with the first and continuing through the extraction train in order, was extracted with 100-ml. portions of the alcohol layer (45% ethanol-45% methanol-10% water). Twenty-three extracts were withdrawn from the extraction train. The results are shown graphically in Figure 2; the last fraction withdrawn is plotted next to the last fraction remaining in the apparatus (11). The combined withdrawn fractions, having a percentage of hydroxyl of 5.07, constituted 31.3% of the charge. The combined residual fractions, having a percentage hydroxyl of 0.46, constituted 68.7% of the charge.

These results clearly indicate the superiority of methyl esters over free fatty acids in these partitions since a higher average percentage of hydroxyl is obtained for the withdrawn fractions and a lower average percentage of hydroxyl is obtained for the fractions remaining in the apparatus.

For production work a much larger quantity of methyl esters (354 g.) was fractionated by using 1-gal. Pyrex bottles and 1200-ml. portions of both layers. As in the previous case, the charge was divided into three equal portions in the first three bottles. However seven bottles rather than three completed the extraction train. Thirty extracts were withdrawn from the train. Fractions 10 to 34 of the withdrawn material, amounting to 84 g., were combined. The percentage of hydroxyl of the combined fractions was 5.5; the saponification number was 163.0; the acid number was 0.7. Fractions 35 to 38 of the withdrawn material, amounting to 18.2 g., were hard, transparent, and highly colored. They were not combined with the hydroxy esters. The residual material amounted to 240.4 g. with an hydroxyl content of 0.9%.

Summary

The fractionation of the methyl esters of wool wax acids by partitioning between two immiscible solvent layers has been described. Three fractions were obtained: a fraction rich in hydroxyl content, a fraction low in hydroxyl content, and a small amount of hard, transparent, highly colored material. The same procedure when applied to the free wool wax acids did not yield a satisfactory hydroxy acid concentrate.

The preparation of wool wax acids with an essentially zero ester number, that is, in a form free of estolides, lactides, and lactones has also been described.

REFERENCES

1. Abraham, E., and Hilditch, T. P., J. Soc. Chem. Ind., *54*, 398T-404T (1935).
2. Barnes, C. S., Curtis, R. G., and Hatt, H. H., Australian J. Appl. Sci., *3*, 88-99 (1952).
3. Bharucha, K. E., and Gunstone, F. D., J. Sci. Food Agr., *6*, 373-80 (1955).
4. Craig, L. C., J. Biol. Chem., *155*, 519-534 (1944).
5. Darmstaedter, L., and Lifschütz, J., Ber., *29*, 1474-7 (1896).
6. Horn, D. H. S., Hougen, F. W., von Rudloff, E., and Sutton, D. A., J. Chem. Soc., *1954*, 177-80.
7. Horn, D. H. S., and Pretorius, Y. Y., Chemistry and Industry B.I.F. Review, *April 1956*, R27-28.
8. Kuwata, T., J. Am. Chem. Soc., *60*, 559-60 (1938).
9. Lewkowitsch, J., J. Soc. Chem. Ind., *11*, 134-45 (1892).
10. von Rudloff, E., dissertation, University of Pretoria (1952).
11. Craig, L. C., and Craig, D., "Extraction and Distribution," in Weissberger, A. (ed.), "Technique of Organic Chemistry," vol. 3, pp. 271-273, Interscience Publishers Inc., New York, 1950.
12. Ibid., pp. 305-6.
13. Weitkamp, A. W., J. Am. Chem. Soc., *67*, 447-54 (1945).
14. Williamson, B., and Craig, L. C., J. Biol. Chem., *168*, 687-97 (1947).

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The Isomerization of Fats During Hydrogenation and the Metabolism of the Component Fatty Acids[1]

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IT HAS LONG BEEN KNOWN that catalytic hydrogenation of an unsaturated fat produces isomeric unsaturated fats. As early as 1919 Moore (1) isolated solid unsaturated fatty acids from hydrogenated ethyl oleate, and in 1929 Hilditch and Vidyarthi (2) showed that these isomers had resulted from both geometrical and positional isomerization. It is however only in the past few years that improved methods have given impetus to research on the mechanisms involved in the isomerization caused by hydrogenation of oils. Furthermore, since the nutritional effects of fatty acid isomers have been questioned (3), a number of studies on this subject have appeared in the recent literature.

The extent of both geometrical and positional isomerization during hydrogenations has been shown by studies on purified fatty acids and their esters. Boelhouwer *et al.* (4) demonstrated the extensive migration of double bonds which took place during the catalytic hydrogenation of methyl oleate. Their data indicated a preference for a shift of the double bonds away from the carboxyl group. However Allen and Kiess (5) showed the migration of the double bonds to be equal in either direction, and their results were later confirmed by Knegtel *et al.* (6).

The hydrogenation and isomerization of linoleic acid are perhaps more important to commercial operations since the hydrogenation of vegetable oils to form shortening or margarine stocks results in the saturation of the linoleic acid to a mono-ene without extensive reduction of the oleate.

The methylene-interrupted diene system in linoleic acid complicates the study of the positional isomerism since either of the two double bonds may be saturated with hydrogen in addition to their migration along the chain. Allen and Kiess (7) found the migration to be confined to one pentadiene system in contrast to the report by Cousins *et al.* (8), who found double bonds in all positions from 6 through 14. Chahine *et al.* (9) studied the effect of operating conditions on the hydrogenation of cottonseed oil; these workers also found a wide distribution of the remaining double bonds. Cousins *et al.* (8) showed that, after partial hydrogenation of methyl linoleate, the greatest con-

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centrations of residual double bonds were in the 10 position and the concentrations in the other positions decreased as the distance from the 10 position increased. These investigators also noted that lowering the temperature of hydrogenation produced a different pattern of distribution of residual double bonds. With nickel catalyst at 110°C., more than 50% of the total residual double bonds were in the natural 9 and 12 positions. Furthermore the use of nickel catalyst led to the production of fewer *trans* isomers than did the platinum or palladium catalysts. Thus it should be possible to establish commercial hydrogenation conditions in which there is minimum positional and geometrical isomerization.

The positional and geometrical isomerization that occurs during hydrogenation was shown by Allen and Kiess (5, 10) to be related. Thus when a double bond migrated, the new double bond could be either *cis* or *trans* unless steric hindrance prevented the geometrical isomerism. These isomerizations are believed to be caused by a half-hydrogenation-dehydrogenation reaction sequence. During the hydrogenation a hydrogen atom can add to either end of the double bond and form a free center probably still attached to the catalyst. If the catalyst is only partially covered with hydrogen, a hydrogen atom can be removed by the catalyst to form a new double bond. This new double bond can be in a new position and can exist in either geometric configuration. Thus it appears that the extent of both positional and geometrical isomerization is related to the selectivity of the reaction. If the reaction is carried out at high pressure with a low amount of catalyst, the selectivity of the reaction is reduced as is the extent of both geometrical and positional isomerization. On the other hand, at low pressure with a high amount of catalyst, the catalyst is not saturated with hydrogen and the selectivity is increased, as is the extent of isomerization.

If we consider the hydrogenation of linoleic acid, which is the main acid reduced during the hydrogenation of the common vegetable oils to produce base stocks, there are at least 18 isomeric mono-enes formed (8). These isomers are the *cis* and *trans* forms of 9 positional isomers. Thus, from the hydrogenation of cottonseed oil, there would be 18 mono-enes, some dienes, palmitic and stearic acids, that is, a minimum of 21 different fatty acids in the mixture. This could result in the formation of approximately 8,000 different triglycerides.

This extensive isomerization of the component fatty acids of edible fats during hydrogenation raises a number of questions regarding the nutritional effects of the isomers. While very little is known about the metabolism or nutritional effects of purified positional isomers, a number of studies have been carried out on purified and mixed geometrical isomers.

Early work by Sinclair (11) and others indicated that the feeding of pure trielaidin or elaidic acid to the rat led to the deposition of the *trans* isomers in the carcass fat and that the cholesterol esters of the liver fat contained a higher percentage of the deposited *trans* than did the liver triglycerides. However this work was done by using the lead-salt alcohol method for iso-oleic acid determination and therefore may not be quantitative. More recent studies have employed the relatively simple infrared method for the determination of *trans* isomers. It has been shown by Holman (12) and Privett *et al.* (13) that the *trans* isomers of octadecadienoic acids do not exhibit any essential fatty acid activity. Furthermore Holman and Aaes-Jorgensen (12) have reported that nonconjugated dienes containing one or more *trans* double bonds inhibit the growth of rats and worsen the skin condition of essential fatty acid deficient rats. These investigators concluded that a single *trans* double bond in a mono-enoic acid does not inhibit growth. On the other hand, neither Alfin-Slater *et al.* (14) nor Johnston *et al.* (15) found any evidence of growth inhibition in rats which had been fed diets with considerable quantities of *trans* fatty acids containing both mono-enes and dienes.

In addition to the nutritional studies, a number of investigations have been carried out to determine the efficiency of metabolism of fatty acid isomers by the animal organism. Allen *et al.* (16) showed that, when synthetic isomeric triglycerides were fed to rats for 16 days, almost 90% of the dietary *trans* isomers was metabolized, approximately 10% was deposited in the tissues, and less than 1% was excreted. The position of the *trans* double bond did not appear to have any effect on the efficiency of metabolism. Johnston *et al.* (15) found that, when rats were fed a diet containing over 40% *trans* fatty acids, the level of *trans* fatty acids in the carcass fat reached a maximum level after one month and that this level remained unaltered, provided that the intake of *trans* isomers was kept constant. When *trans* fatty acids were removed from the diet, they gradually decreased in amount in the tissues (Table I).

Alfin-Slater *et al.* (14) have studied the effect of long-term feeding, and these investigators reported that the feeding of the *trans* diet to rats for 46 generations did not produce any obvious harmful effects. However it has been shown that *trans* fatty acids are not found normally in the depot fat of rats unless they have been fed in the diet (15, 17) and furthermore that *trans* fatty acids are not passed on from a mother rat to her young (18). It has been shown by Johnston *et al.* that female rats which had a high content of *trans* fatty acids in their depot fat (23.5–26.8% *trans*) gave birth to young that contained no *trans* in their fat. If however the young were allowed to suckle the maternal milk, the percentage *trans* in the carcass fat approached that of the mother (24.3–24.8% *trans*).

The human being also appears to be capable of metabolizing *trans* fatty acids since the amounts of *trans* isomers found in various human tissues are not generally high (19). However the presence of *trans* fatty acids in human tissue seems to be a result of the presence of *trans* fatty acids in present-day diets. Johnston *et al.* (20) have shown that while maternal

TABLE I

The Deposition of Dietary *Trans* Fatty Acids in Rat Carcass Fat (15)

Group	% *Trans* fatty acids in carcass fat		
	1 month	2 months	3 months
10% Margarine stock (40% *trans*)	16.7±1.4[a]	18.9±1.3	18.0±0.9
5% Margarine stock—5% olive oil	10.5±0.6	11.3±0.7	10.8±0.9
10% Margarine stock fed 1 month, then *trans*-free diet for 1 or 2 months	.....	6.5±0.68	4.4±0.87
5% Margarine stock—5% olive oil fed for 1 month, then *trans*-free diet for 1 2 months	.....	4.9±0.57	2.8±0.98

[a] Standard deviation of the mean.

depot fat contained considerable amounts of *trans*, little or none was found in the placental fat from the same individual (Table II). *Trans* fatty acids were also found to be absent in fetal and newborn baby tissues.

TABLE II

Percentage of *Trans* Fatty Acids in Six Samples of Placental and Maternal Fat from Same Individual (20)

Placental fat *% trans*	Maternal depot fat *% trans*
0	6.4
<0.5	6.8
0	6.1
0	1.5
<0.5	1.8
0	5.2

Summary

Extensive positional and geometrical isomerization occurs during the catalytic hydrogenation of edible oils and fats. The extent of this isomerization can be controlled by varying the conditions of hydrogenation.

Studies on the nutritional and metabolic aspects of the geometric isomers formed during the hydrogenation of edible fats indicate that the animal organism appears to be capable of metabolizing *trans* isomers. However there is some evidence to indicate that the deposition of *trans* fatty acids in animal tissues may worsen a pre-existing essential fatty acid deficiency. The possible effects of *trans* fatty acids on other metabolites remains unclear.

REFERENCES

1. Moore, C. W., J. Soc. Chem. Ind., *38*, 320 (1919).
2. Hilditch, T. P., and Vidyarthi, N. L., Proc. Roy. Soc. London, *A122*, 552, 563 (1929).
3. Sinclair, H. M., Lancet, *270*, 381 (1956).
4. Boelhouwer, C., Gerckens, J., Ong Tian Lie, and Waterman, H. I., J. Am. Oil Chemists' Soc., *30*, 59 (1953).
5. Allen, R. R., and Kiess, A. A., J. Am. Oil Chemists' Soc., *32*, 400 (1955).
6. Knegtel, J. T., Boelhouwer, C., Tels, M., and Waterman, H. I., J. Am. Oil Chemists' Soc., *34*, 336 (1957).
7. Allen, R. R., and Kiess, A. A., J. Am. Oil Chemists' Soc., *33*, 355 (1956).
8. Cousins, E. R., Guice, Wilma A., and Feuge, R. O., J. Am. Oil Chemists' Soc., *36*, 24 (1959).
9. Chahine, M. H., Cousins, E. R., and Feuge, R. O., J. Am. Oil Chemists' Soc., *35*, 396 (1958).
10. Allen, R. R., J. Am. Oil Chemists' Soc., *33*, 301 (1956).
11. Sinclair, R. G., J. Biol. Chem., *115*, 211 (1936); *ibid. 118*, 123 (1937); *ibid., 111*, 515 (1935).
12. Holman, R. T., and Aaes-Jorgensen, E., Proc. Soc. Exp. Biol. Med., *93*, 175 (1956).
13. Privett, O. S., Pusch, F. J., and Holman, R. T., Arch. Biochem, and Biophys., *57*, 156 (1955).
14. Alfin-Slater, Roslyn B., Wells, A. F., Aftergood, L., and Deuel, H. J. Jr., J. Nutrition, *63*, 241 (1957).
15. Johnston,, Patricia V., Johnson, O. C., and Kummerow, F. A., J. Nutrition, *65*, 13 (1958).
16. Allen, R. R., Kiess, A. A., Johnston, Patricia V., and Kummerow, F. A., J. Am. Oil Chemists' Soc., *35*, 203 (1958).
17. Hartman, L., J. Am. Oil Chemists' Soc., *34*, 129 (1957).
18. Johnston, Patricia V., Johnson, O. C., and Kummerow, F. A., Proc. Soc. Exp. Biol. and Med., *96*, 760 (1957).
19. Johnston, Patricia V., Johnson, O. C., and Kummerow, F. A., Science, *126*, 698 (1957).
20. Johnston, Patricia V., Walton, C. H., and Kummerow, F. A., Proc. Soc. Exp. Biol. and Med., *99*, 735 (1958).

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Calculation of the Distribution of the Saturated and Unsaturated Acyl Groups in Fats, from Pancreatic Lipase Hydrolysis Data[1]

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THE RESULTS of a series of analyses of natural fats by means of the pancreatic lipase hydrolysis technique of Mattson and Beck (1) were published recently by Mattson and Lutton (2). By the Mattson and Beck procedure, fatty acyl groups in the 1- and 3-positions of triglyceride molecules may be preferentially and nonspecifically removed with little displacement of those in the 2-position.

The data include a) the percentage of saturated acyl groups among the acyl groups in the whole fat and b) the percentage of saturated acyl groups among the acyl groups in the 2-monoglycerides which would be produced if all groups in the 1- and 3-positions were removed by hydrolysis. From these data and on the basis of two simple and plausible assumptions, the proportions in some of the fats of the four triglyceride types, GS_3, GS_2U, GSU_2, and GU_3, and those of the symmetrical and unsymmetrical isomers which comprise the GS_2U and the GSU_2 molecules were calculated in a manner to be described.

The two assumptions are: a) that whatever proportions of saturated acyl groups (S) and unsaturated acyl groups (U) are dispersed among the 1-positions, the 2-positions, and the 3-positions, respectively, of the triglycerides, they are distributed therein at random;[2] and b) that the 1- and 3-positions are occupied by identical proportions of S and U.[3]

The method of calculation is as follows:[4]

a = % S among the acyl groups in the whole sample as found by analysis

b = % S in the acyl groups in the 2-positions. It is equal to the % S among the acyl groups in the 2-monoglycerides, found by analysis

c = % of the total S in the sample present therein as S2 = $\frac{(b)\ (100)}{3a}$ (See Table II, Mattson and Lutton)

d = % of the total S in the sample present therein as S1,3 = 100 − c

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[2] The individual acyl groups constituting the S and U are not under consideration except as members of the two classes.

[3] In correspondence with the author in 1957 A. S. Richardson suggested the possibility that there may be a random distribution involving the 1- and 3-positions but not the 2-position in fats. Speaking of plant triglycerides, he emphasized these predictions: "Even distribution, to the extent that it really occurs, will some day be found to be in large measure the result of preferential attachment of unsaturated acid at the 2-position of the glyceryl residue. Chance distribution, to the extent that it really prevails, will be found to be confined mainly to the 1- and 3-positions." One suggested possibility was that the first step in the triglyceride synthesis is selective esterification at the 2-position. Random distribution, he said, if it thereafter occurred, would be calculated by analogy with esterification of glycol, not glycerol. In that case the pattern of distribution outlined in the present paper would result, but the bare assumption of random distribution involving only the 1- and 3-positions would not necessarily lead to this pattern.

[4] The following symbols will be used in the calculations:

S = Saturated acyl groups in general.
U = Unsaturated acyl groups in general.
S2 = Saturated acyl groups in the 2-positions.
S1,3 = Saturated acyl groups in the 1-positions and those in the 3-positions. Positions 1 and 3 are considered identical.
U1,3 = Unsaturated acyl groups in the 1-positions, also those in the 3-positions. Positions 1 and 3 are considered identical.