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Oxidative desaturation of eicosa-8,11-dienoic acid to eicosa-5,8,11-trienoic acid: comparison of different diets on oxidative desaturation at the 5,6 and 6,7 positions

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Abstract The oxidative desaturation of [1-^{14}C]eicosa-8,11-dienoic acid to eicosa-5,8,11-trienoic acid by rat liver microsomes was studied, and the kinetic conditions appropriate to measure the specific activity of the enzyme were determined. A comparative study of the effects of a balanced diet and essential fatty acid-free diets on the oxidative desaturation of oleic and linoleic acids at the 6,7 position and the oxidative desaturation of eicosadienoic acid at the 5,6 position were made. Eicosadienoic acid showed a higher conversion than oleic acid for all the diets. The conversion of oleic and linoleic acids to $\Delta 6$ acids was equally increased by fat-free diets with or without added methyl palmitate, whereas the oxidative 5-desaturation of eicosadienoic acid at the 5,6 position was not changed. The effect was apparently independent of the amount of endogenous free fatty acids. The results suggest that the rate-limiting and principal regulatory step in the biosynthesis of eicosa-5,8,11-trienoic acid is the 6-desaturation of oleic acid. The 5-desaturation of eicosadienoic acid was increased by a protein diet and decreased by alloxan diabetes to a lesser extent than the 6-desaturation of linoleic acid. The 5-desaturation of eicosadienoic acid would constitute a secondary regulatory step.

Supplementary key words microsomes · fatty acid synthesis · linoleic acid · oleic acid · essential fatty acid-deficient diet · protein diet · diabetes

It has been repeatedly shown that the tissue lipids of rats maintained on a balanced diet have high levels of linoleic and arachidonic acids that belong to the n−6 family, whereas polyunsaturated fatty acids of the oleic acid series (n−9 family) are found in very low amounts. However, when young rats are maintained on a fat-deficient diet, the biosynthesis of the fatty acids of the n−6 family does not occur, due to the absence of the required precursor, linoleic acid. Therefore, the amount of arachidonic acid in tissues is low, while high levels of eicosa-5,8,11-trienoic acid (20:3 n−9) are found (1). This acid is synthesized from oleic acid. Refeeding EFA-deficient rats with linoleic or arachidonic acid reverses the process (1). Feeding experiments (1–4) or experiments in which liver microsomes were incubated with different concentrations of oleic and linoleic acids (5, 6) have indicated that these effects are due to competition between linoleic and oleic oxidative desaturation and to different rates of the two reactions.

However, there is still a great need for information on the regulation of reactions that lead to the synthesis of eicosa-5,8,11-trienoic acid. Therefore, the aims of the present experiment were to study the direct biosynthesis of eicosa-5,8,11-trienoic acid from eicosa-8,11-dienoic acid, and the effect of essential fatty acid-free diets on desaturation of oleic, linoleic, and eicosadienoic (n−9) acids.

METHODS

Chemicals

All-*cis*-[1-^{14}C]eicosa-8,11-dienoic acid (0.15 mCi/mmole) was synthesized in the laboratory (7). 1-Bromo-2-undecyne and the Grignard derivative of 1-chloro-7-

Abbreviations: EFA, essential fatty acids; TLC, thin-layer chromatography.

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octyne were first prepared from octane-1-ol and hexane-1,6-diol, respectively. They were coupled and the diacetylenic chloride produced was partially hydrogenated with Lindlar catalyst (quinoline-poisoned palladium) (8), converted into the nitrile with labeled KCN, hydrolyzed, and esterified with HCl–methanol. The ester was purified by gas–liquid chromatography. Radiochemical purity was greater than 95%.

[1-^{14}C]Linoleic acid (41.4 mCi/mmole) and [1-^{14}C]-oleic acid (36 mCi/mmole) were purchased from the Radiochemical Centre, Amersham, England. The radiochemical purity was 99%, estimated by TLC on silver nitrate-impregnated silica gel in hexane–ether 3:2 (v/v). They were diluted with the corresponding unlabeled fatty acid to a final specific radioactivity of 0.15 mCi/mmole. Unlabeled fatty acids were purchased from the Hormel Institute, Austin, Minn. They were greater than 99% pure.

Animals

Male Wistar rats 3 months of age, maintained on Purina chow, were used.

The effects of a fat-free diet or a fat-free diet supplemented with methyl palmitate were studied with three groups of five rats each. The first group, fed a balanced diet for 65 days after weaning, was used as a control. The second group was fed a fat-free diet for the same period of time, and a third group was fed a fat-free diet for 53 days and then the same diet supplemented with 30 g of methyl palmitate/kg of food for a period of 12 days. The composition of the fat-free diet was the same as described previously (9). It contained 19% defatted casein, 77% sucrose, 4% minerals, and vitamins. In the balanced diet, 30 g of sunflower seed oil was added per kilogram of food. Water and food were given ad lib. Rats in all three groups were killed simultaneously.

In another experiment, three groups of four rats each were used to investigate the effects of a protein diet and diabetes on eicosadienoic ($n-9$) oxidative desaturation. The first group was fed a balanced diet. The animals in the second group received the same diet but they were force-fed an isocaloric casein suspension (Casenolin, Glaxo-Argentina) for 24 hr before they were killed (10). The rats in the third group were made diabetic by injection of alloxan (50 mg of alloxan monohydrate/kg of body weight, administered intravenously) as described previously (11). Animals with a fasting blood glucose concentration exceeding 300 mg/100 ml were considered diabetic. The animals received an isocaloric diet (25 kcal/100 g of body weight).

Isolation of microsomes

The rats were killed by cervical fracture, and livers were immediately excised, washed with cold homogenizing solution, blotted on filter paper, and weighed. The whole liver was homogenized with 3 vol of a cold solution consisting of 0.15 M KCl, 0.25 M sucrose, 0.005 M $MgCl_2$, 0.004 M EDTA, 0.004 M acetylcysteine, and 0.05 M phosphate buffer (pH 7.0). The crude homogenate was centrifuged at 12,000 *g* for 10 min at 0°C to remove cell debris and mitochondria. The microsomal fraction was separated by centrifugation at 100,000 *g* for 60 min in a Spinco model L-2 ultracentrifuge. The pellets were resuspended in the homogenizing solution (1:2 v/v) and protein was measured by the biuret reaction (12), with bovine serum albumin as a standard.

Microsomal desaturation of fatty acids

The oxidative desaturation of fatty acids was measured by incubation of 5 mg of the specified amount of microsomal protein with 1-^{14}C-labeled fatty acids at 35°C in air. The flasks contained 2.5 μmoles of ATP, 0.2 μmole of CoA, 2.5 μmoles of NADH, 15 μmoles of $MgCl_2$, 125 μmoles of NaF, 1 μmole of nicotinamide, 4.5 μmoles of glutathione, and 125 μmoles of phosphate buffer (pH 7.0) in a total volume of 3 ml of 0.15 M KCl, 0.25 M sucrose solution.

The fatty acids were added in a propylene glycol solution (20 nmoles/μl). The flasks containing substrate and cofactors were warmed to 35°C during 30 sec, then the reaction was started by addition of the microsomal suspension to the incubation medium. The incubation was continued in a New Brunswick shaker at 110 cycles/min during the specified periods of time, usually 20 min, and then stopped by addition of 2 ml of 10% KOH in ethanol. The lipids were saponified under nitrogen at 85°C for 45 min and the mixture was then acidified and the fatty acids were extracted with petroleum ether (bp 30°C). The fatty acids were esterified with methanolic 3 N HCl (3 hr at 68°C). The esters were dissolved in petroleum ether and stored in a freezer at −25°C under nitrogen until they were analyzed.

The distribution of radioactivity between substrate and product was measured by gas–liquid radiochromatography in a Pye apparatus with a proportional counter (5). With these data, and knowing the nanomoles incubated, the amount of product was easily calculated. All the data are the means of at least two determinations.

Fractionation of lipids

After incubation of 200 nmoles of labeled eicosa-8,11-dienoic acid with 5 mg of microsomal protein for 20 min at 35°C under the described conditions, the lipids were extracted and washed by the procedure of Folch, Lees, and Sloane Stanley (13). The lipids were separated by TLC on silica gel G; the plates were developed in chloroform–methanol–water 65:25:4 (v/v/v). The spots were

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visualized with iodine vapor and identified by means of standards. They were scraped into vials containing Bray's scintillation solution (14). Counting was done in a Packard scintillation counter.

Free fatty acid determination

The free fatty acid content in the microsomes was determined, when necessary, by gradient-thickness TLC (15). The lipids of the microsomes were extracted with $CHCl_3$–CH_3OH 2:1 (v/v). The extract was filtered and evaporated in vacuo, and the residue was dissolved in $CHCl_3$–CH_3OH–H_2O 15:5:0.8 (v/v/v). Aliquots were separated on silica gel G with hexane–diethyl ether–acetic acid 40:60:2.3 (v/v/v). The spots of the free acids were visualized by spraying with a charring reagent that consisted of a solution of 70% sulfuric acid saturated with potassium dichromate (16). The chromatoplate was heated at 180°C for 25 min. The amount of free fatty acid in each spot was determined with a Zeiss PMQ II spectrophotometer specially equipped for direct measurement in the chromatoplate. Standards of palmitic acid were used for comparison.

RESULTS AND DISCUSSION

Oxidative desaturation of eicosadienoic (n−9) acid

The study of the oxidative desaturation of fatty acids in microsomal suspensions is handicapped by the existence of simultaneous reactions that may mask the specific properties of the reaction. Acylation of CoA, elongation, and incorporation of substrate and products take place in the microsomes. As far as is known, the oxidative desaturation of the unsaturated fatty acids in animals occurs on the acyl CoA thioesters and not on the lipids (17). Under the conditions of the experiments, the acylation of CoA and the oxidative desaturation were produced in the same medium. However, it is unlikely that the acylation would be a rate-limiting step, since Bersten (18) and Pande and Mead (19) have found that the acyl CoA synthesis is faster than the oxidative desaturation. The elongation of the fatty acids did not occur under our experimental conditions. This was shown by the absence of higher homologs of the substrate in the gas–liquid radiochromatograms of the incubation products.

Incorporation of fatty acids into microsomal lipids during incubation has been shown previously (20). To avoid as much as possible the competitive effect of fatty acid transacylation, the concentration of the chosen substrate was the highest one that still allowed an accurate measurement of fatty acid desaturation. At the same time, the increase in the concentration of the substrate changed the desaturation reaction to a zero-order reaction.

The results showed that rat liver microsomes actively desaturate 20:2 (n−9) to 20:3 (n−9). The effect of substrate concentration on the rate of the reaction is shown in Table 1. When the concentration of the acid was 34.8 μM (104 nmoles in 3 ml) or higher, the rate was practically independent of the substrate concentration. Therefore, a substrate concentration higher than 34.8 was used. In a recent publication, Ullman and Sprecher (21) also studied 20:2 (n−9) oxidative desaturation and obtained similar results.

Table 2 shows the incorporation of labeled eicosadienoic (n−9) and eicosatrienoic (n−9) acids during a 20-min incubation of the liver microsomes when the concentration of the substrate was 66.6 μM. At this high concentration, 48.2% of the labeled material was found as free acid at the end of the incubation period.

The effect of the concentration of the microsomes on the rate of the reaction when the substrate concentration was 66.6 μM is shown in Fig. 1. These results clearly show that under the conditions of the experiment and in spite of the partial incorporation in the lipids (Table 2), eicosadienoic (n−9) acid desaturation is proportional to the amount of microsomes used. Therefore, the reaction is of zero order and may be considered dependent only on the amount of enzyme present.

The use of high concentrations of eicosadienoic (n−9) acid would also minimize and even rule out the possible

TABLE 1. Effect of [1-^{14}C]eicosa-8,11-dienoic acid concentration on the formation of eicosa-5,8,11-trienoic acid 20:3 (n−9) by liver microsomes

Substrate	20:3 (n−9) Formed
nmoles	
104	43.5
209	53.1
419	49.7

5 mg of microsomal protein was incubated for 20 min at 35°C in a total volume of 3 ml with cofactors described in the text. Results are means of duplicate samples.

TABLE 2. Incorporation of ^{14}C into microsomal lipids after incubation with [1-^{14}C]eicosa-8,11-dienoic acid

TLC Fractions[a]	% of Total ^{14}C Incorporated[b]
Neutral lipids	9.6
Free fatty acids	48.2
X[c]	5.9
Ethanolamine phosphatides	5.4
X[c]	1.9
Choline phosphatides	17.8
X[c]	8.8
Origin	2.4

[a] The recovery of radioactivity was 88.5%.
[b] Means of two experiments.
[c] X, other zones.

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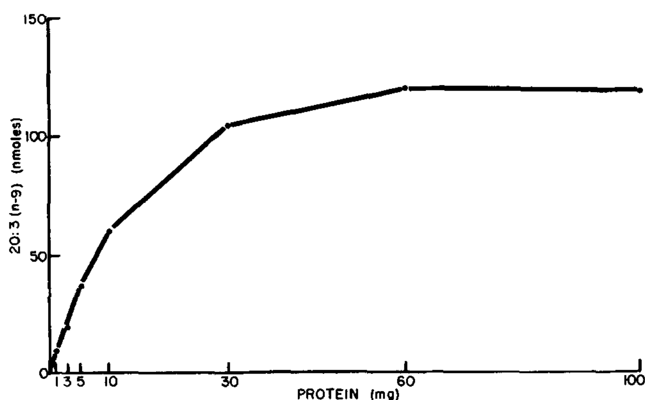

FIG. 1. Effect of the amount of microsomal protein on the oxidative desaturation of eicosa-8,11-dienoic acid. 200 nmoles of substrate was incubated for 20 min at 35°C in a total volume of 3 ml.

competition due to free fatty acids present in the microsomes. This type of effect has been suggested by Uchiyama, Nakagawa, and Okui (22). It would be negligible under the conditions of the experiment, since the content of free fatty acids in the microsomes has been estimated by Abou-Issa and Cleland (23) to be only approximately 10 nmoles/mg of microsomes. In our experiments, we found 12 nmoles of endogenous free fatty acids/mg of microsomal protein expressed as palmitic acid. Besides, the free acids which contain substantial proportions of saturated and monoenoic acids have little inhibitory effect on the oxidative desaturation of unsaturated fatty acids (5, 6).

Effect of fat-deficient diets on the oxidative desaturation of oleic, linoleic, and eicosadienoic (n−9) acids

Alternative pathways may lead to the synthesis of eicosa-5,8,11-trienoic acid through successive oxidative desaturations and elongations (24). However, the main operative sequence would be (25): 18:1 (n−9) → 18:2 (n−9) → 20:2 (n−9) → 20:3 (n−9), in which an oxidative desaturation at the 6,7 position takes place in the first step and an oxidative desaturation at the 5,6 position in the last. Ullman and Sprecher (26) have presented evidence that the alternative route 18:1 (n−9) → 20:1 (n−9) → 20:2 (n−9) → 20:3 (n−9) would not be operative or would be of very little importance in the rat. In this respect, they showed that eicosa-11-enoic acid was not converted into eicosa-8,11-dienoic acid but rather to eicosa-5,11-dienoic acid in small proportions. This last acid would not be converted into eicosa-5,8,11-trienoic acid in the rat.

A similar sequence has also been depicted for conversion of linoleic acid to arachidonic acid (26–29) in which the main route also begins with oxidative desaturation at carbon 6 and ends with oxidative desaturation at carbon 5: 18:2 (n−6) → 18:3 (n−6) → 20:3 (n−6) → 20:4 (n−6). Therefore, it was important to find out which of the reactions were key regulatory steps and to what extent an EFA deficiency might control their activity.

To study the effect of an EFA deficiency on the specific activity of the microsomal desaturases for oleic, linoleic, and eicosadienoic (n−9) acids, a group of rats was maintained on a fat-free diet for 65 days. The degree of EFA deficiency reached was controlled by analyzing and comparing the fatty acid composition of liver lipids of the rats fed the fat-free diet with that of the rats fed a balanced diet. Whereas the 20:3/20:4 ratio for the deficient rats was high (0.6), the proportion of linoleic acid was low (1.6%); corresponding values for the control rats were 0 and 15.9, respectively. Therefore, these results show that the animals used had developed a marked EFA deficiency.

The oxidative desaturation of oleic, linoleic, and eicosadienoic (n−9) acids was studied in experiments in which microsomal suspensions prepared from livers of both groups of rats were incubated for different intervals using a 66.6 μM concentration of substrate. The results are shown in Fig. 2. The data demonstrate that the oxidative desaturation activity for the three acids was 20:2 > 18:2 > 18:1, confirming the results of Ullman and Sprecher (21). A higher conversion of linoleic acid to γ-linolenic acid than of oleic acid to octadeca-6,9-dienoic acid has also been reported for 20-min incuba-

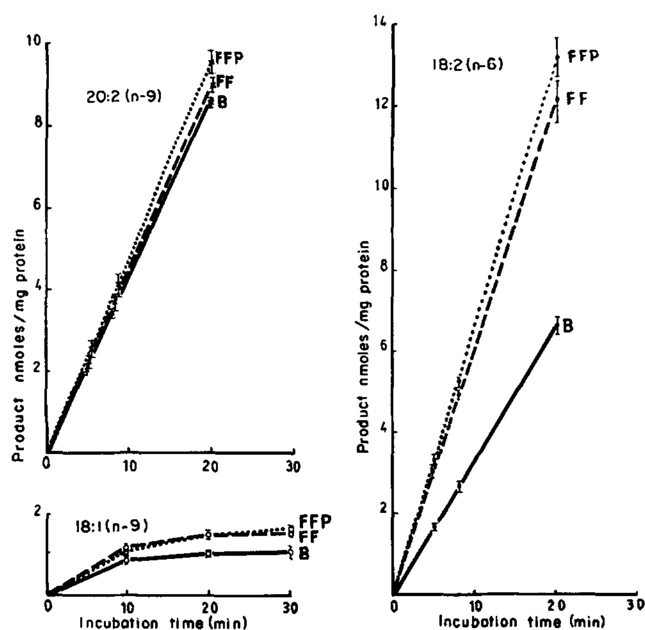

FIG. 2. Effect of EFA-deficient diets on the microsomal desaturation of eicosa-8,11-dienoic acid, oleic acid, and linoleic acid. (——), balanced diet (*B*); (- - -), fat-free diet (*FF*); (···), fat-free diet supplemented with methyl palmitate (*FFP*). Points are means ± SD.

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tions when low concentrations of substrate were used (5).

For the control rats in the present experiments, the desaturation of either linoleic or eicosadienoic (n−9) acid increased linearly up to 20 min. However, for oleic acid the shape of the curve was a hyperbola, and at 20 min there was an apparent plateau. This result is interesting because it would suggest that after 20 min the desaturation of oleic acid had markedly slowed down. The reasons for this phenomenon are not clearly known.

The high rate of desaturation of eicosadienoic acid compared with oleic acid would also suggest that the oxidative desaturation of oleic acid to octadeca-6,9-dienoic acid is the rate-limiting reaction in the n−9 biosynthetic pathway. The rate of oxidative desaturation of eicosadienoic (n−9) acid to eicosatrienoic (n−9) acid is so rapid that the fatty acid formed by the first steps of the series would be converted immediately to eicosatrienoic acid. However, it is quite possible that the later reaction may also regulate the overall synthesis by competition with other fatty acids, as has been shown by Ullman and Sprecher (21). The elongation of 18:2 (n−9) to 20:2 (n−9) could be also a rate-limiting step instead of the 6-desaturation of 18:1 (n−9). However, this is very improbable, considering that 18:2 (n−9) does not accumulate in EFA deficiency.

Fig. 2 also shows that in rats fed a fat-free diet there is an increased rate of desaturation of oleic, linoleic, and eicosadienoic (n−9) acids. However, only the increases for oleic and linoleic acids were statistically significant.

This result is interesting from different points of view. In the first place, it confirms that the main point of regulation in the series is the first step: the oxidative desaturation of oleic acid in the 6,7 position. Secondly, it shows that the increased synthesis of the members of the oleic acid family and deposition of eicosatrienoic (n−9) acid in the tissues of fat-deficient rats are not only due to less linoleic acid available in the tissues to compete with oleic acid desaturation (5) but also to an actual increase in the 6-desaturation activity in the liver.

The fact that the increase in activity of the 6-desaturation of oleic and linoleic acids is similarly evoked in both acids by the fat-free diet (Table 3) is also additional evidence for the hypothesis that a common 6-desaturase desaturates oleic and linoleic acids (5, 24, 30). Besides, the fact that the 5-desaturation of eicosa-8,11-dienoic acid does not follow the same pattern may be the first suggestion of the possible existence of a 5-desaturase different from the 6-desaturase.

The increase of the microsomal specific 6-desaturase activity in rats fed a fat-deficient diet does not necessarily prove that EFA deficiency per se is responsible for this effect. However, as shown in Fig. 2, the oxidative desaturation of oleic, linoleic, and eicosadienoic acids has been also studied in rats fed a fat-free diet supplemented with methyl palmitate. The degree of EFA deficiency was not changed by palmitate addition. Table 3 shows that the reaction rates were not statistically different between the rats fed a fat-free diet and those fed a fat-free diet supplemented with methyl palmitate. Therefore, the activating effect found may be considered to be related to the deficiency of polyunsaturated fatty acids in the diet. Similar results were obtained by Marcel, Christiansen, and Holman (29).

The enhanced microsomal desaturation of oleic and linoleic acids in the rats fed a fat-free diet and those fed an EFA-free diet may be attributed to the presence of different amounts of endogenous free fatty acids in the microsomes. These fatty acids would compete with the substrate acid (5, 6). Uchiyama et al. (22) have also found that the rate of oleic acid synthesis from stearic acid is depressed in starved animals, but it is markedly increased by feeding the animals a fat-free diet. These results have been partially attributed to the different levels of endogenous unsaturated free fatty acids derived from the different diets. However, in our experiments on the 5-desaturase, the EFA-deficient diets evoked a different response from those of 6-desaturase. Therefore, the enhancement of the 6-desaturation produced by EFA deficiency cannot be simply ascribed to the different amount of endogenous free fatty acids competing with the substrate.

Actually, the free fatty acid content of the microsomes of rats fed a balanced diet was shown to be higher (16

TABLE 3. Effects of different diets on the specific desaturating activities of the microsomes for oleic, linoleic, and eicosadienoic acids

Acid	Balanced Diet	Fat-free Diet	% Activation	Fat-free + Palmitate Diet	% Activation
	nmoles/mg prot/min	*nmoles/mg prot/min*		*nmoles/mg prot/min*	
Oleic	0.080 ± 0.003[a]	0.128 ± 0.002 $P < 0.01$	60	0.120 ± 0.005 $P < 0.01$	50
Linoleic	0.335 ± 0.014	0.600 ± 0.026 $P < 0.01$	79	0.625 ± 0.021 $P < 0.01$	86
Eicosadienoic	0.436 ± 0.006	0.448 ± 0.012 $P = 0.12$	2.7	0.479 ± 0.030 $P = 0.07$	10

[a] Means ± SE.

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TABLE 4. Comparative effects of different diets on the fatty acid oxidative desaturation of crude microsomes and microsomes normalized to contain the same amount of endogenous free fatty acids

Acid	Diet				
	Balanced	Fat-free	Fat-free, Normalized	Fat-free + Palmitate	Fat-free + Palmitate, Normalized
Oleic	0.94[a]	1.48	1.30	1.48	1.47
Linoleic	6.53	12.00	11.30	12.50	12.60
Eicosa-dienoic	8.72	8.96	8.73	9.60	9.08

[a] Results are expressed as nmoles of acid converted/mg of microsomal protein during a 20-min incubation at 35°C. Results are means of duplicate samples.

TABLE 5. Effects of dietary protein and diabetes on the oxidative desaturation of eicosa-8,11-dienoic acid and linoleic acid by microsomes

		20:2 (n−9) → 20:3 (n−9)	18:2 (n−6) → 18:3 (n − 6)
		% conversion[a]	
Diet	Balanced	45.5 ± 0.16[b]	7.4 ± 0.36
	Protein	52.9 ± 0.93	11.8 ± 0.18
		$P < 0.01$	$P < 0.01$
Diabetes		32.2 ± 0.51	3.1 ± 0.24
		$P < 0.01$	$P < 0.01$

[a] 216 nmoles of labeled acid was incubated with 30 mg of microsomal protein and the cofactors described in the text for 20 min at 35°C in a total volume of 3 ml.
[b] Means ± SE.

nmoles/mg of microsomal protein) than in rats fed a fat-free diet (13 nmoles/mg of protein) or in rats fed a fat-free diet supplemented with methyl palmitate (11 nmoles/mg of microsomal protein). Table 4 shows the amounts of oleic, linoleic, and eicosadienoic acid desaturated (nmoles/mg of protein) by the liver microsomes of the rats fed the different diets when the amount of endogenous free fatty acids was normalized. Normalization of endogenous free fatty acid content of the microsomes was achieved by addition of proportional amounts of the free fatty acids extracted with $CHCl_3$–CH_3OH 2:1 (v/v) from the microsomes of the rats fed a balanced diet. They were added to the other microsomes to a level of 16 nmoles/mg of protein. The free fatty acids were purified by TLC in hexane–methyl ether–acetic acid 80:20:1 (v/v/v). The desaturation found for crude microsomes or the normalized microsomes was in all cases close enough to eliminate the possibility that in EFA deficiency competition by the endogenous fatty acids is the possible cause of the activation. Therefore, one may conclude that it is the special fatty acid composition produced by an EFA-deficient diet that enhances the specific activity of the 6-desaturases. However, it is not possible at the present to establish the mechanism of the activation.

Effects of a protein diet and diabetes on 5-desaturation of eicosadienoic acid and 6-desaturation of linoleic acid

The enhancement of the conversion of linoleic acid to γ-linolenic acid by a protein diet has been very well documented (9, 31). It has also been shown that alloxan diabetes decreases either the 6-desaturation of oleic, linoleic, and α-linolenic acids or the 9-desaturation of palmitic and stearic acids to palmitoleic and oleic acids, respectively (32, 33). Therefore, in order to investigate to what extent the 5-desaturase compares with 6-desaturase and 9-desaturase, the effects of protein diet and alloxan diabetes on the microsomal conversion of eicosadienoic (n−9) acid to eicosatrienoic (n−9) acid were tested. The results are shown in Table 5. They prove that the 5-desaturation of eicosadienoic (n−9) acid is increased very little by a protein diet and is much less sensitive than the 6-desaturation. Alloxan diabetes decreases either linoleic acid desaturation or eicosadienoic (n−9) desaturation, but again the 5-desaturation is less sensitive. Therefore, these results confirm the previous finding that suggested that the 6-desaturation and not the 5-desaturation is the key control in the synthesis of unsaturated fatty acids. The 5-desaturase probably represents a secondary regulatory point.

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REFERENCES

1. Brenner, R. R., and A. M. Nervi. 1965. Kinetics of linoleic and arachidonic acid incorporation and eicosatrienoic depletion in the lipids of fat-deficient rats fed methyl linoleate and arachidonate. *J. Lipid Res.* **6:** 363–368.
2. Lindstrom, T., and I. J. Tinsley. 1966. Interactions in the metabolism of polyunsaturated fatty acids: analysis by a simple mathematical model. *J. Lipid Res.* **7:** 758–762.
3. Holman, R. T. 1964. Nutritional and metabolic interrelationships between fatty acids. *Federation Proc.* **23:** 1062–1067.
4. Dhopeshwarkar, G. A., and J. F. Mead. 1961. Role of oleic acid in the metabolism of essential fatty acids. *J. Amer. Oil Chem. Soc.* **38:** 297–301.
5. Brenner, R. R., and R. O. Peluffo. 1966. Effect of saturated and unsaturated fatty acids on the desaturation *in vitro* of palmitic, stearic, oleic, linoleic, and linolenic acids. *J. Biol. Chem.* **241:** 5213–5219.
6. Brenner, R. R., and R. O. Peluffo. 1969. Regulation of unsaturated fatty acid biosynthesis. I: Effect of unsaturated fatty acid of 18 carbons on the microsomal desaturation of

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linoleic acid into γ-linolenic acid. *Biochim. Biophys. Acta.* **176:** 471–479.

7. Castuma, J. C., C. S. Arciprete, R. R. Brenner, and A. E. A. Mitta. 1971. Síntesis del éster metílico del ácido eicosa-8,11-dienoico-1-[14]C. Bol. Com. Nac. Energ. Atómica No. 300. 1–12.
8. Lindlar, H. 1952. Ein neuer Katalysator für selektive Hydrierungen. *Helv. Chim. Acta.* **35:** 446–450.
9. Brenner, R. R., and P. José. 1965. Action of linolenic and docosahexaenoic acids upon the eicosatrienoic acid level in rat lipids. *J. Nutr.* **85:** 196–204.
10. Peluffo, R. O., N. T. de Gomez Dumm, M. J. T. de Alaniz, and R. R. Brenner. 1971. Effect of protein and insulin on linoleic acid desaturation of normal and diabetic rats. *J. Nutr.* **101:** 1075–1083.
11. Peluffo, R. O., S. Ayala, and R. R. Brenner. 1970. Metabolism of fatty acids of the linoleic acid series in testicles of diabetic rats. *Amer. J. Physiol.* **218:** 669–673.
12. Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177:** 751–766.
13. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
14. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in liquid scintillation counter. *Anal. Biochem.* **1:** 279–285.
15. Bazán, N. G., Jr., and C. D. Joel. 1970. Gradient-thickness thin-layer chromatography for the isolation and analysis of trace amounts of free fatty acids in large lipid samples. *J. Lipid Res.* **11:** 42–47.
16. Blank, M. L., J. A. Schmit, and O. S. Privett. 1964. Quantitative analysis of lipids by thin-layer chromatography. *J. Amer. Oil Chem. Soc.* **41:** 371–376.
17. Brenner, R. R., A. Catalá, and S. Actis Dato. 1971. Factors that modify the biosynthesis of unsaturated fatty acids in the endoplasmic reticulum. *Acta Cient. Venez.* **22:** 143–146.
18. Bersten, A. M. 1968. Studies on acyl-CoA synthetase. M.Sc. thesis. Sydney University, Australia.
19. Pande, S. V., and J. F. Mead. 1968. Long chain fatty acid activation in subcellular preparations of rat liver. *J. Biol. Chem.* **243:** 352–361.
20. Nervi, A. M., R. R. Brenner, and R. O. Peluffo. 1968. Effect of arachidonic acid on the microsomal desaturation of linoleic into γ-linolenic acid and their simultaneous incorporation into the phospholipids. *Biochim. Biophys. Acta.* **152:** 539–551.
21. Ullman, D., and H. Sprecher. 1971. An *in vitro* study of the effects of linoleic, eicosa-8,11,14-trienoic and arachidonic acids on the desaturation of stearic, oleic and eicosa-8,11-dienoic acids. *Biochim. Biophys. Acta.* **248:** 61–70.
22. Uchiyama, M., M. Nakagawa, and S. Okui. 1967. Effect of free unsaturated fatty acids on fatty acid desaturation by liver preparations. *J. Biochem.* (Tokyo). **62:** 1–6.
23. Abou-Issa, H. M., and W. W. Cleland. 1969. Studies on the microsomal acylation of L-glycerol-3-phosphate. II: The specificity and properties of the rat liver enzyme. *Biochim. Biophys. Acta.* **176:** 692–698.
24. Brenner, R. R. 1971. The desaturation step in the animal biosynthesis of polyunsaturated fatty acids. *Lipids.* **6:** 567–575.
25. Klenk, E., and H. Pflüger. 1963. Über die Biogenese der $\Delta^{5,8,11}$-Eicosatriensäure bei fettfrei ernährten Ratten. *Hoppe-Seyler's Z. Physiol. Chem.* **335:** 53–62.
26. Ullman, D., and H. Sprecher. 1972. An *in vitro* and *in vivo* study of the conversion of eicosa-11,14-dienoic acid to eicosa-5,11,14-trienoic acid and of the conversion of eicosa-11-enoic acid to eicosa-5,11-dienoic acid in the rat. *Biochim. Biophys. Acta.* **248:** 186–197.
27. Mead, J. F. 1961. Synthesis and metabolism of polyunsaturated acids. *Federation Proc.* **20:** 952–955.
28. Klenk, E. 1961. Chemie und Stoffwechsel der Polyenfettsäuren. *Experientia.* **17:** 199–204.
29. Marcel, Y. L., K. Christiansen, and R. T. Holman. 1968. The preferred metabolic pathway from linoleic acid to arachidonic acid *in vitro*. *Biochim. Biophys. Acta.* **164:** 25–34.
30. Brenner, R. R., and A. Catala. 1971. Effect of ATP on the microsomal desaturation of unsaturated fatty acids. *Lipids.* **6:** 873–881.
31. Gomez Dumm, I. N. T. de, M. J. T. de Alaniz, and R. R. Brenner. 1970. Effect of diet on linoleic acid desaturation and on some enzymes of carbohydrate metabolism. *J. Lipid Res.* **11:** 96–101.
32. Mercuri, O., R. O. Peluffo, and R. R. Brenner. 1966. Depression of microsomal desaturation of linoleic to γ-linolenic acid in the alloxan-diabetic rat. *Biochim. Biophys. Acta.* **116:** 409–411.
33. Mercuri, O., R. O. Peluffo, and R. R. Brenner. 1967. Effect of insulin on the oxidative desaturation of α-linolenic, oleic and palmitic acids. *Lipids.* **2:** 284–285.

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