Effects of Dietary *trans* Acids on the Biosynthesis of Arachidonic Acid in Rat Liver Microsomes

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ABSTRACT

Effects of dietary *trans* acids on the interconversion of linoleic acid was studied using the liver microsomal fraction of rats fed a semipurified diet containing fat supplements of safflower oil (SAFF), hydrogenated coconut oil (HCO) at 5 and 20% levels or a 5% level of a supplement containing 50.3% linolelaidic and 24.3% elaidic acids devoid of *cis,cis*-linoleic acid (TRANS). Growth rate was suppressed to a greater extent with the animals fed the 20% than the 5% level of the HCO-supplemented diets and still further by the TRANS diet compared to the groups fed the SAFF diets. Food intake was greater in the groups fed the HCO than the SAFF-supplemented diets, demonstrating the marked effect of an essential fatty acid (EFA) deficiency on feed efficiency. In contrast to an EFA deficiency produced by the HCO supplement, which stimulated the in vitro liver microsomal biosynthesis of arachidonic acid, diets containing the TRANS supplement exacerabated the EFA deficiency and depressed 6-desaturase activity of the liver microsomal fraction. The liver microsomal fraction of the animals receiving this supplement also was more sensitive to fatty acid inhibition of the desaturation of linoleic acid than those obtained from animals fed either the SAFF or HCO diets. It is suggested that dietary *trans* acids alter the physical properties of the 6-desaturase enzyme system, suppressing its activity, which increases the saturation of the tissue lipids and, in turn, the requirement for EFA or polyunsaturated fatty acids.

INTRODUCTION

The inhibitory effect of linolelaidic and elaidic acids on 6-desaturase activity, which is the key reaction in the regulation of the conversion of linoleic to arachidonic acid (1,2), has been well demonstrated by in vitro experiments with the liver microsomal fraction of rats fed a fat-deficient diet by Brenner and Peluffo (3). Nutritional experiments with rats also have shown that linolelaidic acid inhibits the conversion of oleic to 5,8,11-eicosatrienoic acid and linoleic to arachidonic acid (4-7). Elaidic acid also appears to have a similar effect, but to a relatively minor extent compared to linolelaidic acid (4,5). Linolelaidic acid itself is not converted in vivo to polyunsaturated fatty acids as shown in nutritional studies (4-7), and as recently demonstrated by radioactive tracer experiments in rat brain by Karney and Dhopeshwarkar (8). However, linolelaidic acid, as well as *cis,trans* isomers of linoleic and elaidic acids, are incorporated into the lipids of most tissues (4-11), and are readily metabolized (12).

In order to further determine the nutritional effects of *trans* acids, a study was made of the effects of an EFA deficiency and its exacerbation by feeding rats a diet containing a fat supplement of *trans* acids devoid of *cis,cis*-linoleic acid, as the sole source of fat in the diet on the interconversion of linoleic acid by liver microsomes.

MATERIALS AND METHODS

Experimental

Animals. Weanling male Sprague-Dawley rats (Dan Rolfsmeyer Co., Madison, WI) were divided into 5 groups of 10 animals and fed a basic fat-free diet supplemented with 5 or 20% by weight of safflower oil (SAFF), groups I and II, respectively; 5 or 20% of hydrogenated coconut oil (HCO), groups III and IV, respectively; or 5% of an ethyl ester concentrate of *trans* acids containing 50.3% linolelaidic acid (*t,t*-18:2) and 24.3% (*t*-18:1) devoid of *cis,cis*-linoleic acid (TRANS), group V. The diets (Table I) were made isocaloric by adjusting the relative amounts of fat, carbohydrate and fiber. The composition of the fat supplements and the contribution of each fatty acid to the dietary calories are shown in Table II.

The animals were weighed at 2-day intervals, food intake was also measured and daily consumption as well as feed efficiency determined. After the growth rate reached a plateau, the animals in each group were killed under a light ether anesthetic by withdrawal of blood from the retroocular plexes.

Preparation of liver microsomes. Freshly excised livers were perfused with saline and homogenized in a Potter-Elvehjem homogenizer in 2 vol of a solution containing 0.25 M sucrose, 5 mM $MgCl_2$, 0.15 M KCl, 1.5 mM GSH and 50

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TABLE I

Diet Composition

	5% Fat diet (% by wt)	20% Fat diet (% by wt)
Casein (vitamin test)	22.50	22.50
L-cystine[a]	0.20	0.20
Wesson salt mixture plus $ZnCl_2$ and $MnSO_4 \cdot H_2O$[b]	4.03	4.03
Choline mix[c]	1.00	1.00
Vitamin mix[d]	1.00	1.00
Cellulose (Alphacel)	10.25	29.00
Sucrose	56.02	22.27
Fat	5.00	20.00

[a]L-Cystine is added to the diet to bolster the level of sulfur-containing amino acids.

[b]Wesson salt mixture does not contain zinc or manganese, hence these elements are added to the mix as follows: 0.60 g of $ZnCl_2$ and 0.90 g of $MnSO_4 \cdot H_2O$/200 g of salt mixture.

[c]Choline mix consists of 22% choline dihydrogen citrate in vitamin test casein.

[d]One kg of the vitamin mix contains: 2.5 g thiamine HCl, 2.5 g riboflavin, 9.0 g nicotinic acid, 9.0 g calcium pantothenate, 2.0 g pyridoxine HCl, 4.0 g cyanobalamin (B_{12}), 7.5 g *p*-aminobenzoic acid, 0.1 g folic acid, 0.02 g biotin, 20.0 g *meso*-inositol, 0.5 g menadione (vitamin K), 943.0 g vitamin test casein. Fat and vitamins A, D and E are mixed into the diet daily and stored at 0 C overnight. Vitamin D_2, 5.0 mg; retinol acetate, 6.9 mg; α-tocopherol acetate, 300 mg/kg of diet.

mM of potassium phosphate buffer pH 7.0. Homogenates were centrifuged at 800 × g for 10 min to remove cell debris, then at 10,000 × g for 20 min. The microsomal fraction was recovered by centrifuging the 10,000 × g supernatant at 100,000 × g for 1 hr. The pellet was suspended in a solution containing 5 mM $MgCl_2$, 0.15 M KCl, 1.5 mM GSH and 50 mM potassium phosphate buffer (pH 7.0) to give a final concentration of 20 mg/ml protein, determined by the method of Lowry et al. (13).

Incubation conditions. ATP, CoASH, malonyl-CoA, NADH and bovine serum albumin containing less than 0.005% fatty acid were purchased from Sigma Chemical Co., St. Louis, MO, GSH from P-L Biochemical, Inc., Milwaukee, WI, and [1-^{14}C]linoleic acid (50 μCi/μmol) from New England Nuclear, Boston, MA. The [1-^{14}C]linoleic acid was purified by thin layer chromatography, converted to the ammonium salt and bound to bovine serum albumin (14). All incubations were carried out at 37 C in a total vol of 2.0 ml. For measurement of desaturase activity, each incubation was conducted for 10 min in the medium containing the following: 10 μmol of $MgCl_2$, 0.3 mmol of KCl, 3 μmol of GSH, 10 μmol of ATP, 0.6 μmol of CoA, 2.5 μmol of NADH, 100 μmol of potassium phosphate buffer, pH 7.0, 200 nmol of radioactive linoleic acid and 5 mg microsomal protein in 0.001% Triton X-100. The same conditions were used for determination of desaturation chain elongation reactions except that 0.6 μmol of malonyl CoA was added to the incubation mixture.

Incubations were stopped by the addition of 10 ml of dimethoxypropane (DMP) containing 200 μl of concentrated HCl to 1 ml of the incubation medium. After a reaction time of 20 min to allow for conversion of the water to methanol and acetone, these solvents and the excess DMP were evaporated in a stream of nitrogen at room temperature and the lipid was interesterified with methanol as described by Shimasaki et al. (15).

Radioactivities of the methyl esters were determined on fractions isolated by gas liquid chromatography using an Aerograph Model 600-D gas chromatograph equipped with a 9:1 splitter and a 12' × 0.125" id column packed with 10% Silar 10C on 100-200 mesh Gas-Chrom Q at 210 C with a flow rate of N_2 of 20 cc/min. Fractions corresponding to each fatty acid ester (peaks in the chromatogram) were collected in glass tubes attached directly to the outlet of the splitter, coincident with their detection by the flame detector, and transferred to scintillation counting vials by washing the tubes with 15 ml of scintillation fluid (5.5 g of Permablend I/liter of toluene). Radioactivity was counted in a Packard Model 3310 scintillation spectrometer. Recovery of radioactivity by this technique of collection was ca. 75%. The activity of 6-desaturase, determined independently, was calculated from the counts of 18:3 corrected for background. The activities of 6-desaturase, chain elongation and

TABLE II

Fatty Acid Composition and Caloric Level of Dietary Fats[a]

	SAFF supplement			HCO supplement				TRANS supplement		
		Group I (5% wt)	Group II (20% wt)			Group III (5% wt)	Group IV (20% wt)			Group V (5% wt)
Fatty[b] acids	Fat (% wt)	Dietary calories (%)	Dietary calories (%)	Fatty acids	Fat (% wt)	Dietary calories (%)	Dietary calories (%)	Fatty acids	Fat (% wt)	Dietary calories (%)
16:0	9.6	1.2	4.8	10:0	5.7	0.4	1.76	16:0	18:1	2.3
18:0	3.6	0.5	2.0	12:0	45.2	5.9	23.6	18:0	7.3	0.9
18:1	13.4	1.7	6.8	14:0	16:8	2.5	10.0	*t*-18:0	24.3	3.0
18:2	73.4	9.2	36.8	16:0	8.8	1.4	5.6	*tt*-18:2	50.3	6.3
				18:0	13:8	1.9	7.6			
				18:1	1.7	0.3	1.16			
				18:2	0.1-0.5					

[a]SAFF = safflower oil; HCO = hydrogenated coconut oil; TRANS = ethyl ester-concentrate of *trans* acids.

[b]Shorthand designation; number before colon = chain length; number after colon = number of double bonds, *t* = *trans* double bond.

5-desaturase in the overall reaction were determined simultaneously from the total counts of the products corrected for background, the proportion of the counts in the 20:3 + 20:4 acids, and the 20:4 fraction, respectively. The products of the reactions (18:3 and those of chain elongation after a short lag period) under the assay conditions just described were proportional to protein concentrations in excess of 5 mg in the incubation mixture and a reaction time of ca. 15 min.

Fatty acid composition. The fatty acid composition of liver microsomal lipid was determined on methyl esters prepared by interesterification with HCl as a catalyst (15) using a Hewlett Packard Model 5840A gas chromatograph. This analysis also was carried out with a 12' x 0.125" id column packed with Silar 10C on 100:200 mesh Gas-Chrom Q at 200-250 C programmed at 2.0 C/min with a flow rate of nitrogen of 10 cc/min.

The highly purified fatty acid standards (>99%) and the ethyl ester concentrate of *trans* acids which contained 50.3% linolelaidic acid (*t,t*-18:2) and 24.3% elaidic acid (*t*-18:1) (Table II) were obtained from Nu-Chek-Prep, Inc., Elysian, MN.

RESULTS

Weight gains of the animals in each group (Fig. 1) showed that although there was no difference in the growth rates of the 5 and 20% SAFF groups, that of the 20% HCO group was significantly lower than the 5% HCO group. The TRANS dietary regimen gave an even greater suppression of growth rate than the 20% HCO diet as also shown in Figure 1. Measurement of food intake of the animals in each group (Table III) showed that, in spite of differences in growth rate, the animals of the HCO-supplemented groups consumed more food than those of the SAFF groups. The animals in the TRANS group consumed the least food and their growth was suppressed the most. Accordingly, feed efficiency was highest in the SAFF group and greatly diminished in the other groups. The difference in growth rate between the 5 and 20% HCO groups was also reflected by corresponding differences in feed efficiency.

The fatty acid composition of the liver microsomal lipid of the animals in each group reflected generally that of the dietary fat as shown in Table IV. The SAFF supplement, which contained ca. 70% linoleic acid, supplied more than an adequate amount of this fatty acid in the diet to meet the requirement for essential fatty acids even at the 5% dietary

level. Evidence to this effect was that the levels of arachidonic acid in the microsomal lipid of the 5 and 20% SAFF groups were essentially the same, ca. 32%, in spite of the fact that the diet of the 20% SAFF group contained a much larger amount of linoleic acid (Table II). The fatty acid composition of the liver microsomal lipid of these animals (groups I and II) exhibited the normal pattern for animals receiving a diet adequate in essential fatty acids (EFA). The liver microsomal lipid of the animals receiving the HCL supplements exhibited a fatty acid composition typical of an EFA deficiency. Compared to the SAFF groups, the levels of 16:1 and 18:1 were elevated. Those of linoleic and arachidonic acid were decreased and there was an appreciable formation of 20:3, giving a high triene-to-tetraene ratio in the microsomal lipid of the HCO groups (III and IV). The pattern of these changes was more pronounced in the 5% HCO group than the 20% group because the hydrogenated coconut oil

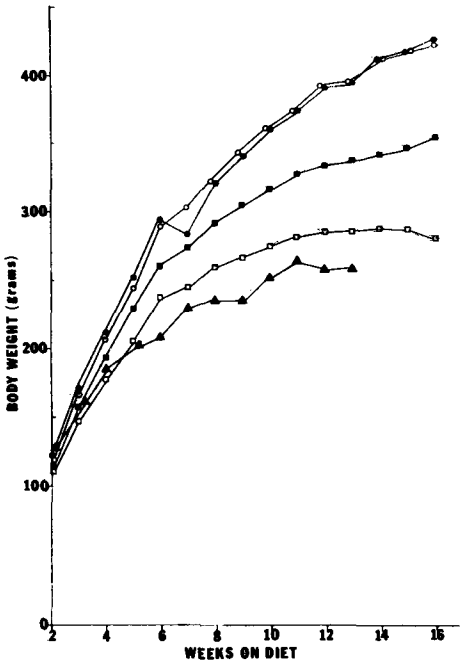

FIG. 1. Growth rate of rats from weaning fed a fat-free diet supplemented with: 5% or 20% safflower oil, groups I and II, respectively, open and solid circles; 5% hydrogenated coconut oil, group III, solid squares; 20% hydrogenated coconut oil, group IV, open square; 5% ethyl ester concentrate of *trans* acids, group V, solid triangles. At 14 weeks, the weights (M ± SD) of the animals of each group were as follows: I, 411 ± 16; II, 411 ± 32; III, 342 ± 22; IV, 286 ± 25 and V, 260 ± 22. III was significantly greater than IV ($P < .001$) and IV was significantly greater than V ($P < .100$).

TABLE III

Feed Efficiency[a]

Age (weeks)	Group[b] 5% HCO			20% HCO			5% SAFF			20% SAFF			5% TRANS		
	Food intake			Food intake			Food intake			Food intake			Food intake		
	Daily	Total[a]	Feed[a] eff.	Daily	Total	Feed eff.	Daily	Total	Feed eff.	Daily	Total	Feed eff.	Daily	Total	Feed eff.
3	6 ± 1.9[c]	6 ± 1.9	–	8.1 ± 3.9	8.1 ± 3.9	–	7.2 ± 1.9	7.2 ± 1.9	–	8.2 ± 1.3	8.2 ± 1.3	–	5.0 ± .71	5.0 ± .71	–
4	13 ± 1.4	67 ± 4.9	1.0 ± .07	14.9 ± 3.3	73.6 ± 19	.98 ± .24	11.4 ± 1.7	73 ± 15	1.4 ± .12	19.8 ± .45	80 ± 8.3	1.2 ± .07	9.6 ± 2.0	62 ± 1.6	1.4 ± .14
5	17 ± 4.2	171 ± 17	.68 ± .05	18.5 ± 1.2	193 ± 30	.61 ± .10	15.2 ± 1.6	169 ± 28	.87 ± .04	15.0 ± 2.8	187 ± 21	.78 ± .03	14 ± 1.4	151 ± 9.2	.84 ± .08
6	19 ± 3.8	287 ± 44	.57 ± .07	20 ± 4.8	321 ± 51	.46 ± .07	16.2 ± 1.6	273 ± 47	.71 ± .09	16.2 ± 1.5	300 ± 28	.62 ± .02	15.6 ± .89	252 ± 11	.64 ± .06
7	18 ± 2.9	422 ± 59	.46 ± .05	21.4 ± 4.4	462 ± 77	.39 ± .06	17.8 ± 1.6	411 ± 53	.59 ± .04	18.4 ± 3.2	423 ± 37	.56 ± .01	15.8 ± 1.9	293 ± 146	.54 ± .08
15	22 ± 1.9	1653 ± 160	.20 ± .02	23.8 ± 4.2	1818 ± 308	.16 ± .02	17.0 ± 2.8	1466 ± 157	.26 ± .01	18.4 ± 6.5	1441 ± 121	.27 ± .01	17 ± 1.2	1254 ± 96	.21 ± .01
16	20 ± 2.9	1799 ± 175	.18 ± .02	21.2 ± 6.4	1979 ± 345	.15 ± .02	16.8 ± 1.3	1589 ± 167	.25 ± .01	17.4 ± 2.9	1540 ± 152	.26 ± .02	15.2 ± 1.3	1358 ± 101	.19 ± .01
19	19 ± 2.3	2339 ± 211	.15 ± .01	19.8 ± 3.9	2539 ± 436	.12 ± .02	15.8 ± 1.1	1938 ± 193	.22 ± .01	15.6 ± 2.07	1902 ± 167	.22 ± .01	–	–	–
20	19 ± 2.7	2471 ± 221	.14 ± .01	23 ± 4.5	2705 ± 467	.11 ± .02	17.2 ± 18.4	2057 ± 203	.21 ± .01	17.2 ± 1.6	2020 ± 179	.21 ± .01	–	–	–

[a]Grams gained/grams of feed consumed; no allowance made for amount of milk consumed before weaning.

[b]HCO = hydrogenated coconut oil; SAFF = safflower oil; TRANS = trans acid concentrate.

[c]M ± SD, 5 animals; feed efficiency of 5% HCO vs 20% HCO significant, $P > .025$ at 20 weeks; feed efficiency of 5% HCO vs 20% SAFF significant, $P < .001$ at 20 weeks; total food intake of 5% HCO vs 20% SAFF significant, $P < .01$ at 20 weeks.

TABLE IV

Fatty Acid Composition of Liver Microsomal Lipid (% wt)

Dietary group[a]	I 5% SAFF	II 20% SAFF	III 5% HCO	IV 20% HCO	V 5% TRANS
16:0[b]	18.7 ± 1.1[c]	15.0 ± 0.5	19.6 ± 0.9	17.3 ± 1.0	15.9 ± 0.2
16:1	3.0 ± 0.7	0.7 ± 0.1	10.3 ± 0.1	4.1 ± 0.6	12.3 ± 1.5
18:0	14.3 ± 0.5	22.6 ± 0.8	18.9 ± 1.1	25.0 ± 0.6	13.0 ± 0.9
18:1	7.3 ± 1.0	4.6 ± 0.1	20.7 ± 1.5	15.6 ± 0.3	29.3 ± 1.9
t-18:1					2.2 ± 0.9
18:2	10.1 ± 1.1	16.1 ± 0.5	2.7 ± 0.1	4.3 ± 0.3	5.3 ± 0.2[d]
20:3ω9			14.0 ± 0.2	7.3 ± 0.2	5.1 ± 0.7
20:4	31.2 ± 1.8	32.2 ± 0.2	6.5 ± 0.7	17.0 ± 0.7	3.8 ± 0.1
22:4	4.4 ± 0.5	3.0 ± 0.4	1.0 ± 0.1	2.5 ± 0.2	0.6 ± 0.1
20:3/20:4			2.2	0.43	1.3

[a]SAFF = safflower oil; HCO = hydrogenated coconut oil; TRANS = concentrate of ethyl linoleate (Table II); see Materials and Methods for additional details of dietary regimens.

[b]Shorthand designation of fatty acids; number before colon = chain length; number after colon = number of double bonds.

[c]M ± SD, n = 4.

[d]Contains a mixture of positional and geometric isomers.

supplement contained a small amount of linoleic acid (Table II) which provided a significant amount of this fatty acid in the diet, particularly in the animals fed at the 20% level, group IV. Accordingly, the levels of 18:2 and 20:4 were higher in 20% than the 5% HCO group and the effect on the composition of the other fatty acids was not so great (Table IV) giving a relatively low triene-tetraene ratio indicative of only a borderline EFA deficiency in this group, IV. However, in spite of the fact that the EFA deficiency was only borderline, the growth rate of the animals of this group was suppressed to a greater degree than that of the animals of the 5% HCO group (Fig. 1).

The TRANS supplement was devoid of linoleic acid (Table II), hence the levels of linoleic and arachidonic acids were very low in the animals of group V, and the severity of the EFA deficiency was greater in the animals of this group than those of groups III and IV. The triene-tetraene ratio was elevated in this group, indicative of an EFA deficiency, but it was not as high as in the 5% HCO group. The triene-tetraene ratio does not give a true indication of the EFA deficiency in animals fed linolelaidate because this acid inhibits the conversion of oleic to 20:3 and 18:2 to 20:4 as shown in previous work (4-7). Accordingly, the level of arachidonic acid in the liver microsomal lipid of the animals of this group was very low compared to that of the SAFF animals (Table IV). Likewise, the level of 20:3 was lower in liver microsomal lipid of these animals than in those of the HCO groups (III and IV). The levels of 18:1 and 16:1 were elevated in this group (V) even above those of the HCO groups, which were already high compared to those in the animals of the SAFF groups. The positional and geometric isomers of the 18:2 acids were not identified in the analysis of the liver microsomal lipid of this group, but this fraction should consist mainly of linolelaidate inasmuch as the dietary fat was devoid of *cis,cis* linoleate. This fraction also might contain some 5,9-18:2 as a product of the desaturation of elaidate (16,17), as well as 6,9-, 5,8- and 8,11-isomers found in EFA-deficient animals (18). However, the actual composition of this fraction will have to await further, detailed analyses. Regardless, the data in Table IV show that the dietary regimens produced animals having liver microsomal lipid of widely different fatty acid compositional patterns; namely, a normal pattern, groups I and II, 2 levels of a typical EFA deficiency, groups III and IV, and a third pattern characteristic of an EFA deficiency complicated by an effect of dietary *trans* acids, group V.

The effect of the dietary regimens on the interconversion of linoleic acid by liver microsomes in vitro is shown in Table V. These analyses showed that the simple EFA deficiency developed in groups III and IV by feeding the HCO supplement produced a marked elevation in the activity of the 6-desaturase chain elongation enzyme system by comparison with groups I and II. In these experiments, the activities of the chain elongation and 5-desaturase enzyme systems are dependent on substrates produced in the interconversion of linoleic acid, and do not

TABLE V

Liver Microsomal Enzyme Activities (nmol/min/mg protein)

Dietary group[a]	I 5% SAFF	II 20% SAFF	III 5% HCO	IV 20% HCO	V 5% TRANS
6-Desaturase[b]	0.315 ± .078[c]	0.436 ± .010	0.661 ± .004	0.644 ± .028	0.238 ± .004
6-Desaturase	0.343 ± .076	0.470 ± .016	0.654 ± .031	0.686 ± .055	
Chain elongation	0.234 ± .049	0.305 ± .020	0.451 ± .013	0.470 ± .050	
5-Desaturase	0.062 ± .009	0.121 ± .001	0.147 ± .007	0.137 ± .018	

[a]SAFF = safflower oil; HCO = hydrogenated coconut oil; TRANS = concentrate of ethyl linolelaidate, (Table II), see Materials and Methods for additional details of dietary regimens.

[b]Determined independently.

[c]M ± SD (n = 4). Group I vs group II, 6-desaturase $P < .025$; group I vs group V, 6-desaturase $P < .01$; group II vs group IV, 6-desaturase $P < .001$.

represent maximal reaction rates.

In contrast to groups III and IV, the 6-desaturase activity of the liver microsomal fraction of the TRANS group (V) was significantly lower than that of the SAFF groups. The suppression of 6-desaturase activity in the animals of the TRANS group (V) was further demonstrated by comparison of different concentrations of ATP on the activity of this enzyme system in the liver microsomal fraction of animals from the 3 different dietary groups as shown in Figure 2. These experiments demonstrated that, while an EFA deficiency of the type produced by depriving the animals of adequate linoleate elevated 6-desaturase activity (group IV), that produced by the effect of the TRANS supplement (group V) suppressed the activity of this enzyme system. This experiment also showed that the lower activity of the microsomal fraction of the TRANS group was not due to impaired activation of substrate.

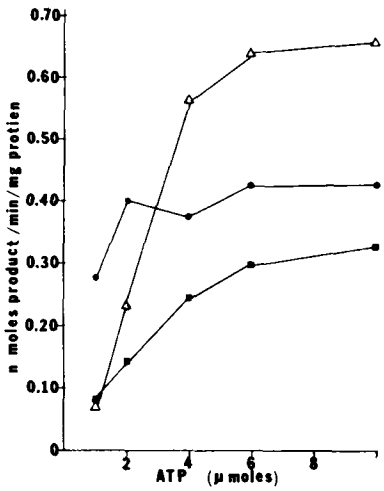

FIG. 2. Effect of ATP concentration on the rate of desaturation of linoleic acid in vitro by rat liver microsomal fractions obtained from rats fed diets supplemented with: 20% hydrogenated coconut oil, group IV, open triangles; 20% safflower oil, group II, solid circles; 5% ethyl ester concentrate of *trans* acids, group V, solid squares.

A comparison of the inhibitory effects of fatty acids on the desaturation of linoleic acid by the liver microsomal fraction of animals from the different dietary regimens is shown in Figure 3. In these experiments, the substrate consisted of a mixture of 20 or 200 nmol of palmitic, stearic, oleic, elaidic or linolelaidic acids plus 200 nmol of [1-^{14}C]linoleic acid. These experiments showed that inhibition of 6-desaturase activity by these fatty acids, particularly in the palmitic and stearic acids, was much greater with the liver microsomal fraction obtained from the animals fed the TRANS supplement than of those fed the HCO or SAFF supplements.

DISCUSSION

In this study, the in vitro experiments showed that 6-desaturase activity of liver microsomal preparations was enhanced in an EFA deficiency produced by the HCO diet but was suppressed in animals fed the TRANS-supplemented diet, although this diet also produced an EFA deficiency. These experiments are in accord with the nutritional effects of these dietary supplements inasmuch as oleate is readily converted to 20:3 in an EFA deficiency produced by feeding HCO-supplemented diets, and the reaction is suppressed in animals in which an EFA deficiency is produced upon feeding diets containing supplements of linolelaidic acid (4-7). Although elaidic acid also suppresses the conversion of oleic to 20:3, its effect is minor compared to linolelaidic acid as indicated in previous studies (4,5). Thus, the effect of the TRANS dietary supplement

appears to primarily result from its content of linolelaidic acid.

As a result of the suppression of 6-desaturase activity in the animals fed diets containing linolelaidate, the level of 20:3, which accumulates to high levels in an EFA deficiency, is relatively low in the liver microsomal fraction of these animals. As shown here and in previous work (4-7), the level of *cis* 18:1 is elevated simultaneously with a decrease in 20:3 in animals fed linolelaidate. Guo and Alexander (16) have shown that dietary linolelaidic acid contributes to the pool of *cis* 18:1 via de novo synthesis from acetate produced in its catabolism as opposed to a pathway involving biohydrogenation. Thus, the elevated level of 18:1 indicates that linolelaidate does not inhibit de novo fatty acid synthesis or impair 9-desaturase activity in animals fed diets containing *trans* fatty acids.

It appears paradoxical that linolelaidic acid inhibits 6-desaturase activity but enhances the formation of 18:1 in vivo, that is, 9-desaturase activity, particularly as the activity of both enzymes are elevated in an EFA deficiency produced by feeding either a fat-free or an HCO-supplemented diet (19-21). However, as pointed out by Peluffo et al. (22) and demonstrated by others (23,24), the 6- and 9-desaturase enzyme systems respond differently to a number of dietary and hormonal stimuli. Several investigators (25-27) have suggested that desaturase activity is regulated, in part, by the synthesis of enzyme protein, whereby enzyme concentration is a factor. In accord with this hypothesis, it has been shown that the level of 9-desaturase is elevated in an EFA deficiency (25). The elevation of desaturase activity in animals with an EFA deficiency has been studied extensively by Peluffo et al. (22) and by Holloway and Holloway (25). The former investigators found that 6-desaturase activity correlated with changes in the triglyceride-phospholipid ratio. Both groups of investigators considered that the increase in desaturase activity might be related to membrane fluidity in which enzyme activity was increased to maintain the ratio of unsaturated to saturated fatty acids. However, unsaturation of the tissues is decreased by the TRANS supplement more than by the HCO dietary fat. Thus, *trans* acids apparently have a direct effect on the 6-desaturase enzyme system. Moreover, it seems unlikely that the effect of *trans* acids on 6-desaturase can be explained on the basis of decreased protein synthesis inasmuch as 9-desaturase activity apparently is elevated when these acids are fed as the sole source of fat in the diet.

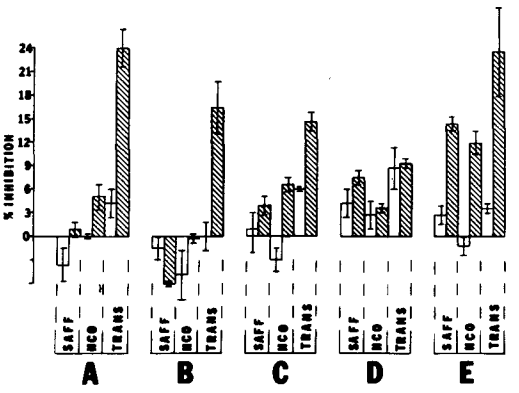

FIG. 3. Inhibition of the desaturation of linoleic acid by liver microsomal fractions obtained from rats fed diets supplemented with safflower oil (SAFF), hydrogenated coconut oil (HCO) or a concentrate of *trans* acids (TRANS) with A, palmitic acid (16:0); B, stearic acid, (18:0); C, oleic acid (*c*-18:1); D, elaidic acid (*t*-18:1); E, linolelaidic acid (*t,t*-18:2). Open bars, 20 nmol, and hatched bars, 200 nmol of each acid with 200 nmol of [1-^{14}C]linoleic acid. Inhibitions were significant for 200 nmol of all fatty acids with the microsomal fraction of the TRANS group as follows: 16:0, $P < .001$; 18:0, $P < .025$; *c*-18:1, $P < .001$; *t*-18:1, $P < .001$; *t,t*-18:2, $P < .005$.

Brenner and Peluffo (3) showed that both linolelaidate and elaidate exhibited an inhibitory effect in vitro on the desaturation of linoleic acid by the rat liver microsomal fraction of EFA-deficient animals. Recently, Mahfouz et al. (28) demonstrated that *trans* monounsaturated acids inhibit 9- as well as 5- and 6-desaturase activity of the liver microsomal fraction of EFA-deficient rats in in vitro experiments. The in vitro experiments reported herein show that *trans* acids inhibit 6-desaturase activity of the liver microsomal fraction of animals fed a diet containing adequate linoleate (SAFF group) and animals fed the TRANS supplement, devoid of EFA and presumably highly deficient in EFA as well as animals deprived of adequate linoleate by feeding hydrogenated coconut oil. However, of particular significance is the observation that the liver microsomal fraction of the animals receiving the TRANS-supplemented diet are more sensitive to the inhibitory effects of fatty acids than that of either the HCO or SAFF groups. Thus, it is apparent that these fatty acids alter the physical properties of the 6-desaturase enzyme system in some manner. It has been shown that acyl desaturases have a lipid requirement (29-31), hence the effect on the 6-desaturase system by *trans* acids might result from an alteration of the composition of the lipid cofactor.

The nutritional data indicate that low growth rate in the animals of the HCO and TRANS groups is related to low feed efficiency, which in turn is associated with the EFA deficiency in these groups of animals. The fact that the growth rate was lower in the animals of the 20% than in the 5% HCO group indicated that the high level of saturated dietary fat increased the EFA requirement in this group. However, the high level of saturated fat did not inhibit the conversion of linoleic to arachidonic acid as the animals of the 20% HCO group exhibited only a borderline EFA deficiency as evidenced by the levels of linoleate and arachidonate in the microsomal tissue lipid. The increased requirement of EFA in diets containing high levels of saturated fat is generally explained on the basis of the importance of dietary linoleate for the utilization of saturated fatty acids (32-35). However, Privett et al. (36,37) observed that both fish oils and concentrates of eicosapentaenoic and docosahexaenoic acids which did not cure an EFA deficiency stimulated the growth of EFA-deficient animals. Moreover, Kurata and Privett (38) observed that dietary Menhaden oil inhibited the conversion of linoleate to arachidonate which apparently was due to the suppression of 6-acyl desaturase activity by eicosapentaenoic and docosahexaenoic acids. Thus, the nutritional effects of *trans* acids probably is largely due to inhibition of the 6-acyl desaturation system which increases the saturation of the tissue lipids and, in turn, the requirement for EFA or polyunsaturated fatty acids.

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