THE COMPONENT FATTY ACIDS OF HUMAN DEPOT FAT

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The fatty acid composition of human depot fat has been the subject of few previous researches. Jaeckle (1) in 1902 reported quantitative values for palmitic, stearic, and oleic acids. Since in the methods employed other saturated and unsaturated acids now known to occur in this fat were not recognized, the results of his work must be considered as qualitative only. The first quantitative data based on the somewhat more accurate procedures of two decades ago were described by Eckstein in 1925 (2). Eckstein's results were far from complete. He found 26.6 per cent of saturated fatty acids and 63.6 per cent of unsaturated acids. By the use of certain soap procedures and partial fractional distillation of the methyl esters of the component fatty acids he reported for the first time a trace of lauric acid, about 1.00 per cent of myristic acid, and less than 1 per cent each of linoleic, linolenic, and arachidonic acids. Identification of the last three acids was made through study of the bromine addition compounds. Wagner (3) 1 year later confirmed the presence of linoleic and arachidonic acids, but not linolenic. Several more recent contributions to the chemistry of human fat have been made by Heiduschka and Handritschk (4), Cathcart and Cuthbertson (5), Cuthbertson and Tompsett (6), and Stolfi (7), but no further detailed work to evaluate the component fatty acids has appeared in spite of the obvious importance of adipose tissue in the chemistry of the human body as brought out in the interesting discussion of this subject by Wells (8).

In the present work, we have distilled the methyl esters of five specimens of human depot fat through a highly efficient electrically heated column packed with glass helices. By this procedure in each instance we had available three main fractions composed essentially of C_{14}, C_{16}, and C_{18} esters respectively, and in addition, small intermediate and residual fractions. The main fractions from two of the specimens were then studied by crystallization procedures at low temperature, developed in this laboratory, in order to identify the esters present. Tetradecenoic and hexadecenoic acids were demonstrated in this fat for the first time. Repeated crystallization of the octadecenoic acid (oleic) present gave evidence for the presence of other octadecenoic acids than oleic. The linoleic acid in

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this fat also appears to be a mixture of isomeric octadecadienoic acids, although ordinary linoleic acid (*cis, cis*) is the principal component of this mixture. On the basis of the acids identified as a result of this work, calculations of the component fatty acids in human depot fat were made and are shown in Table I.

TABLE I

Per Cent of Fatty Acids in Human Fat

Specimen No	XIII	XII	III	XX	H-XIV
Lauric	0.1	0.6			0.9
Myristic	2.7	5.9	2.6	2.6	3.9
Tetradecenoic	0.2	0.6	0.4	0.4	0.5
Palmitic	24.0	25.0	24.7	25.4	25.7
Hexadecenoic	5.0	6.7	7.3	5.6	7.6
Stearic	8.4	5.8	7.7	7.7	5.2
Octadecenoic	46.9	45.4	45.8	44.8	46.6
Octadecadienoic	10.2	8.2	10.0	11.0	8.7
Arachidonic	1.0	1.0	0.4	0.3	0.6
Other C_{20}	1.5	0.8	1.1	2.2	0.3

EXPERIMENTAL

Description of Fat Specimens—The five specimens of human fat were obtained for us at autopsies made by the Department of Pathology. The pathology in each case was not believed to be of significance in the history of the fat specimen. In other words, we consider these specimens to be reasonably normal human body fats. Autopsy records show the following: Specimen XIII, female, 53 years, cardiac hypertrophy; Specimen XII, male, 74 years, arteriosclerosis; Specimen III, male, 61 years, prostatic hypertrophy; Specimen H-XIV, male, 66 years, arteriosclerosis; Specimen XX, history unknown.

Specimens were taken mainly from the abdominal regions, pannicular, mesenteric, perirenal, etc.

Preparation and Distillation of Methyl Esters—The fresh adipose tissue, after being washed with cold water and cooled to −20°, was hashed and heated on the steam bath for 2 to 5 hours. The fat was then pressed out in a lard press. Water was completely removed by warming under reduced pressure. Methyl esters were prepared in the usual manner and distilled. Care was taken that the yield of esters was practically complete, usually over 97 per cent of the weight of fat taken, so that they are representative of the fatty acids of the specimen in question. Analytical data on the original fats and their methyl esters are shown in Table II.

Specimens of the above esters were distilled through an electrically heated column, 90 cm. in length, packed with glass helices. The esters of Specimens XIII and XII were worked up in distillations of four batches and two batches, respectively, in order to have available sufficient amounts of the main fractions for intensive investigation. The procedure followed for Specimen XII was as follows: A charge of 460 gm. was distilled into six fractions and residue. The head fraction (C_{12}–C_{14}) and two small intermediates were returned to the distilling flask with a new charge of 342 gm. of esters and the mixture was distilled with special care to obtain a head fraction with any C_{12} ester present. The main fractions and residues from the two batches were combined. There were thus available from this specimen three combined main fractions, a head, and two small intermediates and residue. Final distillation and analytical data on the five specimens are presented in Table III.

TABLE II

Analytical Data on Specimens of Human Fats and Their Methyl Esters

Specimen No.	Neutral fat		Methyl esters	
	I No.	Saponification No.	I No.	Mol. wt.
XIII	68.9	196.5	67.8	282.3
XII	67.4	194.0	64.9	289.9
III	68.8	197.9	67.8	283.5
XX	64.8	197.3	64.4	280.4
H-XIV	68.5	195.4	69.2	285.4

Experience with this still has shown that, as a rule, the main fractions are over 95 per cent of the carbon series in question; intermediate fractions are usually small in proportion to the care taken in the distillation. These intermediate fractions have been calculated from their molecular weights as binary mixtures of the two adjacent main fractions.

The C_{18} main fractions were discontinued as soon as definite evidence of rising boiling point was observed. No attempt was made to continue the distillation up to the C_{20} main fraction which was later found to amount to only about 2 per cent of the total esters. As a result, the residual fractions were about three-fourths C_{18} esters and one-fourth C_{20} esters.

Fractional Crystallization Studies of Nature of Main and Residual Fractions—The main and residual fractions of Specimens XII and XIII were studied in detail by crystallization procedures at low temperature. Only the experiments on Specimen XIII are described below on account of space limitations. However, the results on the two specimens agreed very closely.

TABLE III

Final Distillation Data on Five Specimens of Methyl Esters of Human Fat

Specimen No.	Charge	Fraction C series	Weight	Mol. wt. of esters	Mol. wt. of acids	I No.	Thiocyanogen No.	Polybromide No.
	gm.		*gm.*					
XIII	2065	12–14	16.8	239.7	225.7	11.1		
		14	12.9	242.2	228.2	6.4		
		14–16*	21.3	245.5	231.5	14.5		
		14–16	21.2	255.0	241.0	22.7		
		16	527.9	267.6	253.6	17.4		
		16–18	108.7	281.9	267.9	49.3		
		18	1167.1	296.7	282.7	87.9	75.3	
		Residual 18–20	188.7	305.5	291.5	119.6		10.0
XII	802	12–14	9.0	228.1	214.1	16.6		
		14	35.3	239.3	225.3	12.0		
		14–16	18.4	251.7	237.7	24.5		
		16	227.2	269.4	255.4	20.0		
		16–18	34.5	282.2	268.2	59.1		
		18	438.1	297.3	283.3	88.9	77.7	
		Residual 18–20	37.6	309.0	295.0	144.0		18.5
III	420	14–16	35.4	260.4	246.4	20.3		
		16	87.9	272.6	258.6	24.4		
		16–18	55.0	286.3	272.3	59.1		
		18	216.6	294.8	280.8	88.6	75.9	
		Residual 18–20	24.3	305.0	291.0	102.5		6.2
H-XIV	450	14–16	10.9	245.0	231.0	10.9		
		14–16	8.7	253.2	239.2	12.4		
		14–16	15.5	260.0	246.0	35.0		
		16	116.6	268.0	254.0	21.6		
		16–18	34.4	279.0	265.0	71.8		
		18	231.0	296.9	282.9	90.5	78.9	
		Residual 18–20	34.5	301.0	287.0	98.2		6.6
XX	635	14–16	20.0	244.0	230.0	13.7		
		16	152.5	268.5	254.5	17.4		
		16–18	52.0	279.5	265.5	55.0		
		18	350.7	297.0	283.0	91.0	77.0	
		Residual 18–20	36.0	310.0	296.0	100.5		5.3

* This mixture of C_{14}–C_{16} esters was not added to the still pot with the next batch; hence, the two C_{14}–C_{16} fractions here.

C_{14} Fraction

The C_{14} esters were crystallized by the procedure described in Chart I. By the procedure described in Chart I there were obtained 8.5 gm. of methyl myristate which is shown to be practically pure by iodine number, molecular weight, and melting point. The combined filtrate fraction, amounting to only 1.5 gm., consists of 52 per cent of methyl tetradecenoate

and 48 per cent of methyl myristate. Since the thiocyanogen number is very close to the iodine number, the presence of acids with more than one double bond is unlikely. From the iodine number, the C_{14} main fraction is 8.1 per cent methyl tetradecenoate. On account of the small amount of material available, no attempt was made to identify the tetradecenoic acid further.

C_{16} Fraction

The crystallization procedure for the separation of the constituents of the C_{16} fraction is described in Chart II.

When the original methanol solution was cooled to $-25°$, only a small amount of methyl palmitate precipitated, but when cooled further to $-50°$ a total of 83.0 gm. of this ester crystallized out; from the iodine number this was 97.3 per cent pure. The filtrate from this crystallization was further broken up into two fractions, the crystals of which were 94 per cent and the

CHART I

Crystallization of C_{14} Esters of Specimen XIII

10 gm. C_{14} esters
Mol. wt. 242.2, I No. 6.4,
dissolve in 400 cc.
methanol, cool to $-60°$

P_1 | F_1

Dissolve in 370 cc. methanol,
cool to $-60°$

P_2 | F_2

8.5 gm., mol. wt. 240.8,
I No. 0.0,
m.p. 18.4°

F_1 and F_2 combined, 1.5 gm.,
mol. wt. 239.9, I No. 57.3,
thiocyanogen No. 54.8

filtrate 97.5 per cent methyl hexadecenoate. This is an almost unbelievably sharp separation and demonstrates the ease with which the crystallization procedure separates simple ester mixtures of this type. The methyl hexadecenoate was further demonstrated by reduction to methyl palmitate, and by oxidation of the acid to dihydroxypalmitic acid of known melting point. The melting point of the ester, $-42.4°$ to $-41.5°$, is practically identical with that of a specimen of methyl hexadecenoate prepared in this laboratory from menhaden oil by Frank Smith which has been shown by disruptive oxidation to be 9,10-hexadecenoate. Since the thiocyanogen number of the ester is very close to the iodine number, it seems likely that acids more unsaturated than hexadecenoic are not present in significant amounts.

C_{18} Fraction

The C_{18} esters of Specimen XIII were assumed to consist of methyl stearate, oleate, and linoleate. The polybromide number of this fraction (0.0007) in comparison with that of the succeeding residue fraction (10.0)

CHART II

Fractional Crystallization of C_{16} Methyl Esters of Specimen XIII

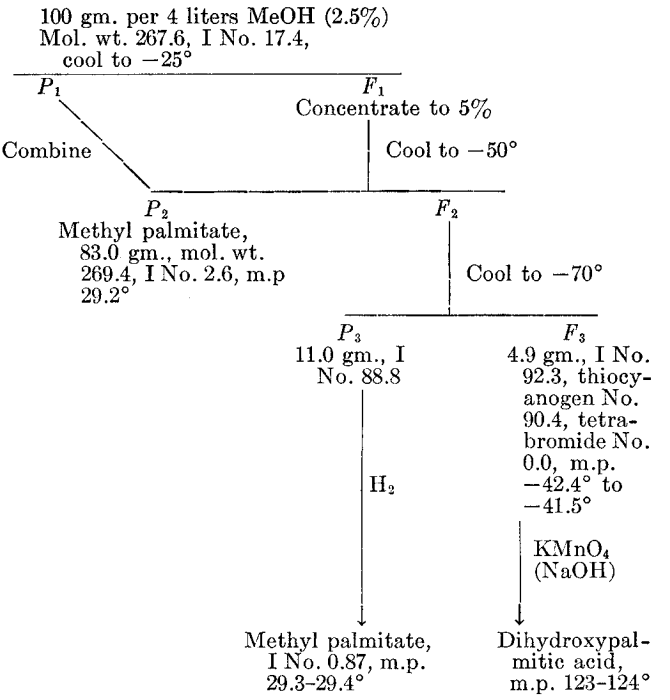
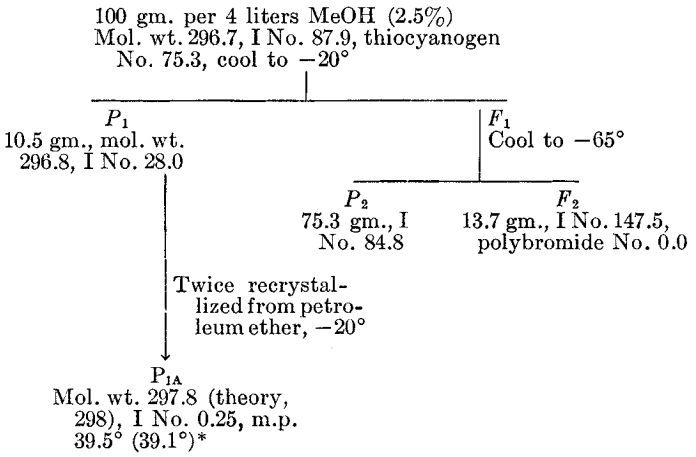


CHART III

Fractional Crystallization of C_{18} Methyl Esters of Specimen XIII



* Reported by Francis and Piper (9).

shows that no appreciable amount of methyl arachidonate or of methyl linolenate is present in this fraction. This is also further evidence of the degree of separation attainable with the distillation apparatus used in this work.

Preliminary separation of the fraction is shown in Chart III.

The crystallization in Chart III separated the C_{18} esters into fractions composed mainly of methyl stearate (P_1), methyl oleate (P_2), and a mixture of oleate with linoleate (F_2). For the further work on the methyl oleate fraction we are indebted to Dr. Carl Millican who worked up larger amounts of the C_{18} esters as part of a more general investigation in progress at the time this work was done (10). The crystallization was carried out in acetone in the earlier operations in Chart IV, because filtration is easier from this solvent than from methyl alcohol. For full details, see Chart IV.

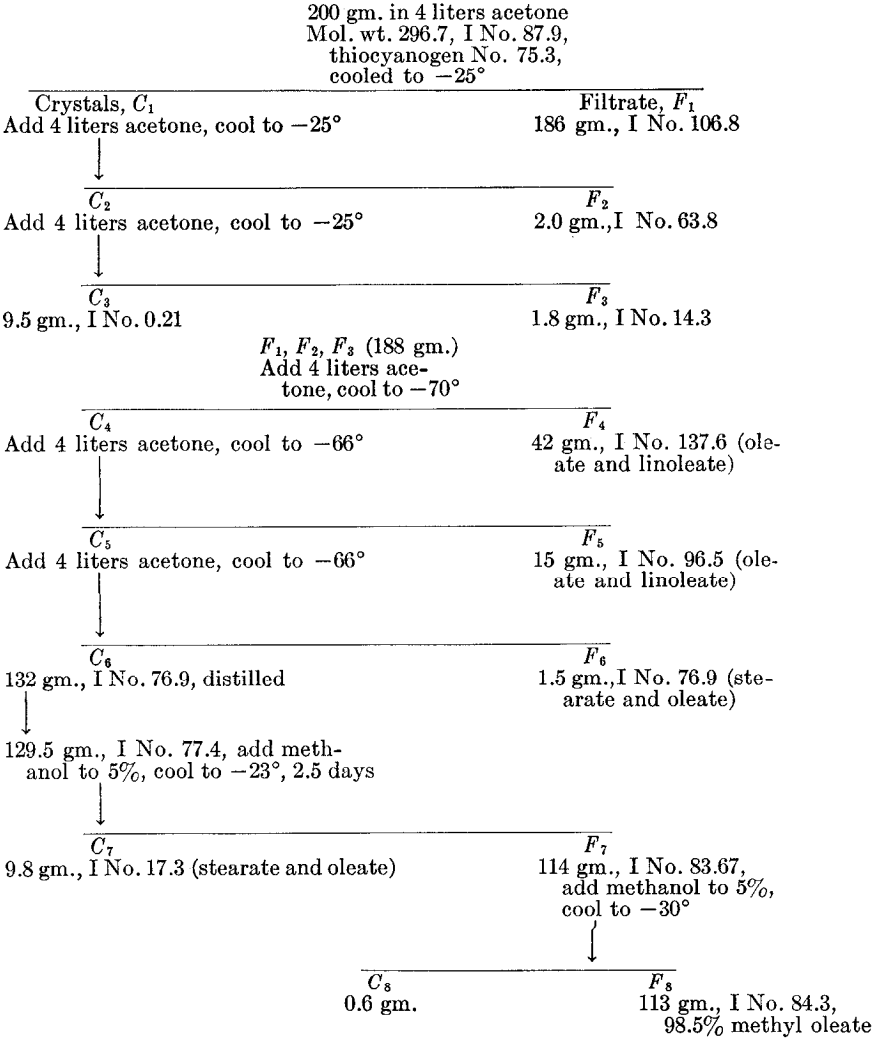
By the above procedure 113 gm. of methyl oleate of 98.5 per cent purity were isolated from 200 gm. of C_{18} esters, or about 80 per cent of the oleate in the C_{18} esters used. Oleic acid, prepared from the ester, gave the following constants: iodine number 88.8, thiocyanogen number 87.8, m.p. 12.4–12.7°. This oleic acid was recrystallized six times from petroleum ether at −60°, in order to remove any contaminating linoleic acid. The six times crystallized oleic acid gave the following results on analysis: iodine number 89.0, thiocyanogen number 87.2, mol. wt. 282.2, m.p. 12.8–13.0°. Pure oleic acid from olive oil melts at 13.3°. Both values for thiocyanogen number above are regarded by us with suspicion, because it is inconceivable that as much as 2 per cent of linoleic acid is present, as is indicated by calculation from the iodine-thiocyanogen number equation. The six consecutive filtrate fractions, amounting to 0.8 to 3.0 gm., melted at 1°, 2°, 8°, 9°, 10°, and 11°. Several of these were combined, crystallized once, and the resulting crystal fraction oxidized with alkaline permanganate by the procedure of Lapworth and Mottram (11). The resulting dihydroxystearic acid melted at 122–123°. The mixed melting point with an authentic specimen of dihydroxystearic acid prepared in a similar manner was 125–126°.

We interpret these results as follows: The octadecenoic acid of human depot fat is principally ordinary oleic acid. There are present with this oleic acid appreciable amounts of isomeric octadecenoic acids. These account for the decidedly low melting point observed in the original specimen and the 0.3–0.5° low melting point even after recrystallization. The contaminating isomers may consist, in part, of vaccenic acid (12) which has been identified in several animal fats in small amounts, or of one or more other isomers in the double bond position. The filtrate fractions in the recrystallization of the oleic acid give mixed dihydroxy derivatives of oleic and the isomeric acids. These isomers are concentrated in the filtrate frac-

tions which, as a result, have considerably lower melting points. This gradual removal of the isomers by recrystallization accounts for the rise in melting points of the several filtrates and for the appreciable rise in melting

CHART IV

Crystallization of C_{18} Esters of Human Fat



point of the oleic acid in the final crystal fraction. Since the postulated isomeric octadecenoic acids have not been actually isolated in a sufficiently pure state for identification, this interpretation of data is still open to

question. Millican, in applying these crystallization procedures to several seed oils, had little difficulty in preparing oleic acid of the same melting point as the purest specimen from olive oil. However, similar preparations from a number of animal sources almost invariably resembled the oleic acid preparation from human fat.

Several of the filtrate fractions from the crystallization of the C_{18} esters which from their high iodine number were assumed to be concentrates of methyl linoleate were converted to the free acids and crystallized according to the procedure described in Chart V.

If the original fatty acid mixture used in Chart V had been composed of ordinary oleic and linoleic acids, we should have been successful in effecting a much higher concentration of the linoleic acid. Frankel, Stoneburner, and Brown (13) by the use of crystallization procedures were able to isolate

CHART V

Fractional Crystallization of Linoleic Acid Concentrates of Human Fat

32.5 gm. in 425 cc. acetone
Mol. wt. 281.2, I No. 125.0, cooled to −50°

P_1 1.2 gm. | F_1 Cool to −70°

P_2 14.8 gm., I No. 122.3, tetrabromide No. 29.1, thiocyanogen No. 93.0 | F_2 16.2 gm., I No. 143.6; 12.5 " in 200 cc. acetone, cooled to −70°

P_3 5.7 gm., I No. 145.7; 5.6 " in 400 cc. petroleum ether, cooled to −60° | F_3 6.9 gm., I No. 139.8

P_4 | F_4 5.3 gm., I No. 146.0, tetrabromide No. 53.3, m.p. −14.5°, m.p. bromides 113.5–114°

linoleic acid by crystallization of the fatty acids of a number of semidrying oils from acetone and petroleum ether. With olive oil, however, difficulty was encountered and the results were interpreted as due to the presence of mixtures of isomeric octadecadienoic acids.

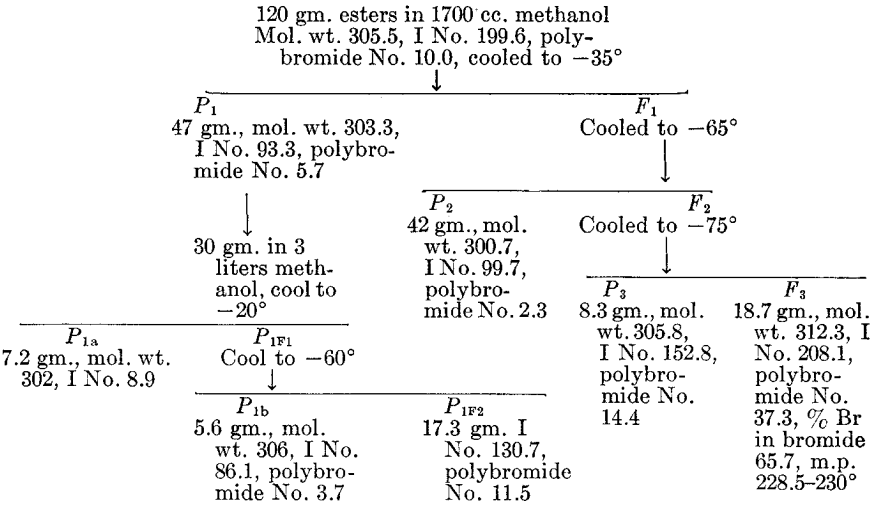
F_4 calculated from the iodine number as a mixture of oleic and octadecadienoic acids contains 61.5 per cent of the latter (iodine number 181.0). On the basis of the tetrabromide number 102.9 the content of linoleic acid in this fraction is 51.8 per cent. The difference between these values, 9.7 per cent, is octadecadienoic acid which does not yield the usual petroleum ether-insoluble tetrabromides. In other words, about five-sixths of the octadecadienoic acid of this fraction is linoleic acid; a similar calculation for P_2 shows a ratio of the two types of acids of about four-fifths.

Quantitative calculation of the composition of the C_{18} fraction can be made from iodine number-thiocyanogen number equations. These

equations are based on iodine numbers of 85.6 and 172.4 for methyl oleate and linoleate respectively, and on thiocyanogen numbers of 85.6 and 91.4 for these esters. The thiocyanogen number 91.4 for methyl linoleate was the value obtained on a specimen of pure ester prepared especially for the purpose; the determination was made by the procedure of Matthews, Brode, and Brown (14) at 16°. The equations and results given in per cent are as follows: methyl oleate = 2.488 × thiocyanogen number − 1.317 × iodine number = 71.57; methyl linoleate = 1.235 (iodine number − thiocyanogen number) = 15.56; methyl stearate = 100 − (oleate + linoleate) = 12.87.

CHART VI

Fractional Crystallization of Residual Fractions of Specimen XIII



These values have been expressed in Table I as stearic, oleic, and linoleic acids. The failure to obtain more complete separation of the unsaturated esters of this fraction is due to the probable complexity of their nature, whereby clear cut separations do not result.

C_{20} *Fraction*

The total residual fraction of Specimen XIII amounted to 188.0 gm. which, from the molecular weight, consists of 138 gm. of C_{18} esters and 50 gm. of C_{20} esters (2.5 per cent of the original specimen). From the polybromide number of this fraction (10.0) the arachidonate content is calculated to be 1.0 per cent, leaving 1.5 per cent of other C_{20} esters. The esters of the residual fraction were crystallized according to the procedure in Chart VI.

It is clear from the data in Chart VI that satisfactory separations were not obtained. The principal objective of the fractional crystallization, the

isolation of a concentrate of methyl arachidonate, was fairly successful in that final filtrate fraction F_3, amounting to 18.7 gm., contained 40 per cent of this highly unsaturated ester. This is calculated from the corrected factor, 91.8, of Mowry, Brode, and Brown (15). The identity of the methyl arachidonate is based on the bromine content of the polybromide, 65.7 per cent (theory for methyl arachidonate, 66.78 per cent), and its melting point, both of which values are comparable with those previously obtained by one of us (16) on similar products from a number of glandular tissues. It is likely that much better separations of C_{20} esters would be obtained if a pure C_{20} ester fraction could be prepared. However, we hesitated to try to separate these residual fractions by distillation through an electrically heated and packed column because of previous experience in this laboratory in the separation of methyl arachidonate by this method (15). The further identification of methyl arachidonate was therefore not possible. Also, it was impossible to separate and identify other possible C_{20} acids. However, we have calculated indirectly that portion of the iodine number of the C_{20} fraction which is not due to methyl arachidonate and found it to be approximately 85. This we believe to be due to the presence of an acid or acids of lesser unsaturation than arachidonic, possibly eicosenoic. It seems likely, therefore, that both this acid and arachidic acid are minor components of this fat.

DISCUSSION

For the first time, crystallization methods at low temperature have been applied to the study of the component fatty acids of two specimens of human body fat. Practically pure specimens of methyl myristate, palmitate, stearate, and oleate have been isolated from the esters of this fat, thus confirming previous work in this field. In addition, the presence of tetradecenoic and hexadecenoic acids in this fat has been demonstrated for the first time, these results being in line with several comparatively recent investigations on animal body fats. The oleic and linoleic acids have been shown to occur along with other isomers of these acids. Our results on the C_{12} and C_{20} fractions are incomplete. It will be necessary to work up considerably larger amounts of fat to investigate these fractions further, and positively identify the acids present with the exception of arachidonic acid, the presence of which was confirmed from analysis of its octabromide. Our results furthermore do not prove the absence of higher acids than C_{20} although these must be present, if at all, in only traces.

The demonstration of the presence in human fat of isomeric octadecenoic and octadecadienoic acids is of special interest. The synthesis of oleic acid in the animal body has been proved by numerous investigations in the past. It seems likely that the fatty acid synthesis by the animal organism results largely in the formation of ordinary oleic acid. This may be the case in

the human body. If Millican's results on the octadecenoic acids of animal origin are not in error, the isomeric acids in human fat may originate from synthesis and from the food. The finding of acids isomeric with linoleic, which is an essential fatty acid at least for rats, is worthy of special note. The most likely explanation of its occurrence is that it has been derived from similar acids in the dietary fat.

SUMMARY

1. The methyl esters of the fatty acids from two specimens of human depot fat were separated by distillation through an efficient column into six or seven relatively simple fractions; the main fractions representing esters of single carbon series were studied by crystallization procedures at low temperature.

2. Methyl myristate, palmitate, stearate, and oleate were isolated and identified as practically pure compounds.

3. The presence of tetradecenoic and hexadecenoic acids was demonstrated in this fat for the first time.

4. The oleic and linoleic acids of human fat are the principal C_{18} unsaturated acids present, but they are found along with isomeric octadecenoic and octadecadienoic acids.

5. The presence of arachidonic acid is confirmed.

6. From the data obtained from crystallization studies on two specimens and from distillation data on three more, the fatty acid compositions of five specimens of human fat have been calculated and recorded.

7. In the five specimens studied the linoleic (total octadecadienoic) acid contents ranged from 8.2 to 11.0 per cent; the values for arachidonic acid fell between 0.3 and 1.0 per cent.

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