Assimilation of Dietary Eicosenoic and Erucic Acid Esters[1,2]

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THE BODY FAT of land animals consists largely of glycerides of fatty acids of chain length C_{16} and C_{18}. In the higher animals (excluding fish, birds, and reptiles) fatty acids of this molecular size make up more than 90% of the total fatty acids.

Two factors contribute to this concentration of C_{16} and C_{18} acids. First, the predominant fatty acids in most terrestrial diets are of this chain length. Second, the endogenous fat, synthesized by the animal from carbohydrate and protein and deposited in the tissues, is composed almost entirely of C_{16} and C_{18} fatty acid glycerides in those species which have been studied.

A probable third factor is partial rejection of acids of other chain lengths by preferential metabolism or excretion (5). Thus, when coconut oil is fed to rats, it is almost completely absorbed and there is some deposition of the short chain acids, but the proportion of C_{16} and C_{18} acids deposited is still much higher than in the coconut oil (7). Saturated acids of 20 carbon atoms and higher are poorly absorbed by rats, that is to say, they are rejected to a considerable degree by excretion (1, 4).

There is evidence that the absorption of monoenoic acids also diminishes for those above C_{18} (2, 3, 6), but little is known concerning the degree to which they are stored in the body. Consequently the present work was undertaken to determine the extent of deposition of the common monounsaturated C_{20} and C_{22} acids in comparison with oleic acid, when fed to albino rats as the methyl esters.

Experimental

Materials and Diets. The basal diet was as follows: corn starch 58, vitamin-free casein 20, yeast (fat-free) 8, cellulose (Alphacel) 5, salt mixture (U.S.P. XIV) 4, fat (when added) 5. It was almost but not entirely fat-free since there were traces of fat in the starch and the vitamin mixture. Each rat received the following amounts of vitamins administered weekly in 0.1 ml. of corn oil: A, 84 I.U.; D, 84 I.U.; E, 0.84 mg. (d-α-tocopherol).

The fatty acid esters were prepared in the laboratory from natural vegetable oils by fractional distillation of the mixed methyl esters, followed by fractional crystallization of the appropriate fractions from acetone at temperatures down to —45°C. Methyl oleate was prepared from filbert oil and peanut oil, methyl-11-eicosenoate from jojoba oil (*Simmondsia californica* Nuttall) (8), and rapeseed oil, and methyl erucate from rapeseed oil and jojoba oil. The esters were of high purity. The analysis of one lot is shown in Table I.

TABLE I
Analysis of Dietary Methyl Esters

Methyl ester	Iodine value		Total dienes	Total trienes
	Found	Theory		
			%	%
Oleate	85.1	85.6	0.23	0.01
Eicosenoate	78.4	78.2	0.21	0.08
Erucate	71.7	72.0	0.37	0.05

The determinations were made according to the Official and Tentative Methods of the American Oil Chemists' Society.

Fifty inbred albino rats of the Wistar strain were divided into five groups, each group composed of five males and five females. The fat or lipide portions of the diets for the respective groups were as follows: corn oil, no fat, methyl oleate, methyl eicosenoate, and methyl erucate. All groups received the basal diet. The fats were added to the extent of 5 parts of fat to 95 parts of basal ration in each fat-containing diet. Starch was added instead of fat for the no-fat diet.

The rats were selected at weaning (weight 30–40 g.) and were divided into groups equally distributed according to weight and litter. They were individually caged. The feed was provided *ad libitum,* and a record was kept of the amount eaten.

Feeding was continued for 12 weeks. At the end of that time the males were sacrificed. The females were bred and continued on the same diets until their litters were weaned. The total feeding period was 20 weeks.

Extraction of Body Fat. The bodies were separated into three portions, *viz.,* pelt, viscera, and carcass. Each portion was heated with 10% aqueous alkali to release the fat. After acidifying, the fat and fatty acids were combined and saponified by alcoholic alkali. The soap solutions were extracted with petroleum ether to remove unsaponifiable matter, then acidified to recover the total fatty acids. The yield of fatty acids is shown in Table II.

These determinations were done by groups and not by individual animals. Hence it was not possible to make an analysis of variance. The fat-free diet gave the lowest yield of fat from both males and females, as would be expected. Otherwise the differences between diets are not consistent and are probably not significant. The feeding of eicosenoic and erucic esters as 5% of the diet does not appear to have caused any reduction in body fat as compared to oleate feeding at this level.

Characteristics of the Body Fat. Iodine value and equivalent weight determinations were made on the pooled fatty acids of each group. In addition, 10 determinations of each characteristic were made on

TABLE II
Average Yield of Fatty Acids

Dietary fat	Fatty acids per rat (g.) from				Total fatty acids, % of rat wt.
	Pelt	Carcass	Viscera	Total	
Males					
Corn oil	4.36	3.66	3.58	11.6	5.9
None	3.44	2.68	1.36	7.5	3.9
Oleate	3.98	2.90	2.16	9.0	5.1
Eicosenoate	4.52	2.46	3.52	10.5	5.5
Erucate	3.56	2.62	2.68	8.9	5.2
Mean	3.97	2.86	2.66	9.5	5.1
Females					
Corn oil	2.58	2.25	1.88	6.7 (1)[a]	4.2
None	2.10	1.46	1.54	5.1	3.5
Oleate	3.44	2.64	2.22	8.3	6.2
Eicosenoate	3.74	2.30	1.86	7.9	5.7
Erucate	3.40	2.70	2.47	8.6 (2)[a]	6.2
Mean	3.05	2.27	2.00	7.3	5.2

[a] Figures in parentheses represent the number of animals that died during the feeding period.

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TABLE III
Iodine Value of Body Fatty Acids

Dietary fat	Fatty acids from			Total Fat
	Pelt	Carcass	Viscera	
Males				
Corn oil	101.8	102.3	101.2	101.8
None	66.9	66.1	62.5	65.8
Oleate	77.8	77.6	73.0	76.6
Eicosenoate	77.4	74.8	76.0	76.2
Erucate	73.5	69.2	70.8	71.3
Females				
Corn oil	101.6	99.8	97.7	99.8
None	67.6	70.4	69.0	68.8
Oleate	77.4	76.3	74.1	76.1
Eicosenoate	77.9	74.2	71.6	76.6
Erucate	73.6	73.2	70.2	72.4

the fatty acids from individual animals in one group in order to obtain an estimate of the variation in the data. On the basis of the variation found in these values it was calculated that differences of 5.0 units or more in iodine value and 5.3 units or more in equivalent weight were significant at the 95% level.

Iodine values of the fatty acids of the body fats are given in Table III. As would be predicted, the corn oil diet produces a fat of high iodine value and the fat-free diet a fat of low iodine value. There is little difference between the fat from the three ester diets although the erucate diet gives a slightly lower iodine value for both sexes. There is no significant difference between the values for fat from males and females.

Mean molecular weights (equivalent weights) of the body fatty acids were determined by titration. The equivalent weights of the pelt fatty acids from the male rats fed eicosenoate and erucate were significantly higher than the corresponding equivalent weight of acids from the oleate diet. They were also higher for the carcass and visceral fatty acids, but the increment was not statistically significant for all. The same trend was evident in the fatty acids from female rats.

The equivalent weights of the body acids from the erucate diet were not significantly higher than those from the eicosenoate diet, suggesting that less erucate was deposited than eicosenoate. The values were somewhat higher for the acids from the pelt (including subcutaneous tissue) than for the acids from the remainder of the body.

Determination of Polyenoic Acids. The content of dienoic, trienoic, and tetraenoic acids in the body fatty acids was determined by ultraviolet absorption after isomerization.

The content of diene acid is naturally much the highest on the corn oil diet (Table IV). The diene acid level on this diet is about twice that found in rats on an ordinary mixed diet. Although the fat level in the diet is fairly low (5% by weight), the animal evidently stores linoleic acid to a large extent. Females appear to retain somewhat less than males.

It is evident that more diene acid was retained in the body fat on a fat-free diet than on any of the three ester diets. A similar effect has been noted by others in feeding fats which lack linoleic acid. Possibly the added fat dilutes the linoleate in the system, and some is then lost by excretion. The males had more diene acid in the pelt and the viscera than in the carcass fat on all diets except corn oil.

There were no consistent differences in the content of triene acids. There did not appear to be a rise in triene acid on the linoleic-acid-deficient diets as has been reported to occur in blood lipides.

The tetraene acid content was noticeably higher on the corn oil diet than on the others, otherwise there were no significant differences. The eicosenoate diet (C_{20}) has evidently not caused any appreciable increase in the arachidonic acid content of the body fat. Thus the animal did not convert eicosenoate to arachidonate.

Distillation of Methyl Esters of Body Fats. The acids from the pelt, carcass, and viscera of each group (with one exception) were combined and esterified with methanol. The pelt acids of males on the eicosenoate diet were esterified and distilled separately. The esters were fractionally distilled at 0.5-mm. pressure through a spinning band column. The proportions of C_{16}, C_{18}, C_{20}, and C_{22} acids were estimated from the distillation curves, weight of the distilled fractions, and the analysis of the fractions.

As expected, the fats from the first three groups (diets containing corn oil, no fat, and methyl oleate)

TABLE IV
Polyenoic Acids in Body Fat

Dietary fat	Diene acid, % of total fatty acids				Triene acid	Tetraene acid
	Pelt	Carcass	Viscera	Total fat		
Males					%	%
Corn oil	24.2	27.0	25.9	25.7	0.3	1.3
None	3.9	3.1	3.9	3.6	0.1	0.2
Oleate	2.2	1.5	1.8	1.8	0.1	0.1
Eicosenoate	2.9	1.8	2.3	2.3	0.2	0.2
Erucate	3.6	1.6	2.9	2.8	0.4	0.3
Females						
Corn oil	20.4	19.0	(11.5)	19.7	0.2	1.2
None	2.2	2.8	3.5	2.8	0.2	0.4
Oleate	2.1	2.0	1.8	2.0	0.1	0.1
Eicosenoate	3.2	1.8	1.4	2.1	0.2	0.2
Erucate	2.5	1.7	1.4	1.9	0.1	0.1

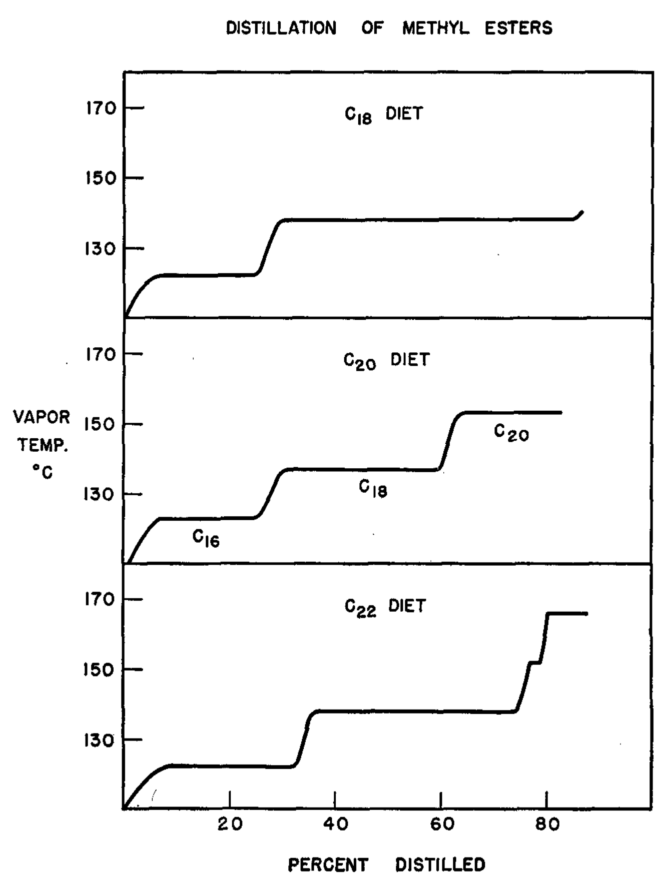

FIG. 1. Distillation curves for methyl esters of body fats of male rats on diets of oleate, eicosenoate, and erucate.

gave almost entirely C_{16} and C_{18} acids. However there was evidence of a small amount of longer-chain acid(s) in each of these three.

The fat from the group on methyl eicosenoate diet yielded a considerable amount of C_{20} acid (Figure 1). Similarly the fat from the methyl erucate group gave an appreciable distillate fraction of C_{22} acid and also a little C_{20} acid. Identification of the C_{20} and C_{22} acids is described below.

Eleven lots of body fat esters were distilled in all. Distillation curves are shown in Figure 1 for three of these. The distillation curves for fatty esters from female rats followed the same pattern as those for males. The esters from the pelts of males on the eicosenoate diet differed from the combined carcass and visceral esters in having more C_{18} and less C_{16} acids, thus accounting for the differences in mean molecular weight. The content of C_{20} acid did not differ.

Identification of Eicosenoic and Erucic Acids in the Body Fats. Distillation data for the esters from the body fat of the groups fed methyl eicosenoate are shown in Table V. Fraction 1 (males), mainly palmitic ester, when saponified and hydroxylated by alkaline permanganate, gave some 9,10-dihydroxypalmitic acid, m.p. 122–123°, alone and mixed with an authentic sample. This fraction contained 9-hexadecenoic acid as well as palmitic acid. Fraction 2 consisted chiefly of oleic acid with a little stearic and linoleic acids.

TABLE V
Distillation of Methyl Esters from Body Fat of Group Fed Methyl Eicosenoate

Fraction	Steady temp. °C. at 0.5 mm.	Wt of fraction	Iodine value	$n_D^{25°}$	Chain length
Males, fat of pelt only		g.			
1	121–122	5.0	25.8	1.4428	C_{16}
2	136–138	8.0	81.5	1.4508	C_{18}
3	150–153	4.7	79.6	1.4530	C_{20}
Females, total fat					
1	121–123	10.0	25.4	1.4426	C_{16}
2	135–136	6.9	84.6	1.4507	C_{18}
3	136–137	6.4	80.5	1.4507	C_{18}
4	151–153	8.7	80.5	1.4533	C_{20}

Fraction 3 (males) was almost pure methyl eicosenoate as judged by the iodine value (theory 78.2) and refractive index. It was identified by saponifying and hydroxylating with permanganate, whereupon it gave a dihydroxy acid which melted at 129–130°, unchanged by admixture with 11,12-dihydroxyarachidic acid. This fraction therefore contained 11-eicosenoic acid, the same as the dietary acid. Fraction 4 from the female fat yielded the same acid.

TABLE VI
Distillation of Methyl Esters from Body Fat of Group Fed Methyl Erucate

Fraction	Steady temp. °C. at 0.5 mm.	Wt. of fraction	Iodine value	$n_D^{25°}$	Chain length
Males		g.			
1	121–122	12.6		1.4420	C_{16}
2		1.8		1.4440	
3	137–138	10.7	85.8	1.4508	C_{18}
4		7.5	78.7	1.4502	C_{18}
5	151–152	1.8	81.7	1.4547	C_{20}–C_{22}
6	166	3.1	71.4	1.4550	C_{22}
Females					
1	115	1.5	21.8	1.4402	
2	115–119	5.9	21.9	1.4420	C_{16}
3	128–132	8.6	78.2	1.4504	C_{18}
4		2.5	73.3	1.4555	
5	159–161	1.4	71.5	1.4559	C_{22}

Table VI gives the distillation data for the groups fed methyl erucate. Fraction 5 (males) was hydroxylated and gave 11,12-dihydroxyarachidic acid, m.p. 130°, confirmed by mixed melting point. Fraction 4 (females) yielded the same dihydroxy acid. Fraction 6 (males) was almost pure methyl erucate (theor. iodine value 72.0). On hydroxylation it was converted to 13,14-dihydroxybehenic acid, m.p. 129–130°, alone and mixed with an authentic sample. Admixture with 11,12-dihydroxyarachidic acid lowered the melting point by 5–7°. Thus the fat from this group contains both eicosenoic and erucic acids. The amount of erucic acid which distilled (as ester) is estimated as 9% of the total fatty acids, and there was doubtless a further amount in the distillation residue.

Saturated Acids in the Body Fats. Palmitic acid is the chief constituent of the saturated acids of rat fat. The methyl esters of palmitic and higher saturated acids are solid at room temperature. They distill at the end of the unsaturated fraction of corresponding chain length and, if present in appreciable quantity, solidify in the outlet tube. Thus they were readily detected during the distillation. The quantities were estimated by low-temperature crystallization and iodine value determinations.

There was a substantial amount of palmitic acid in the fat from all groups in this experiment. Small amounts of stearic acid (about 2 or 3% of the total acids) were observed in the fat from the groups on corn oil and no fat diets. Still less stearic acid was seen in the corresponding distillates from the oleate, eicosenoate, and erucate diet groups. Arachidic ester was not seen to crystallize in the distilled ester fractions from any of the groups, even when the

TABLE VII
Estimated Composition of Fatty Acids from Body Fat

Dietary fat	Percentage of total fatty acids								
	Hexadecenoic	Palmitic[a]	Linoleic[b]	Oleic	Stearic	Eicosenoic	Erucic	Trienoic	Tetraenoic
Males:									
Corn oil	6	23	26	40	<3			0.3	1.3
None	13	32	4	47	<3			0.1	0.2
Oleate	6	18	2	70	<2			0.1	0.1
Eicosenoate	7	18	2	43	<2	28		0.2	0.2
Erucate	7	24	3	49	<2	2	12	0.4	0.3
Females:									
Corn oil	5	26	20	45	<3			0.2	1.2
None	11	31	3	52	<3			0.2	0.4
Oleate	6	17	2	71	<2			0.1	0.1
Eicosenoate	7	20	2	42	<2	26		0.2	0.2
Erucate	7	25	2	45	<2	2	15	0.1	0.1

[a] Probably includes a little myristic acid.
[b] May include some isomers of linoleic acid.

fractions were cooled to 5°. The distillation residues were saponified and the acids recrystallized. They were mostly unsaturated, but the analysis indicated a small content of arachidic acid, estimated to be less than 2% of the total acids in all groups. No more than a trace of behenic acid was found in the fat from the erucate group.

Estimated Composition of the Body Fatty Acids. The estimated proportions of the chief acids of the body fats are shown in Table VII. These results are based on the pooled fat of five males and five females on each diet. Arachidic acid is not included because it was not formally identified and in any case does not exceed 2% of the total acids.

Discussion of Results

The results show conclusively that eicosenoic and erucic acids were deposited in the body fat to a considerable extent when fed as the respective methyl esters. The body fat of the rats fed corn oil, no fat, and methyl oleate may have contained small amounts of these acids, but it was estimated from examination of the distillation residues that the total of the eicosenoic and erucic acids in the fat would not exceed 2% of the fatty acids. In contrast, eicosenoic acid amounted to about 28% of the acids in the fat of male animals fed eicosenoate, and erucic acid to about 12% in the corresponding erucate group. The two acids were formally identified by conversion to dihydroxyarachidic and dihydroxybehenic acids, respectively.

Large amounts of oleic acid were found in the body fat of rats fed methyl oleate. A direct comparison cannot be made however with the deposited eicosenoic and erucic acids since a proportion of the oleic acid was undoubtedly synthesized from carbohydrate when the dietary fat level was only 5%. The amount of linoleic acid deposited by rats fed corn oil was similar to the amount of eicosenoic acid deposited when eicosenoate was fed. However, as the intake of linoleate from corn oil was less than the intake of pure esters, the proportion of dietary linoleate deposited was greater. Since little, if any, linoleic acid is synthesized by the rat, this comparison is valid.

These results cannot be explained on the basis of differences in digestibility. Determination of the excreted fatty acids in this experiment showed that the digestibility of eicosenoate and erucate was 90–95% (10).

It is interesting to note that the amounts of these acids stored by female rats were nearly the same as those stored by males even though the females were maintained on the diets for an additional eight weeks.

Proportions of the various acids synthesized on the "no fat" diet are shown in Table VII and are generally in agreement with the results of Longenecker (9). The content of palmitic acid, 32% (males), fell to 18% when oleate or eicosenoate was supplied in the diet but was reduced only to 24% on the erucate diet. This suggests that the animal finds it preferable to increase its synthesis of fat rather than to deposit more of the dietary erucic acid.

Possible Conversion to Shorter Chain Length Acids. A small proportion of eicosenoic acid (about 2%) was identified in the fat of the rats fed erucate. In the distillation of the esters of this group all of the eicosenoate is driven over by the methyl erucate, whereas in the fat from the first three groups in Table VII the eicosenoate remained in the distillation residue and was difficult to estimate. Thus it is not possible to decide whether this is a normal content of eicosenoate or whether part has been formed by removal of a two-carbon unit from the dietary erucate. In either case the amount is relatively small.

There does not appear to have been any conversion of dietary eicosenoic acid to oleic acid since the proportion of oleic acid (43%) in the body fat is even less than on the no-fat diet. The sum of the oleic and eicosenoic acids deposited on the eicosenoate diet is almost the same as the amount of oleic acid deposited on the oleate diet, suggesting that the extent of deposition of dietary monoenoic acid (over and above the synthesized fat) is the same whether the diet is oleate or eicosenoate.

There is no evidence that the unsaturated esters tested were converted to saturated acids for deposition. The palmitic acid content was less and the stearic acid content no more on the ester diets than on the no-fat diet. Furthermore the content of arachidic acid is judged to have been less than 2% throughout, and there was not more than a trace of behenic acid from the erucate diet. It may be that interconversion of fatty acids in the body is less extensive than hitherto believed.

No serious nutritional or metabolic disturbances were noted in the rats on diets of methyl oleate, eicosenoate, and erucate although there were some symptoms of essential fatty acid deficiency and excretion of lipide was increased on the erucate diet. This part of the work will be reported in another communication.

Summary

1. Eicosenoic acid, fed to rats as methyl ester to the extent of 5% by weight of the diet, was deposited in substantial amount in the body fat.

2. Erucic acid, fed in the same way, was deposited in considerably lesser amount.

3. Neither of these acids appeared to be as readily deposited in body fat as the linoleic acid of corn oil.

4. The three monounsaturated acids, oleic, eicosenoic, and erucic, were apparently deposited without alteration in chain length or degree of saturation. There was no evidence of conversion of eicosenoate to arachidic or stearic acids, or of erucate to behenic, arachidic, or stearic acids in any appreciable amount. Similarly there appeared to be little or no conversion of eicosenoate to oleic acid or of erucate to eicosenoic acid.

5. The eicosenoate in the diet was not converted to arachidonic acid by the animal.

6. The amount of total fat deposited and the proportion in carcass, skin, and viscera were approximately the same on diets of oleate, eicosenoate, and erucate.

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