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Positional Isomerism of Unsaturated Fatty Acids in the Rat Quantification of Isomeric Mixtures

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Abstract

Mono- and dienoic acids of lipids from rat milk, rat sucklings, and rats on a fat-deficient diet were investigated. The percentage of uncommon isomers of palmitoleic acid was highest in milk, the newborn and the suckling rats, but receded after weaning. Isomers of linoleic acid were found only in traces in sucklings but became pronounced in rats on diets lacking essential fatty acids. The proportion of 8,11-diene among octadecadienoic acids increased markedly under such conditions within one week and two additional isomers became prominent after longer periods of fat-deficient diet.

A supplement of hydrogenated coconut fat did not influence the occurrence of these isomers.

Dietary petroselinic acid is incorporated by the rat into tissue lipids. 4-Hexadecenoic and a small amt of 8-eicosenoic acid arise from it.

Quantification of isomeric mixtures by ozonization-hydrogenation and subsequent gas-liquid chromatography is discussed in detail.

Introduction

IT IS KNOWN THAT LIPIDS of rats reared on a fat-deficient diet contain unsaturated acids which are isomeric with those in rats on a normal diet (9,18,22,24). In particular, the fatty acids of rats in an advanced state of essential fatty acid (EFA) deficiency have been investigated and 5,8,11–20:3 was found prominent among polyunsaturated acids. Quantifications of this acid relative to arachidonic has served as a parameter for defining the degree of EFA deficiency (16). In most of these studies the acids from liver or liver phosphatides were analyzed since there the level of polyunsaturated acids is relatively high regardless of the nutritional state of the animal (5).

The present report concerns mainly the isomers of monoenoic and dienoic acids found in the total lipids of the rat and the progressive change which these acids undergo during early life and in the course of dietary regimens. In an exploratory experiment, various diets were fed for 3 weeks to rats 25 days old. Isomers of palmitoleic acid present at the beginning of this period had diminished, regardless of the dietary treatment. Within the same time, the amt of isomers of linoleic acid had become significant in rats on a fat-free diet. On the basis of these results a more elaborate experiment was designed.

The dietary period was extended to 6 months and fatty acid analyses were made at time intervals. Analyses were extended to the feed and milk of dams and the progeny during the first 3 weeks after birth. A fat-free diet and the same diet supplemented with either hydrogenated fat or corn oil were used. The data of the preliminary experiment were closely verified by the results of the comprehensive experiment; therefore, only the latter are reported in detail.

Positional isomers of monoenoic esters are not separated well by common gas-liquid chromatography (GLC) procedures and this difficulty is also often en-

TABLE I
Fatty Acids of Vitamin-free Casein [a]

Acid Chain length: double bonds	% of total fatty acids
6	1.5
8	1.4
10	3.1
12	3.3
14	10.7
14:1	1.4
15	1.0
16	25.4
16:1	2.2
17	0.9
18	18.9
18:1	27.3
18:2	0.8
18:3	0.8
19	+
20	1.0

[a] "Vitamin-free Casein," from Nutritional Biochemicals, Cleveland, Ohio.

countered with dienoic esters. More recent methods make use of silver salts as complexing agent and separation of certain isomers has been achieved (4,32). Still, these methods are insufficient for analyzing biological mixtures of isomers by separation. The usual procedure was followed here by separating first the mixture of isomers from other fatty acids. Purity in regard to chain length and number of double bonds is easily checked and the mixture of isomers is then subjected to oxidative splitting of the double bonds. Identification and quantification of fragments enables one to determine the isomeric structures and their relative amts. The method of quantification is elaborated in the experimental section.

Experiments

Male rats of the Sprague-Dawley type were obtained from commercial sources and taken under dietary control at an age of 25 days. They were divided into nutritional group, *1, 2* and *3*, of 66, 45 and 36 animals, respectively. The fat-free diet *1* was a semisynthetic diet with vitamins (6,23). Diet *2* was the basal diet *1* with 5% Hydrol (hydrogenated coconut fat from Durkee Co., Chicago 47, Ill.) which provides fat lacking EFA. Diet *3* was the same basal diet with 5% corn oil. The fatty acid analyses of Hydrol and corn oil are given in the pertinent tables.

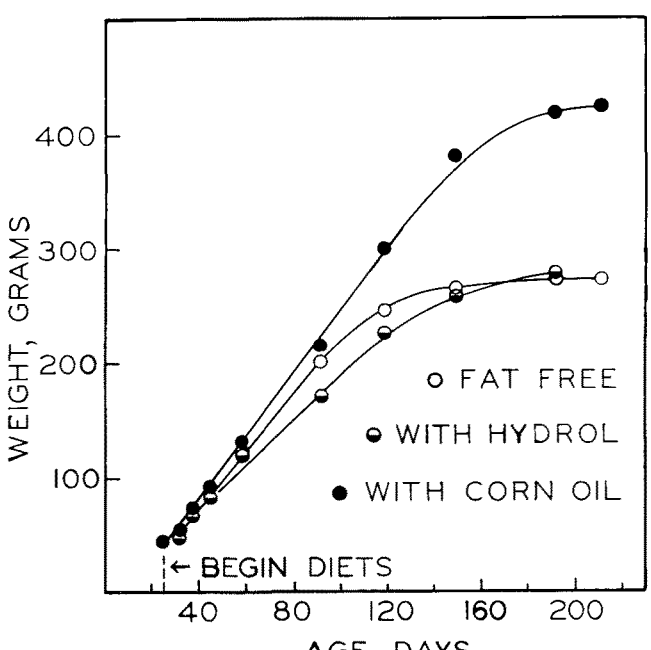

FIG. 1. Weight of rats on fat-free diet, ○; supplemented with Hydrol, ◒; and supplemented with corn oil, ●.

It was expected that some isomers would appear in small amts. Therefore, it was necessary to analyze also the fatty acids of the casein used in the diets. Aliquots of 1 kg from three lots of commercial casein were extracted in a Waring blendor repeatedly with 2:1 chloroform:methanol, and 3.0 [1], 2.5 [2] and 3.2 [2] g of soluble material were obtained. TLC indicated that triglycerides were the major portion of the lipids. The total extracts yielded 2.2, 1.1 and 1.1 g fatty acids which according to TLC contained very little autoxidized material. GLC analyses of the esters were rather similar for all three samples and are exemplified by the data of Table I. The esters of 18:1, 18:2 and 16:1 were isolated on larger scale and their structural composition determined as described below. The fraction 18:1 contained 10% *trans* double bond, and consisted of 90% 9- and 9% 11–18:1. Fraction 18:2 contained 76% of the normal 9,12–18:2, but also 17% and 6% of esters unsaturated in position 11 and 5, respectively. The latter compounds have not been further identified. Ozonization of 16:1 showed 60% 9–16:1 and the presence of a wide variety of isomers among which 12% 7–16:1 was prominent.

The daily intake of feed by an adult rat may be estimated as 20 g. This contains 3.6 g of vitamin-free casein (6,23). The rat is then supplied with about 5 mg of long-chain fatty acids per day of which less than 0.05 mg is linoleic acid.

The analyses of fatty acids in vitamin-free casein have been made without precautions against loss of short-chain fatty acids since long-chain mono- and dienoic acids were of major interest here. The structures of casein acids is not reflected in the acids of the EFA-deficient rat. However, further study of 16:1 and 18:2 acids in such dietary preparations of casein may be of interest in some other context.

Diets and water were offered ad libitum. The animal quarters were at a constant temp of 27C without humidity control. Seven, five and two animals, of the respective groups, died in the course of 6 months. The time-weight curves, which are given in Figure 1, are characteristic for the particular diets and the condition of the animals may be evaluated from them.

At each time interval, six animals from each group were killed under ether anesthesia. The carcasses were stored at −17C and were ground in frozen condition. The pulp was repeatedly extracted cold with chloroform + methanol, 2:1, in an Omni-mixer. The fatty acids were obtained from the extract by saponification with KOH in boiling ethanol and subsequent common procedures. Aliquots of 5 g of acids were esterified with diazomethane for analyses.

Suckling rats were received in dry ice from two commercial sources and each shipment furnished rats 1, 7 and 14 days old. According to age, 60, 40 and 35 carcasses were worked up to obtain the fatty esters. Regardless of source, the fatty acid composition of rats of the same age were in close agreement.

Feed of mother animals was secured from one of the commercial sources together with sucklings. The material was extracted and the fatty esters prepared by procedures similar to those used with the rat esters.

Rat milk was obtained as described by Glass (13,20) from dams which had littered 16 days earlier (Minnesota strain, Department of Biochemistry, Institute of Agriculture, University of Minnesota). Twenty grams

[1] "Vitamin-free Test Casein (assayed)" from General Biochemicals, Chagrin Falls, Ohio. The samples were taken from different lots.
[2] "Vitamin-free Casein" from Nutritional Biochemicals, Cleveland, Ohio.

of milk from 6 animals yielded 1.4 g fatty acid methyl esters.

Analytical procedures involved GLC of small samples for quantification; liquid-liquid chromatography (LLC) of larger aliquots followed by GLC to obtain individual esters or mixtures of isomers in quantity; ozonization-hydrogenation-GLC to determine the structures of these compounds and to quantify the isomers.

Conditions for analysis and quantification of total esters by GLC were as previously described (27). Tests with several synthetic mixtures had shown that the proportionality of peak area to weight of ester is different with saturated and polyunsaturated esters. Correction factors, determined experimentally, were 1.1 for linoleate, 1.3 for linolenate and 2.1 for arachidonate when chromatographing in a Beckman GC2 instrument with thermal conductivity detector. Stationary phases were β-CDX acetate and propionate in a ¼ in × 12 ft aluminum column at 230C and 75 ml flow of He/min. It was assumed that the correction factors for the aforementioned authentic compounds are valid also for their isomers.

Fractionation procedures on a larger scale were chosen so that selective enrichment of isomers would be minimized. The esters were fractionated by LLC (25,33) in portions of 1–2 g and it was necessary to run several LLC columns to obtain 18:2 in sufficient amt from animals which had been on diets *1* or *2* for more than 2 months. The recovered esters were contaminated with some silicone oil and were purified from it by alembic distillation at 110–130C/0.1 mm (11). The esters superimposed in LLC fractions were then further separated by GLC. The previously reported procedure for collecting GLC fractions (28) was simplified by use of a ground joint connection between detector outlet and collector tubing (11). Samples of 50–100 mg were applied and the collected fractions were checked for purity by GLC and hydrogenation-GLC. Several samples were subjected to IR spectroscopy and did not show *trans* double bonds to any appreciable extent.

Quantification of Isomeric Unsaturated Esters

Ozonization and subsequent procedures for identification have been described and assay of isomeric unsaturated esters has been outlined in connection with analyses of other materials (11,26,33). In the present case, the precision of quantification of isomers was more crucial and, therefore, procedures were evaluated in greater detail.

Beckman GC2 and GC2A equipment with thermal conductivity or H_2-flame detector was used and both aldehyde esters and aldehydes were identified. The quantification of isomers was based on the ratio of areas of aldehyde ester peaks in the thermal conductivity recording of one chromatogram. Aldehyde esters of different mol wt result from isomeric fatty esters. This was taken into account by multiplying the respective areas with the ratios of MW fatty ester/MW aldehyde ester.

It is necessary to inject the aldehydic compounds within 30 min after the reduction of ozonides since during prolonged storage they undergo changes which cause new peaks in GLC. Although the aldehydes are always found, their quantification leads to erratic results, the trend of which conforms to the relative volatilities of the homologous series. Therefore, quantification was based only upon the aldehyde esters; they are less volatile than the aldehydes.

TABLE II

Quantification of Mixtures by Ozonization-Hydrogenation-GLC

Methyl ester	Composition %	Composition from GLC areas of Ald-esters, %
Petroselinate	1.00	1.1
Oleate	99.00	98.9
Petroselinate	2.03	2.1
Oleate	97.97	97.9
Petroselinate	4.9	4.2
Oleate	95.1	95.8
Petroselinate	25.3	25.8
Oleate	24.5	25.9
11–Eicosenoate	25.0	24.4
Erucate	25.1	24.0
6,9–15:2	36.4	35.4
9,12–15:2	63.5	64.4
6,9–16:2	16.8	16.6
9,12–16:2	83.1	83.3

The results listed in Table II show that the principle of the procedure is valid for monoenoic and for dienoic esters. The former mixtures were synthetic and the latter (33) are separable by GLC on β-cyclodextrin (β-CDX) acetate or propionate and were quantified this way.

The analyses given in Table II concern mixtures which are very favorable for quantification. For example, oleate, authentic (Hormel Foundation) or prepared from rat fat does not give rise to any trace of C_6 aldehyde ester by ozonization. However, some C_8 aldehyde ester and an unidentified compound between C_8 and C_9 aldehyde esters appeared in all cases and amounted to 0.5–1.0% of the GLC area of C_9 aldehyde ester. The values of Table II are corrected against such background.

Over-oxidation is an inherent problem in all oxidative procedures for fragmentation at the double bond and leads to illegitimate shorter chain products. Several reactions are known which minimize the side products but our attempts to eliminate their formation completely were not successful, although numerous methods for converting ozonides into short-chain alcohols, their acylated derivatives, into aldehydes or into acids were tested. Furthermore, it is obvious that evaporation of high boiling organic solvents is undesirable for quantification of fragments and, similarly, aqueous extraction for removal of reagents or separation of fragmentation products may introduce error. The methods referred to above gave the least amt of side products. They are rapid and afford relative quantifications.

Difficulties in developing a method which avoids any side products begin with obtaining an authentic sample where the presence of minimal amts of isomers is ruled out with certainty. Presently, the best method known for checking purity of structure is ozonization, but it is the method under investigation.

The objection that saponification or autoxidation may create minor contaminants is valid with experimental samples as well as with authentic samples. A rather convincing argument for the reality of isomers at low percentage is given when they occur during some phase of the experiment at a high level and when high values coordinate with low ones in a regular way. This is the case here with several monoenoic and dienoic acids. Some other isomers appear also higher than the background level of the method but a trend is not indicated.

Several analyses have been repeated after 6 months with identical results. Therefore, artifacts due to unrecognized procedural changes in the course of the lengthy investigation are unlikely.

TABLE III
Fatty Acids (%) of Rat Feed, Milk and Rats Age 0–25 Days

Compound	Structure	Feed	Milk	Age of rats (days)			
				1	7	14	25
8		+	4.6	0.4	1.1	0.7	—
10		+	14.9	1.0	6.9	5.2	2.9
12		+	14.4	0.7	10.3	8.9	7.3
14		1.3	13.9	1.6	8.6	8.3	6.9
16:0		19.3	21.9	28.8	24.9	24.6	23.1
16:1		2.3	1.9	5.1	2.3	3.0	3.0
	6–	1.1	7.5	7.3	3.6	2.8	2.4
	7–	9.7	12.8	14.1	12.4	14.0	17.9
	9–	87.1	77.5	76.9	78.9	78.1	73.3
18:0		8.9	2.4	6.4	4.7	5.1	7.3
18:1		33.2	12.6	33.4	27.1	28.6	33.4
	9–	92.8	88.9	85.9	86.2	89.1	89.1
	11–	4.1	7.5	8.9	8.0	7.7	10.9
18:2		31.8	12.3	10.0	10.8	12.7	12.4
	5,8–	—	+	+	+	+	+
	6,9–	+	+	+	+	+	+
	8,11–	1.4	1.1	1.1	1.0	1.5	1.7
	9,12–	98.6	98.9	98.9	98.1	98.5	98.3
18:3		3.2	1.1	0.7	1.4	1.6	0.6
20:4		—	+	11.8	3.3	2.9	3.4

Results

Esters of 14:0, 14:1, 15:0, 16:2, 17:1 and 20:1 have been tentatively identified by GLC. Their total amt is 1–2% and they are omitted in the tabulations. Values listed in the tables of <2% are considered as estimates and values of <0.3% are denoted by "+". The results are given in Tables III to VI, but only those isomers are listed where a progressive change was observed.

The esters 8–16:1, 7–18:1 and 8–18:1 were found in all samples of rat lipids at levels of 1–3% which is above the background level of the ozonization method with 9–16:1 and 9–18:1. Similarly, the presence of 1%, or less, 7,10–18:2 was indicated in all analyses of rat acids. The latter acid has been reported by Fulco and Mead (8) from fat-deficient rats. None of the diets produced a trend of these isomers so the individual values are not listed.

A 20:1 fraction from heart, kidney, liver and spleen was analyzed in conjunction with other experiments (30). It consisted of 7–, 11– and 13–20:1 with only a very minor amt of 9–20:1. Klenk and Tschöpe (18) have reported the isomers 11– and 13–20:1 from liver lipids of fat-deficient rats.

Similarly, a 14:1 fraction consisted of 9–14:1 with about 10% 7–14:1.

C_{17} was most abundant among odd-numbered fatty acids. Major components were 9– and 8–17:1, but saturated and dienoic C_{17} were detected by GLC.

Petroselinate, 6–18:1, was supplied for 3 weeks at a level of 3% in one of the preliminary experiments with diet *1* which had also been supplemented by 1% each of linoleate and linolenate. After 3 weeks, the 18:1 fraction of the total fat contained 30.4% 6–18:1 so this isomer represented 13% of all fatty acids in the rat; 16:1 contained 9.6% 4–16:1, or 1% of all acids; 20:1 contained 80% 8–20:1 but the amt of the whole fraction is less than 0.3%. The acid 6–16:1 is natural to the rat at least at certain times (Table III), but 6–18:1 and 4–16:1 have never been found under other circumstances. The amt of 6,9–18:2 was not elevated after supplying 6–18:1, although the usual desaturation of such compound in position 9 would lead to this isomer of linoleic acid.

TABLE IV
Fatty Acids (%) from Rats on Diet *1* (Fat-free)

Compound	Structure	Days on diet							
		0[a]	7	14	21	35	67	95	188
10		3.1	1.8	0.6	0.3	0.2	+	+	+
12		7.2	4.6	1.6	1.0	0.5	0.2	+	+
14		6.9	5.8	3.8	3.1	2.8	2.2	2.1	1.9
16:0		24.4	30.7	32.6	33.4	33.9	32.0	30.6	27.8
16:1		3.6	8.3	13.1	12.7	15.5	15.7	14.9	15.3
	6–	3.2	3.6	+	1.8	1.3	2.0	1.4	+
	7–	12.8	5.5	2.6	3.6	2.7	3.9	3.9	3.8
	9–	79.7	87.6	94.9	92.3	94.8	93.4	93.1	95.1
18:0		7.3	6.8	5.8	5.8	4.6	3.3	2.9	2.6
18:1		30.9	33.9	39.0	41.4	41.4	46.4	49.5	52.4
	9–	87.8	86.9	86.9	86.0	86.9	86.8	84.0	79.9
	11–	8.4	8.4	8.6	9.9	9.5	11.2	12.4	15.8
18:2		13.1	8.1	3.5	2.4	1.1	0.4	+	+
	5,8–	+	1.4	1.3	3.2	2.0	1.4	10.7	16.0
	6,9–	+	0.6	1.9	2.5	3.2	6.0	10.6	12.7
	8,11–	0.6	4.4	5.0	8.6	16.2	46.9	45.6	49.4
	9,12–	99.4	93.6	91.8	85.7	78.5	45.6	33.1	21.9
20:4		3.5	+	+	+	+	+	+	+

[a] The age of these rats was 25 days (cf. values in Table III from other rats 25 days old).

Progressive Changes of Fatty Acid Composition

Medium Chain Length Fatty Acids. The equally high level of C_{10}, C_{12} and C_{14} acids is in good agreement with earlier reports on the composition of rat milk (1,2,7,12) and is in contrast to other milk fats (10). It should be noted that our analyses of milk do not pertain to the colostrum. The medium change length acids are not at an unusually high level in the newborn rat but the intake of milk is well reflected by their increase in sucklings (Table III). When the supply of such acids is maintained after weaning in form of Hydrol, diet *2*, their percentages are much lower in the adult animal than in the suckling although the level of C_{12} and C_{14} is much higher in Hydrol than in milk (Table V). Possibly the metabolic rates of medium chain length acids are higher in the adult than in the suckling rat.

Isomerism of 16:1. In contrast to the foregoing, the relative amts of 16:1 isomers are virtually the same in milk and newborns (Table III). The occurrence of 6–16:1 is unexpected. The percentage decreases as synthesis *de novo* of other acids increases in the suckling and the isomer virtually disappears later on (Tables IV to VI). Very little 6–16:1 acid was found in the dam's feed and its synthesis in the rat may be pronounced during pregnancy and lactation.

The percentage of 7–16:1 may increase slightly with the supply of this isomer in the milk. Regardless of diet, the amt decreases after weaning. The isomer is, however, genuine to the higher animals since it has been found in pig brain (17) and experiments with C^{14}-labeled acids have shown the relation 7–16:1 ⟷ 9–18:1 (29,31).

Isomerism of 18:1. The composition of the 18:1 fraction is very similar in rat milk and the newborn (Table III). In agreement with other reports, the amt of 18:1 increases with fat-free diet *1* (Table IV) and this was found also with Hydrol diet *2* (Table V).

cis-Vaccenic acid, 11–18:1, is always present, and its percentage increases somewhat under prolonged fat-free regimen. The conversion, 9–16:1 → 11–18:1 has been reported (15,29,31). The isomers, 7– and 8–18:1 are always indicated as minor components and their amt exceeds the blank value of the method with authentic oleate or petroselinate.

Isomerism of 18:2. The percentage of linoleic acid is about equal in milk and the newborn and it stays constant to weaning age (Table III). From then on the level increases when linoleic acid is supplied (Table VI) or decreases sharply with diets lacking this acid (Tables IV and V). A very minor amt of linoleic acid is supplied with the casein of the diet (Table I) and the acid is tenaciously retained by the animal.

The isomers, 5,8–, 6,9– and 8,11–18:2 are found only in traces in young or in fully supplemented adult animals. Their percentages become significant and finally outrank that of linoleic acid during a prolonged EFA-deficient diet. Quantification of 18:2 from carcasses

TABLE V
Fatty Acids (%) from Rats on Diet *2* (Hydrol Hydrogenated Coconut Fat)

Compound	Structure	Days on diet			
		0[a]	95	125	Hydrol
10		3.1	0.1	+	4.3
12		7.2	3.8	3.5	47.5
14		6.9	4.9	4.3	19.0
16:0		24.4	29.0	30.0	10.5
16:1		3.6	13.5	12.1	—
	6–	3.2	+	3.4	
	7–	12.8	4.3	4.6	
	9–	79.7	93.3	90.4	
18:0		7.3	3.8	3.2	15.0
18:1		30.9	44.9	46.9	3.3[b]
	9–	87.8	84.5	87.8	44.6
	11–	8.4	10.7	9.8	14.6
18:2		13.1	+	+	—
	5,8–	+	6.8	9.4	
	6,9–	+	8.9	10.6	
	8,11–	0.6	37.3	36.3	
	9,12–	99.4	47.0	43.7	

[a] See footnote on Table IV.
[b] Percentages of other isomers in 18:1 of Hydrol are 6–, 1.5; 7–, 3.1; 8–, 8.8; 10–, 17.0; 12–, 10.3.

at the beginning of diet *1* showed an average of 170 mg linoleic and of about 1 mg 8,11–18:1 per animal; the same quantification after 67 days on diet *1* showed 70.4 mg. 18:2 per animal and they consisted of about 30 mg linoleic, 30 mg 8,11–18:2, 4 mg 6,9–18:2 and 1 mg 5,8–18:2 acids. According to our analyses of 18:2 from casein a total of about 0.4 mg 8,11–18:2 may have been furnished with the diet during that period.

Conversion of 9–18:1 into 6,9–18:2 has been demonstrated with rat liver microsomes (14) and this isomer is a likely precursor of 5,8,11–20:3 which accumulates with EFA deficiency. Similarly the acid with one additional double bond, 6,9,12–18:3 (γ-linolenic), is precursor of 5,8,11,14–20:4 (arachidonic). Both products are found in much larger amts than their C_{18} precursors. The latter have in common a proximal structure (carboxyl—first double bond) of 6 carbon atoms. Apparently, such proximal length in polyenoic C_{18} acids lends itself to rapid further conversion.

Isomerism of 20:3 and 20:4. Polyunsaturated acids are at a higher concn in organ than in body fat and the polyunsaturated isomers characteristic of fat deficiency do not differ in this respect from the polyunsaturated acids of the healthy animal (5). Routine analyses were not made but the data of Table IV were supplemented by analyses of acids from rats after 6 months of diet *1*. 5,8,11–20:3 was isolated from the total fat and contained about 1% 7,10,13–20:3. Although the percentage of trienoic acids was lower in carcass than organ fat their amt was larger in the former. However, arachidonic acid was isolated only from organ lipids. It contained about 1% of the isomeric 4,7,10,13–20:4 which has been reported by several investigators (18,24).

Discussion

Slow growth was among the first symptoms observed with rats on fat-deficient diet (3). In our experiments, growth plateaus were approached after 120 days with fat-free diet *1*, somewhat later and higher with Hydrol diet *2*, and after 180 days with fully supplemented diet *3*.

In the growing rat, a diet lacking EFA is indicated already after one week by a decrease in linoleic acid while a relative increase of 8,11–18:2 becomes significant. It may be expected that this effect is more pronounced when analyzing the liver lipids rather than the total. The need for early evaluation of EFA deficiency was, in part, the reason for quantifying 5,8,11–20:3 and using its ratio to arachidonic acid as a measure (16). Quantification of 8,11–18:2 in conjunction with linoleic acid may serve the same purpose, and comparison with the "triene-tetraene ratio" in this regard would be of interest. However, with present methods, analysis of 8,11–18:2 obviously is less practical than of 5,8,11–20:3.

TABLE VI
Fatty Acids (%) from Rats on Diet *3* (Corn Oil)

Compound	Structure	0[a]	7	14	21	95	188	Corn oil
10		3.1	1.4	0.4	0.4	+	+	—
12		7.2	3.1	1.3	1.1	+	+	—
14		6.9	4.0	2.9	2.7	1.8	1.7	—
16:0		24.4	25.4	28.9	28.1	27.3	24.3	11.5
16:1		3.6	6.3	7.0	6.9	10.7	10.3	0.4
	6–	3.2	1.5	+	+	+	+	1.9
	7–	12.8	5.5	3.9	3.9	2.7	3.8	4.7
	9–	79.7	91.2	93.2	93.9	95.9	94.9	93.4
18:0		7.3	6.8	5.6	5.3	3.1	2.7	1.8
18:1		30.9	31.8	31.3	31.0	32.9	36.5	26.7
	9–	87.8	87.5	89.2	90.8	90.8	89.1	91.2
	11–	8.4	7.5	6.8	6.1	7.9	9.9	1.2
18:2		13.1	17.0	19.8	21.0	24.2	24.4	58.0
	5,8–	+	+		+	+	—	+
	6,9–	+	+		+	+	—	1.0
	8,11–	0.6	1.1		1.6	0.7	1.0	1.2
	9,12–	99.4	98.9		98.4	99.3	99.0	96.0
18:3		—	—	+	+	+	+	1.6
20:4		3.5	4.1	2.9	3.5	+	+	—

[a] See Footnote on Table IV.

Leaving aside linoleic and linolenic types, the isomeric acids found in rats can be correlated as shown in Table VII which gives also references to other reports on these isomers. Grouping is made under the assumption that desaturation takes place exclusively between the monoenoic double bond and the carboxyl group. The results with 6–18:1 indicate that this postulate is valid also for monoenoic acids with proximal moieties shorter than 9 carbon atoms.

The monoenes, 8–18:1, 9–18:1, 8–16:1 and 9–16:1 (or 11–18:1), listed in the order of Table VII, are possible precursors of the four unusual 18:2 acids, but only two of the monoenes, 9–16:1 and 9–18:1, extend conversion to C_{20} trienes, 7,10,13–20:3 and 5,8,11–20:3. Only one isomer, 4,7,10,13–20:4, of arachidonic acid occurs and most likely it derives from 9–16:1 *via* 11–18:1. With a required minimum of 4 carbon atoms as proximal moiety (21), a 20:4 acid can be formed from 9–16:1 without interference, whereas not more than 3 methylene-interrupted double bonds find place in a C_{20} chain when 9–18:1 is the precursor. When comparing oleic and palmitoleic acid in regard to their further conversions, it appears reasonable to evaluate the respective amounts of 20:3 acids. This level is accessible to both monoenes and represents the major products from both precursors. Still, 9–16:1 must undergo one more elongation step than 9–18:1 in order to arrive at 20:3. Ratios in total animal fat under severe EFA deficiency were 2:1 for oleic:palmitoleic + *cis*-vaccenic and in order of 100:1 for the respective

TABLE VII
A. Correlations of Monoenoic and Polyenoic Acids

Acid	Structure				
14:1					7–
16:1	6–	7–[b]	8–		9–[a]
18:1	8–	9–[a]			11–[a]
18:2	5,8–	6,9–	7,10–[d]		8,11–[b,c,d,e]
18:3					5,8,11–[e,f]
20:2		8,11–[d,f]			
20:3		5,8,11–[a]			7,10,13–[b,c,d]
20:4					4,7,10,13–[b,c]

B. Monoenoic Acids Not Listed Under A

9–14:1	9–18:1[a]	(11–18:1)[a]	7–18:1	7–20:1
	11–20:1[c]	13–20:1[c]	9–20:1	

[a] Structures commonly known.
[b] Privett, et al., (24).
[c] Klenk and Tschöpe (18).
[d] Fulco and Mead (8).
[e] Fulco and Mead (9).
[f] Compound not identified in this investigation.

Discussion

Slow growth was among the first symptoms observed with rats on fat-deficient diet (3). In our experiments, growth plateaus were approached after 120 days with fat-free diet *1*, somewhat later and higher with Hydrol diet *2*, and after 180 days with fully supplemented diet *3*.

In the growing rat, a diet lacking EFA is indicated already after one week by a decrease in linoleic acid while a relative increase of 8,11–18:2 becomes significant. It may be expected that this effect is more pronounced when analyzing the liver lipids rather than the total. The need for early evaluation of EFA deficiency was, in part, the reason for quantifying 5,8,11–20:3 and using its ratio to arachidonic acid as a measure (16). Quantification of 8,11–18:2 in conjunction with linoleic acid may serve the same purpose, and comparison with the "triene-tetraene ratio" in this

trienes. On this basis, oleic greatly outranks palmitoleic acid as a precursor. Mead (21) suggested that the enzyme systems are not prominently selective since the ratios oleic:palmitoleic were in his experiments of the same magnitude as those of 5,8,11–20:3 : 7,10,13–20:3. Klenk and Tschöpe (18) referred to the acids of rat liver phosphatides in EFA-deficient rats and found a ratio of about 3:1 for the polyenoic acids of oleic and palmitoleic type.

In a broader comparison of monoenoic acids it is well known that Δ^{odd} monoenes are by far prominent in amts above Δ^{even} and that their conversion products play a much greater role. One-carbon degradation of fatty acids has been demonstrated for certain animal tissues and compositional data suggests that it applies also to unsaturated acids (17). In such case, it leads from Δ^{odd} to Δ^{even} acids and explains in all likelihood the occurrence of 8–17:1 in the rat. However, one-carbon degradation has little importance for the composition of the total rat fat.

The preference for biosynthesis of Δ^{odd} monoenes extends to odd-numbered acids for which those of mullet oil are a recently reported example. Among the unsaturated C_{15}, C_{17} and C_{19} acids of this fish oil, neither Δ^{even} monoenoic nor any polyenoic acids possibly derived from them have been found in distinct amts (33).

Biological pathways other than desaturation of saturated long-chain acids lead also to Δ^{odd} monoenes. Such direct synthesis of monoenoic acids has been shown for some micro-organisms and 10–16:1 (in *Mycobacterium phlei*) is the only exception known to us where a Δ^{even} monoene represents an appreciable portion of the acids (19). The occurrence of a Δ^{even} monoene, 6–16:1, in the rat is surprising. However, Kishimoto and Radin (17) found 6–16:1 to be a significant portion of 16:1 isomers from pig brain. The authors concluded that there is a mechanism for synthesis of acids unsaturated in position 6 which so far had not been recognized in animals, and our findings indicate that such synthesis is not limited to brain tissue.

Neither 10–18:1 nor 10–16:1 were found in rats, nor have they been identified from pig brain (17). Very minor amts of 10–20:1 and 10–22:1 are the only Δ^{even} monoenes of even-numbered chain length identified in the latter. Strong preference for desaturating carbons 9 and 10 prevails above desaturation of the neighboring carbons 10 and 11 and minimizes the same for carbons 8 and 9. The zig-zag form of the aliphatic chain may not permit a suitable steric conformation of these carbon atoms in reference to the carboxyl group or its coenzyme A ester.

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Dietary Fat and the Fatty Acid Composition of Tissue Lipids

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Abstract

Some characteristics of the fatty acid composition of animal tissue lipids are described and the origins of tissue fatty acids are discussed briefly. The effect of dietary fat on composition of tissue lipids is discussed. Types of dietary fatty acids for which experimental work is described include polyunsaturated fatty acids, short-chain fatty acids, fatty acids with chain length greater than C_{18}, *trans* unsaturated fatty acids, fatty acids with conjugated double bonds, acetylenic fatty acids, branched-chain fatty acids and oxygenated fatty acids. The individuality of fatty acids is discussed in relation to their roles as components of tissue lipids.

FATTY ACID COMPOSITIONS of animal tissue lipids have been investigated extensively over the past 50 years, first with methods such as fractional crystallization and fractional distillation and more recently by chromatographic techniques. The older analytical work was generally limited to lipids which were available in large quantities and most analyses were made on depot fats although some studies were done on lipids of tissues such as liver and brain (1,2). The advent of newer techniques has made it possible to work with much smaller amts of material. This has resulted in an ever-increasing volume of analytical data, which is no longer restricted to tissues having large quantities of lipids.

The picture that emerges from this wealth of analytical information is one of characteristic fatty acid patterns for the different lipid classes of each tissue. These patterns are subject to alteration by a variety of influences but under ordinary circumstances they are remarkably reproducible in different animals of the same species. Consideration will therefore be given first to some of the characteristic fatty acid patterns found in different lipid classes of the tissues of animals on their normal diets as a preliminary