occurrence of oleo-dipalmitin.

Previous studies, using radiotracers (5), have shown that the effect upon the partition coefficient for glycerides of increasing unsaturation by one double bond, *e.g.*, oleic acid replacing stearic acid, is approximately equal to the effect of shortening the carbon chain by two methylene groups, *e.g.*, palmitic acid replacing stearic acid. This approximate equality explains the distinctly inferior separations obtained here in cocoa butter glycerides containing C_{16} and C_{18} compared with fats composed primarily of C_{18} fatty acids. Based upon this assumption of approximate equality, the 20 possible glycerides (constitutional isomers of cocoa butter) permitted under random pattern may be arranged in six groups of increasing polarity or functionality compared with tristearin (Table I).

Previous (9) and present data show that oleic acid occurs at least once and to only a small extent twice in each glyceride molecule of cocoa butter; evidence by other workers shows that this oleic acid is selectively in the 2 position (15,16,17). Therefore the major glycerides are limited to a) 1,3 distearo-2-olein (SOS); b) 1 stearo-2-oleo, 3-palmitin (SOP); and c) 1,3-dipalmito-2-olein (POP) in polarity categories of 1, 2, and 3. This simplification encouraged an attempt to account for the observed weight distribution curve in terms of three component theoretical curves and a difference curve for components of 4 and higher polarity. In constructing the theoretical component curves of Figure 3, the amount of SOS was first calculated from the fatty acid data of Figure 2 and then was plotted (open circles). The partition coefficient was determined, and the theoretical distribution curve (broken line and open circle No. 1) was calculated and plotted. The area under this curve corresponds to 26.5% of the total area and to a 26.5% content of SOS. Similarly the amount of SOP was calculated to be 38.7% (broken line No. 2). For

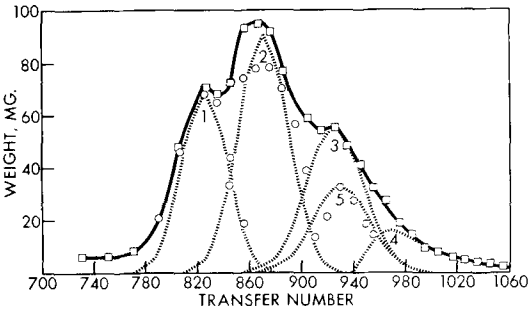

FIG. 3. Component analysis of weight distribution curve of natural cocoa butter as guided by glyceride composition data.

TABLE 1

Calculated Glyceride Composition of Cocoa Butter for Random; Even; Kartha; 1,3 Random Palmito-Stearo-2-Olein Distribution Compared with Experimental Results (%)

Glyceride	Polarity	Theoretical patterns				Experimental results			
		Random	Even	Kartha	1,3 Random palmito-stearo-2-olein	Scholfield (9)	Present	Hilditch-Stainsby (1)	Meara (2)
SSS	0	4.14	.....	0.41	.....	0.16	0.1	.....	.....
SPS	1	9.44	.....	1.01	.....	.....	.....	.....	.....
SOS	1	12.75	0.6	21.97	26.94	22.00	26.5	18.4	22.0
SPP	2	7.18	.....	0.82	.....	.....	.....	.....	.....
SOP	2	19.38	84.5	36.25	40.79	41.00	38.7	51.9	57.0
SSL	2	1.29	.....	1.89	.....	.....	.....	.....	.....
SOO	2	13.08	4.0	7.33	3.53	.....	.....	12.0	5.8
SPL	3	1.97	.....	3.79	.....	.....	.....	.....	.....
PPP	3	1.82	.....	0.23	.....	.....	.....	.....	.....
POP	3	7.37	.....	14.92	15.44	12.00	15.1	6.5	3.7
POO	3	9.94	.....	5.96	2.67			8.4	7.4
OOO	3	4.47	.....	0.46	0.12		11.4	.....	1.1
SOL	3	2.65	10.9	1.52	5.6			.....	.....
PPL	4	0.75	.....	1.82	.....			.....	.....
POL	4	2.02	.....	1.24	4.24			.....	.....
SLL	4	0.13	.....	0.11	.....			.....	.....
OOL	4	1.36	.....	0.14	0.37		8.4	.....	.....
PLL	5	0.10	.....	0.09	.....			.....	.....
LOL	5	0.14	.....	0.02	0.29			.....	.....
LLL	6	0.005	.....	0.001	.....			.....	.....

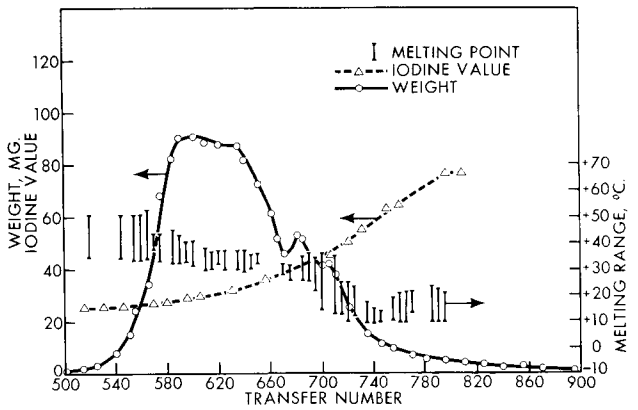

FIG. 4. Countercurrent distribution of natural cocoa butter showing iodine values and melting-point ranges of the fractions.

polarity group 3, of which POP is a member, 26.5% was calculated (broken line No. 3).

The residuum composed of glycerides of categories 4, 5, and 6 between this theoretical curve and the weight curve is given in broken line No. 4 and amounts to 8.4%.

Calculation of POP from the analytical data of Figure 2 gave the data points shown in Figure 3 as open circles. The area under the theoretical curve, calculated by using these data (broken line No. 5), corresponds to 15.1% POP, which leaves 11.4% (26.5–15.1) to be accounted for by other members of the category 3 glycerides.

Results of this type of analysis for glyceride composition (Table I), which involves the construction of component theoretical curves guided by analyses for fatty acids, differ little from our earlier published results (9), which were calculated solely from the fatty acid analysis data. The present analysis has an advantage over past work because gas chromatography was used to determine fatty acid compositions, more frequent fraction analyses were obtained, and the binomial behavior of glyceride types on countercurrent distribution could be exploited.

Results differ from those of Hilditch and Stainsby (1) and of Meara (2) principally in the lower content of palmito-oleo-stearin and in the greater amount of dipalmito-olein.

Of the theoretical patterns for fatty acid distribution yet proposed, random and even appear most divergent from the experimental data. Kartha's (18) procedure randomly proportions all the trisaturates greater than an experimentally determined value among remaining glycerides. This pattern, when extended to the individual triglycerides, accounts closely for the amounts of compositionally different glycerides. It does not account for the observation that the saturated acids are concentrated in the 1 and 3 positions and oleic acid is found mainly in the 2 position (15,16,17). Vander Wal however pointed out that modified random distribution for cocoa butter does not necessarily obviate the possibility of a specific configuration in the glycerides (19).

A variant pattern of glyceride structure formulated here is also consistent with experimental data. It assumes the experimentally-supported observation that oleic acid occurs once in each cocoa butter glyceride and exclusively in the 2 position (15,16,17). It further assumes a random distribution of palmitic, stearic, linoleic, and the residual oleic over 33% on the 1,3 positions of glycerol. This structure, brefly described as "1,3 random palmito-stearo-2-olein," has the virtue of direct calculation, if not significance to the biological mechanism of synthesis.

Evidence for a degree of randomness is found in the compositionally-distinguishable glycerides of vegetable oils. In all liquid oils yet examined by countercurrent distribution (3,4,5,6,7) the glycerides which can be separated by this technique were present in amounts approximating a random pattern. However enzymatic hydrolysis (15,16) shows the saturated acids of these unsaturated oils to be concentrated in the 1 and 3 position. Mattson and Lutton's data also show an "element of randomness" to these liquid oils if not complete randomness in the specific case of olive oil. The effect of specificity upon glyceride composition appears to be most pronounced in high-melting, highly-saturated fats like animal fats and "vegetable tallows," including cocoa butter.

Prior to completion of this manuscript, a paper by Vander Wal (20) has been published, in which he calculated the distribution of saturated and unsaturated acyl groups in fats from pancreatic lipase hydrolysis data. Since this computation does not differentiate between individual saturated and unsaturated fatty acids and the compositionally distinguishable glycerides, direct comparisons of his figures and those in Table I are possible only by putting the figures for the individual glycerides into glyceride types. For his calculated values of GS_3, GS_2U, GSU_2, and GU_3 of 7.1%, 67.5%, 23.3%, and 2.1%, the corresponding values for the 1,3 random palmito-stearo-2-olein hypothesis are 0.0%, 83.17%, 16.01%, and 0.78%. The corresponding experimental values of Hilditch and Stainsby (1) are 2%, 77%, 21%, and 0.0%; and of Meara (2) 0.0%, 82.7%, 13.2%, and 0.0%. The amount of GS_2U found in the present analysis is 83.2%. Thus both Vander Wal's calculation and the present 1,3 random palmito-stearo-2-olein pattern account for the observed glyceride types, but the latter hypothesis has the advantage of describing the composition of the compositionally-distinguishable glycerides in terms of their individual component saturated and unsaturated fatty acids.

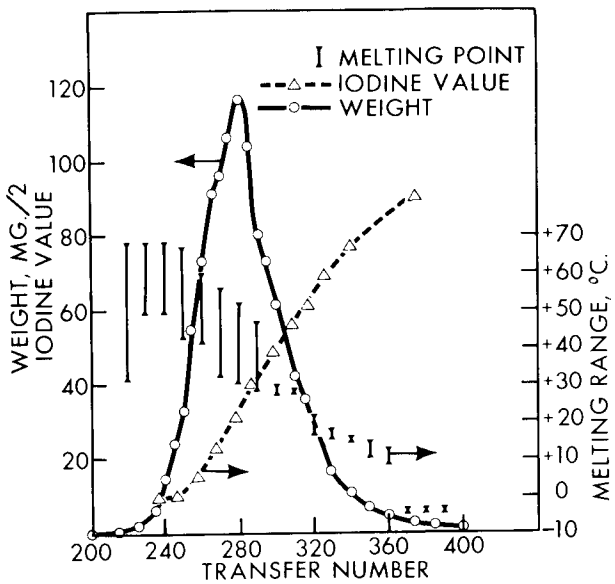

FIG. 5. Countercurrent distribution of interesterified cocoa butter, showing iodine values and melting point of the fractions.

Data in Figure 4 for a similar countercurrent distribution of natural cocoa butter, but with a lesser number of transfer stages, are included since melting points for individual tubes were recorded. The temperatures range from +10° to +50°C.

Interesterified Cocoa Butter. The weight distribution curve for interesterified cocoa butter in Figure 5 differs from that for natural cocoa butter in Figure 1. The curve shows no evidence of individual glyceride peaks but rather shows the merging of 20 individual glyceride distribution curves as expected under a random pattern. This curve was obtained without recycling in order to prevent the "head" of the band from overlapping the "tail." Such a situation would be expected for glycerides as divergent in partition coefficients as tristearin and trilinolein under recycle conditions. Otherwise the run is comparable in operating conditions, solvents used, and volume ratios to that on natural cocoa butter. Comparing data of Figure 5 and for the corresponding fatty acid composition in Figure 6 with Figures 1, 2, and 4 for

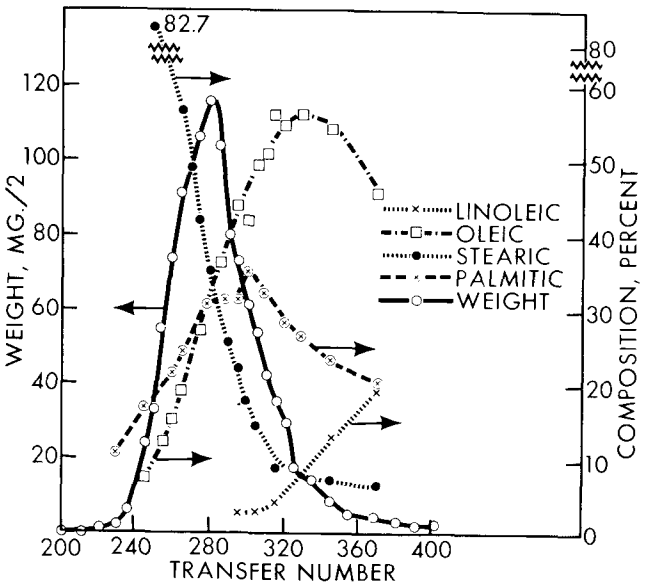

FIG. 6. Fatty acid composition of fractions from the countercurrent distribution of interesterified cocoa butter presented in Figure 5 (gas chromatographic data).

natural cocoa butter reveals marked differences in shape of the weight curve, the melting point of the fractions, the range of the iodine value curve, and the curves for the percentage composition of the fatty acids. Pertinent comparisons are given in Table II. Interesterification evidently alters the countercurrent distribution pattern of natural cocoa butter greatly; furthermore the reaction brings about those changes anticipated for a random structure.

An unique opportuntiy was provided to compare the fatty acid analyses of countercurrent distribution fractions of interesterified cocoa butter by three recent analytical methods. None were available to fat chemistry two decades ago: a) gas chromatography, b) double labelling with radio-active isotopes, and c) spectro-iodine value. A surprising coherence is apparent (Figures 7 and 8). When plotted, as in Figure 6, the three sets of data constitute similar graphs. Of the three, spectro-iodine value is the most laborious procedure. It requires a month for one

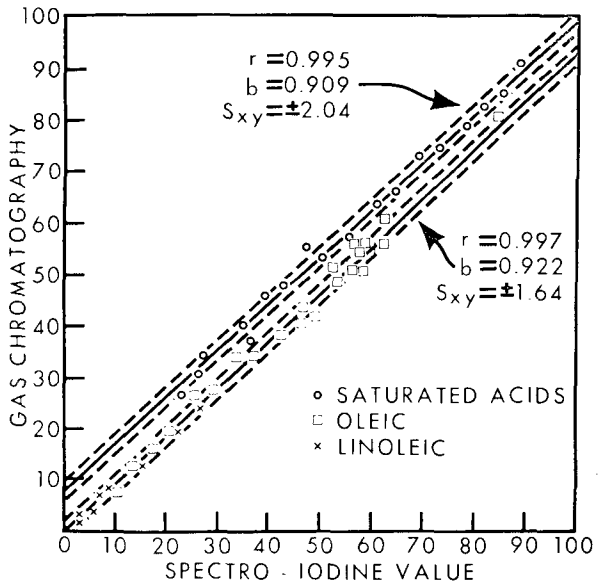

FIG. 7. Comparison of data by gas chromatography and spectro-iodine value procedures.

TABLE II

Characteristics of Natural, Interesterified, and Synthetic Cocoa Butter Fractions from Countercurrent Distribution

	Natural	Interesterified	Synthetic
Melting point, maximum, °C	+50	68	+50
Melting point, minimum, °C	+10	−5	+30
Stearic acid, maximum, %	70.5	82.7	64.5
Oleic acid, maximum, %	39.8	58	31
Iodine value, maximum	75	90	27

worker to procure data. Gas chromatography, the most complete in its analysis of saturated and unsaturated fatty acids, requires weeks to perform. In contrast, radiochemical assay is a matter of hours when it is applicable.

The amount of saturated acids, calculated by difference according to spectro-iodine value, shows a uniform error compared to data from gas chromatography of oleic and linoleic acids. Radiochemical

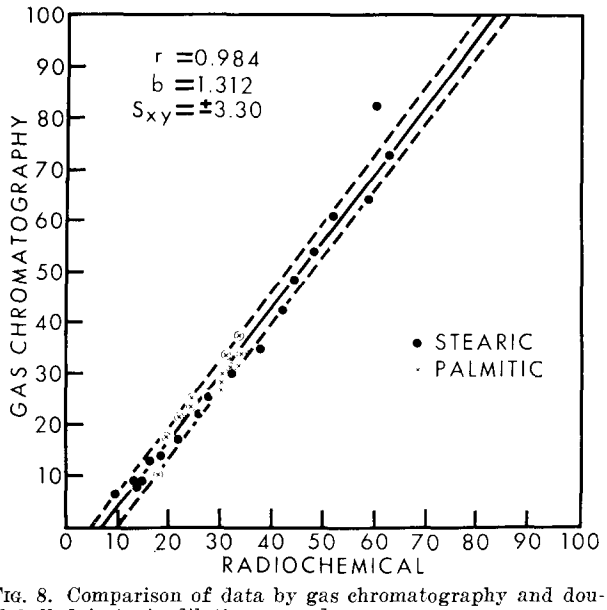

FIG. 8. Comparison of data by gas chromatography and double-labelled isotopic dilution procedures.

data for palmitic and stearic acids did not deviate greatly from gas chromatographic data.

"Synthetic" Cocoa Butter. Based upon the proposed structure for cocoa butter, a triglyceride was synthesized by esterifying the random 1,3 diglycerides of palmitic and stearic acids with oleyl chloride. A typical preparation had a weight percentage composition of 18.2% palmitic acid, 40.8% stearic acid, and 41% oleic acid. This synthetic product (m.p. 31–32°C.) had a slight effect on the melting point of natural cocoa butter (32–34°C.) when mixed in equal proportions (m.p. 31.5–32.5°C.). Both alone, and in mixtures, the synthetic displayed the double melting-points characteristic of natural cocoa butter in its polymorphic forms (25°C. and 32–34°C.). After "refining" the synthetic product with sodium carbonate, "bleaching" with Darco G60, and "deodorizing" with nitrogen gas at 200°C., its taste was bland.

Countercurrent distribution patterns for weight (Figure 9) and for fatty acid composition (Figure 10) of the synthetic are comparable to those of natural cocoa butter (Figures 1, 2). Oleic acid in both varies as anticipated within narrow limits (therefore the iodine value) whereas stearic and palmitic acids reach 65 and 53% levels, respectively. Since no liquid fatty acids were included in the random diglyceride, the synthetic product contains only disaturated oleins; no low-melting components appear on countercurrent distribution as for the natural fat (Table II).

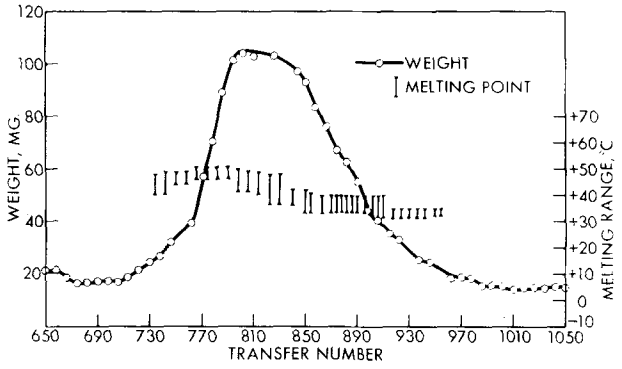

FIG. 9. Countercurrent distribution of synthetic cocoa butter, showing melting points of the fractions.

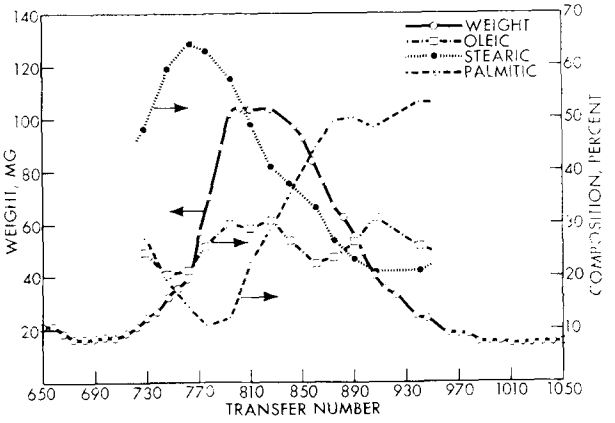

FIG. 10. Fatty acid composition of fractions from the countercurrent distribution of synthetic cocoa butter presented in Figure 9.

Summary

More concise information on the glyceride structure of natural cocoa butter has been obtained by the use of gas chromatography and isotopic dilution methods of analysis. In addition, the structures of a randomized and a synthetic "cocoa butter" have been studied. A "1,3 random palmito-stearo-2-olein" pattern for the glyceride structure of natural cocoa butter has been proposed and found to agree quite closely with the experimentally-determined structure for natural cocoa butter.

Cocoa butter randomized with the use of an interesterification catalyst displays changes in physical properties, including countercurrent distribution patterns for weight, melting points, and fatty acid compositions which are anticipated under the random pattern and which are sharply distinguished from those of natural cocoa butter.

To confirm the validity of the proposed concept for the structure of cocoa butter, a mixed glyceride was synthesized from approximately equal amounts of oleic, palmitic, and stearic acids. The synthetic material displayed the double melting-point of cocoa butter, had slight effect upon the mixed melting-point, and separated on countercurrent distribution into fractions similar to cocoa butter glycerides.

Acknowledgment

The authors are indebted to J. C. Cowan for his interest and encouragement; to L. H. Mason and Janina Nowakowska for gas chromatographic analyses; to R. F. Nystrom (University of Illinois) for certain radiochemical analyses; and to E. A. Emken for technical assistance.

REFERENCES

1. Hilditch, T.P., and Stainsby, W.J., J. Soc. Chem. Ind. (London), *55*, 95T (1936).
2. Meara, M.L., J. Chem. Soc., 2154–2157 (1949).
3. Dutton, H.J., and Cannon, J.A., J. Am. Oil Chemists' Soc., *33*, 46–49 (1956).
4. Dutton, H.J., Lancaster, C.R., and Brekke, O.L., *ibid.*, *27*, 25, 30 (1950).
5. Scholfield, C.R., and Hicks, Mary A., *ibid.*, *34*, 77–80 (1957).
6. Scholfield, C.R., and Dutton, H.J., in press.
7. Scholfield, C.R., and Dutton, H.J., J. Am. Oil Chemists' Soc., *35*, 493–496 (1958).
8. Hilditch, T.P., "The Chemical Constitution of Natural Fats," 3rd ed., John Wiley and Sons, New York, 1956.
9. Scholfield, C.R., and Dutton, H.J., J. Am. Oil Chemists' Soc., *36*, 325–328 (1959).
10. Eckey, E.W., Ind. Eng. Chem., *40*, 1183–1190 (1948).
11. Nystrom, R.F., Mason, L.H., Jones, E.P., and Dutton, H.J., J. Am. Oil Chemists' Soc., *36*, 212–214 (1959).
12. Okita, G.T., Kabara, J.J., Richardson, Florence, and Le Roy, G.V., Nucl onics, *15*, (No. 6), 111 (1957).
13. Baur, F.J., and Lange, W., J. Am. Chem. Soc., *73*, 3926–3928 (1951).
14. Youngs, C.G., J. Am. Oil Chemists' Soc., *35*, 416–417 (1958).
15. Mattson, F.H., and Lutton, E.S., J. Biol. Chem., *233*, 868–871 (1958).
16. Savary, P., Flanzy, J., and Desnuelle, P., Biochim. et Biophys. Acta., *24*, 414–423 (1957).
17. Chapman, D., Crossley, A., and Davies, A. C., J. Chem. Soc., 1502–1509 (1957).
18. Kartha, A.R.S., J. Am. Oil Chemists' Soc., *30*, 326 (1953).
19. Vander Wal, R.J., J. Am. Oil Chemists' Soc., *35*, 483 (1958).
20. Vander Wal, R.J., *ibid.*, *37*, 18 (1960).

[Received July 14, 1960]